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Microplastics reduce eelgrass tolerance to heat stress with implications for restoration and blue carbon

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ABSTRACT

Seagrass beds are key blue-carbon ecosystems, yet their resilience is increasingly challenged by microplastic (MP) pollution and marine heatwaves (MHWs). We experimentally tested how these stressors, alone and combined, affect the seagrass *Zostera marina* (eelgrass) using a controlled mesocosm experiment grounded in multiple-stressor and trait-based ecological theory. Plants were grown for 43 days in sediments with or without polyethylene/polypropylene MPs and a simulated MHW, (+5 °C for 15 days) was imposed in the final phase. MP exposure significantly reduced rhizome elongation (−35%), total root length (−65%), and below-ground biomass, and depleted non-structural carbohydrates (NSC) in leaves and rhizomes (−35% to −40%). Warming alone stimulated leaf growth but further reduced NSC, while the MP × MHW interaction produced the lowest below-ground growth and carbohydrate reserves, consistent with synergistic stress predicted by multiple-stressor theory. MP exposure also reshaped the microbiome enriching putative sulfur-cycling taxa in the rhizosphere and indicating more reducing sediment conditions. With a carbon-balance and holobiont framework, MPs appear to constrain resource supply (oxygen and nutrients) and increase maintenance costs, whereas warming amplifies metabolic demand. The resulting carbon deficit limits below-ground growth, traits that underpin restoration success and blue-carbon function. These findings show the importance of incorporating microplastic monitoring into seagrass management to anticipate cumulative stress under a warming ocean.

1. Introduction

Coastal blue carbon ecosystems (CBE), tidal marshes, mangroves and seagrass meadows, play a key ecological role by providing key ecosystem services such as nutrient cycling, shoreline protection, sediment stabilization and long-term organic carbon sequestration (Macreadie et al., 2021; Infantes et al., 2022; Jiménez-Ramos et al., 2025).Among these, seagrasses are globally distributed marine angiosperms with one of the highest capacities for organic carbon accumulation among vegetated ecosystems (de los Santos et al., 2023; Monnier et al., 2022).However, the capacity of CBE to sustain these services is increasingly challenged by anthropogenic stressors that reduce plant performance and compromise restoration success (Mazarrasa et al., 2021; van Katwijk et al., 2016; Coals et al., 2025). Environmental stressors reducing seagrass growth often also weaken the blue carbon sink function of these systems (Ren et al., 2024; Jiménez-Ramos et al., 2024a; Dahl et al., 2018). This occurs through both direct and indirect pathways, decreased primary production reduces autochthonous carbon inputs (Champenois and Borges, 2021; Kindeberg et al., 2024) and the loss of canopy density increases sediment resuspension and organic matter remineralization (Egea et al., 2023a; Yamuza-Magdaleno et al., 2024). Thus, stressors affecting plant growth and survival can trigger trait-mediated feedbacks that alter sediment stability and long-term carbon storage.

Coastal seagrass meadows are increasingly recognized as sinks for plastic pollution, including microplastics (MPs; 1 μm - 5 mm; Kershaw, 2015). Composed mainly of polyethylene (PE) and polypropylene (PP), MPs enter coastal systems from land and ocean-based sources, and can be trapped by the structural complexity of seagrass canopies (de los Santos et al., 2021; Egea et al., 2023b). While the ecological effects of

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MPs on marine animals are well documented (Gall and Thompson, 2015; Li et al., 2016), their impacts on seagrasses and associated carbon-cycling processes remain comparatively understudied (Li et al., 2023).This gap partly reflects the challenges of studying emerging contaminants in foundation species that form complex interactions with associated macro- and microorganisms (i.e., microbiome; Crump et al., 2018), while simultaneously being exposed to multiple, co-occurring stressors whose combined effects are often difficult to disentangle (Egea et al., 2026a). Addressing these challenges requires experimental approaches that integrate multiple stressors and explicitly consider host–microbiome interactions, which are increasingly recognized as critical for seagrass resilience, conservation, and restoration under future global change scenarios (Nguyen and Winters, 2025).

At the sediment-root interface, MPs can modify physical and biogeochemical properties by altering porosity and permeability, reducing porewater exchange and oxygen delivery to roots (Kalčíková et al., 2017). These changes can enhance reducing conditions, increase sulfide accumulation, and impair root elongation and nutrient uptake. MPs can also release additives and dissolved organic carbon (DOC), stimulating microbial activity and altering biogeochemical cycling (Egea et al., 2024a). According to eco-physiological and carbon-balance theory (Chapin et al., 1990; Kooijman, 2010), these constraints elevate maintenance costs (e.g., detoxification, membrane repair) while limiting resource acquisition, leading to depletion of non-structural carbohydrate (NSC) reserves and slower growth.

In parallel, MPs can restructure the seagrass microbiome in their aboveground (phyllosphere) and belowground (rhizosphere), a key component of the seagrass holobiont that contributes to nutrient cycling, disease suppression, and stress tolerance (Ugarelli et al., 2017; Wang et al., 2021). The seagrass microbiome carries functions that benefit the host plant, including enhancing nutrient availability and growth (Tarquinio et al., 2019), increasing community production (Zhang et al., 2023), and even strengthening the resistance to environmental stressors (Crump et al., 2018). By altering redox conditions and nutrient gradients, MP exposure may favor sulfur-cycling microbial taxa (Desulfobacterota, Campylobacterota), typical of more anoxic environments (Walker et al., 2024), consistent with holobiont theory and stress-induced microbiome instability, as described by the "Anna Karenina" principle of increased variability under stress (Zaneveld et al., 2017).

Alongside pollution, marine heatwaves (MHWs), discrete periods of anomalously high seawater temperature, are becoming more frequent and intense (Hobday et al., 2016). In temperate seagrasses such as *Z. marina*, moderate warming can temporarily stimulate productivity, whereas prolonged or extreme warming often causes respiration to exceed photosynthesis, resulting in negative carbon balance and depletion of carbohydrate reserves (Lee et al., 2007; Egea et al., 2018, 2024b; Moreno-Marín et al., 2018; Kaldy et al., 2022). Although multiple-stressors experiments in seagrasses have gained relevance because they better reflect real-world conditions (Stockbridge et al., 2020; Egea and Jiménez-Ramos, 2025a), the effects of sequential stressor combinations, remain poorly understood. Sequential stress designs are particularly appropriate for episodic stressors MHW which occur over days to weeks in contrast, to chronic stressor such as MPs that persist in sediments for years (Oliver et al., 2019; Ostrowski et al., 2025; Chaudhary et al., 2025; Egea et al., 2026b). Understanding how acute thermal events interact with ongoing pollution stress is therefore critical for predicting seagrass responses under future global change.

According to the Metabolic Theory of Ecology, MTE (Brown et al., 2004), temperature increases metabolic rates following the Q_{10} relationship, accelerating both photosynthesis and respiration, but respiration usually rises faster, reducing net carbon gain. Simultaneously, oxygen solubility in seawater declines, limiting diffusion into sediments and roots. These combined effects create a metabolic squeeze, where energy demand and oxygen limitation converge, conditions that are particularly detrimental for rooted plants in soft sediments (Lee et al., 2007). Warming also affects sediment microbial processes, enhancing sulfate reduction and sulfide generation, which can damage root meristems and inhibit nutrient uptake (Holmer and Hasler-Sheetal, 2014; Walker et al., 2024). Within a multiple-stressor framework, such changes can intensify the negative effects of MPs as both stressors reduce oxygen availability: MPs by restricting supply and MHWs by increasing demand. Therefore, rather than simple additive responses, these stressors can potentially trigger synergistic effects.

To interpret these processes mechanistically, we use three complementary theoretical perspectives. First, multiple-stressor theory predicts that co-occurring stressors interact through additive, synergistic, or antagonistic effects (Crain et al., 2008). Detecting whether the combined effects of MPs and MHWs are worse-than-additive provides a diagnostic for underlying physiological interactions. Second, carbon-balance and Dynamic Energy Budget (DEB) theory describe how stress shifts resource allocation between maintenance, growth, and storage. Under stress, rising maintenance costs and falling resource uptake deplete NSC reserves, constraining growth and recovery potential (Kooijman, 2010). Third, trait-based response-effect frameworks (Suding et al., 2008) link measurable plant traits (e.g., rhizome elongation, root length, below-ground biomass) to ecosystem functions such as sediment stabilization and carbon burial. Declines in these traits can therefore be interpreted as early indicators of reduced ecosystem resilience and blue-carbon potential. Finally, we consider the holobiont perspective, recognizing the seagrass-microbiome association as a functional unit. Microbial shifts toward anaerobic or sulfur-cycling taxa under MPs and MHWs may represent feedbacks to redox stress or altered carbon fluxes, influencing plant resilience and recovery (Martin et al., 2018; Vilaplana et al., 2025).

In this study, we used a 43-day mesocosm experiment to test how microplastic pollution and a simulated marine heatwave affect *Z. marina* growth, carbon reserves and associated microbiome structure. Specifically, we addressed: (1) How MPs and elevated temperature, alone and in combination, affect eelgrass growth, biomass allocation and carbohydrate reserves? (2) Do these stressors alter the structure of the eelgrass associated microbiome, particularly sulfur-cycling taxa in the rhizosphere?(3) How these responses can be interpreted through multiple-stressor, carbon-balance, trait-based, and holobiont frameworks to infer implications for seagrass resilience, restoration, and blue-carbon function? This study advances understanding of how global change stressors interact to influence the resilience of a key foundation species in coastal blue-carbon ecosystems by integrating morphological, physiological, and microbial responses within a sequential stree-exposure design. By combining multiple-stressor, carbon-balance, trait-based, and holobiont perspectives, it provides a mechanistic assessment of how microplastic pollution may alter seagrass performance, restoration potential, and blue-carbon functioning under ocean warming.

2. Material and methods

2.1. Experimental design

The experiment was conducted in indoor mesocosms at the Kristineberg Center for Marine Research and Innovation (Sweden) between June-September 2024 to test the interactive effects of microplastic (MP) pollution and a marine heatwave (MHW) on the seagrass *Zostera marina* (eelgrass). The design followed a controlled factorial approach, with MPs as a sediment stressor and a simulated MHW applied during the final phase to assess whether both stressors act additively or synergistically, a prediction derived from multiple-stressor theory (Crain et al., 2008; Piggott et al., 2015). Surface sediment (top ~20 cm) was collected from Bökevik Bay in the Güllmar Fjord, Swedish Skagerrak coast (58°14′N, 11°26′E), using a 0.35 m × 0.35 m stainless-steel box from a vessel at 11-12 m depth (Fig. 1a). Environmental and sedimentary characteristics of the site are detailed in the Supplementary Material. Eelgrass shoots were collected at ~1 m depth by SCUBA diving,

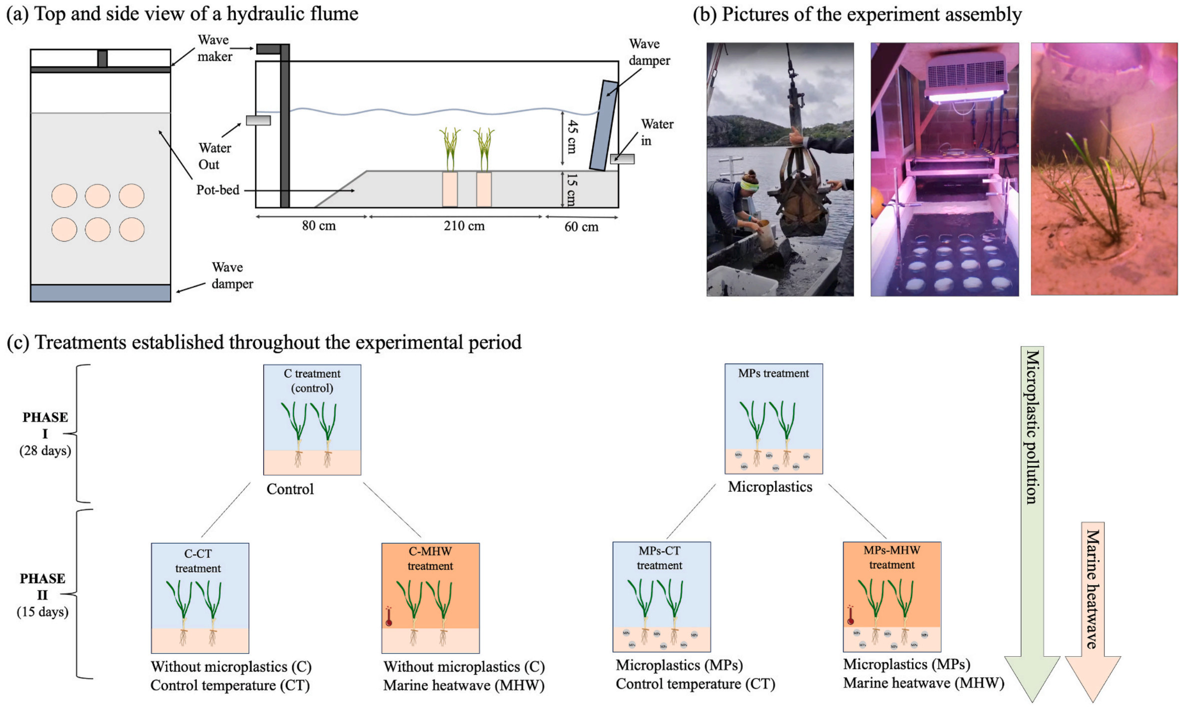

Fig. 1. Experimental setup. (a) Schematic diagram of the hydraulic flume tank used in the mesocosms experiment. (b) Photographs showing (from left-right) the box-corer during sediment collection, the "pot-bed" arrangement within a flume tank, and the standardized eelgrass shoots during experiment period. (c) Temporal sequence of the 43-day experiment showing 2 phases and treatments applied.

transported to the laboratory in aerated seawater, and standardized by trimming rhizomes to 4 cm (3-4 internodes) and roots to ~2 cm length each (4 roots per shoot). This ensured uniform initial biomass (0.69 ± 0.014 g fresh weight FW, per shoot) and minimized confounding size effects, an important step when comparing physiological responses across stressors (Lee et al., 2007).

The mesocosm system consisted of four open-flow wave flumes (350 × 60 × 80 cm) each connected to a continuous seawater supply (3.6 L min^{-1}) from the fjord. Each flume was equipped with an electronic piston wave generator (0.5 Hz frequency) and a wave dampener (20° slope) to reduce reflections, thereby simulating natural hydrodynamic conditions at the low-energy sites where *Z. marina* typically occurs (Infantes et al., 2021, Fig. 1). Gentle background oscillatory flow (5 cm/s) and controlled light photoperiod were maintained using Helio-Spectra LED lamps providing 140 μmol photons $\text{m}^{-2}\ \text{s}^{-1}$ for 14 h daily (≈7.1 mol photon $\text{m}^{-2}\ \text{d}^{-1}$). Light levels were chosen to match optimal eelgrass photosynthesis rates under summer conditions in the region (Olesen and Sand-Jensen, 1993).

Hydrodynamic uniformity was verified using an acoustic Doppler velocimeter (ADV, Nortek Vectrino) sampling at 25 Hz, with a 7 mm measurement volume positioned 10 cm above the bottom and 5 cm in front of the planted pots to prevent leaf interference (Zenone et al., 2022). Measured near-bed flow velocities confirmed consistent conditions across flumes, ensuring that observed differences were attributable to the experimental treatments rather than hydrodynamic artifacts. Incoming seawater chemistry remained stable throughout the 43-day period, with mean (±SD) nutrient concentration of 0.3 ± 0.17 μM NH_4^+, 1.13 ± 0.87 μM NO_3^- and 0.22 ± 0.04 μM PO_4^{3-} (n = 32) showing no significant differences among tanks. This mesocosm configuration provided a controlled yet ecologically realistic environment in which hydrodynamic forcing, light, and temperature could be independently manipulated. The design ensured that any treatment-induced changes in seagrass traits, rhizome elongation, root growth, or NSC content, could be attributed to the imposed stressors rather than environmental variations.

We established 24 experimental units, each consisting of a PVC pot (10 cm diameter x 12.7 cm height, 1 L volume), filled with homogenized sediment and two standardized eelgrass shoots (see Section 2.3). Pots were inserted into custom-built "pot beds" (240 × 60 cm PVC platform with 8 equidistant holes, 10 cm diameter) fixed along the bottom of each flume tank, ensuring even spacing and consistent hydrodynamic exposure (Fig. 1b).

To simulate environmentally relevant microplastic concentration levels, half of the pots were amended with a mixture of polyethylene (PE) and polypropylene (PP) particles. The MPs were generated from transparent LDPE and PP plastic containers (Rotviksbro Handel AB, Uddevalla, Sweden) using a bullet blender to produce irregular spherical fragments representative of environmentally weathered plastics. Particle size analysis was conducted using an Olympus BX50 microscope coupled with cellSens software (Olympus, Tokyo, Japan) yielded mean diameters of 562 ± 34 μm for LDPE and 856 ± 61 μm for PP, (n = 5) at a concentration of ~870 particles kg^{-1} dry sediment, which is in the upper range of MPs concentrations reported in seagrass meadow sediments (e.g., Huang et al., 2020; Dahl et al., 2021) and comparable to values observed in sediments from the Baltic and North Seas (mean ± SD: 863 ± 1371 particles kg-1 dry sediment; Chubarenko et al., 2022). MPs were gently mixed into the sediment before planting to achieve a homogeneous distribution. Pots without MPs served as controls. All pots were randomly distributed across flumes (8 pots per flume, 50% with MPs) to minimize positional bias.

A simulated marine heatwave was applied during the second half of the experiment following the widely accepted definition of Hobday et al. (2016), a period of anomalously warm seawater exceeding the local 90th percentile of historical temperature observations for at least five consecutive days. Prior to the experiment MHW in the area, characteristics for the Gullmar Fjord were quantified using the Marine Heatwaves Tracker app (Schlegel, 2024). Based on these climatological data, a realistic MHW temperature of 23.3 ± 0.8 °C was selected, representing a +5.1 °C anomaly above the local summer mean (18.2 ± 1 °C) and corresponding to 'severe' category heatwave experienced in the region within the last 5 years (Fig. S1 in Supplementary Material).

The 43-day experiment consisted of two sequential phases (Fig. 1c).

Phase I (Days 0-28) evaluated the effects of MP exposure under ambient summer temperature (18.2 ± 1 °C) by comparing control pots containing clean sediments with those with MPs. **Phase II** (Days 28-43) simulated a marine heatwave (MHW) by gradually increasing the seawater temperature in two of the four flumes to 23.3 ± 0.8 °C, while the remaining two flumes were maintained at ambient temperature. This factorial design resulted in four treatment combinations: a Control treatment (no MPs under ambient temperature), an MP treatment (MP-amended sediment under ambient temperature), an MHW treatment (no MPs under elevated temperature), and a combined MPs + MHW treatment (MP-amended sediment under elevated temperature).

At the end **Phase I**, two pots (one Control, one MP) were collected from each of the four flumes, giving four independent replicates per treatment ($n = 4$). During **Phase II**, four pots per flume were sampled (two Control, two MPs), yielding eight pots per temperature regime. Although the design involved some degree of pseudoreplication at the pot level, tanks served as the true replicates for the temperature factor, while pots within tanks were treated as nested subsamples (Underwood, 1997). This approach is common in mesocosm ecology, where logistical constraints limit full replication (Hurlbert, 1984). The four flume tanks were identical in dimension, materials and environmental control, ensuring consistent light, temperature and water flow across treatments. Continuous seawater renewal (3.6 L min^{-1}) from the Güllmar fjord maintained natural nutrient levels and prevented accumulation of metabolic byproducts. Key physical and chemical parameters (seawater temperature, salinity, pH and flow velocity) were measured daily at the tank edges and centers, confirming negligible variability among flumes and verifying that treatment differences resulted from the experimental factors rather than uncontrolled tank effects.

In total, 24 pots (two plants per pot) were distributed evenly across flumes to ensure balanced replication of treatments and sufficient material for response variable measurements (growth, NSC, and microbiome composition). For the analysis of Phase II, data from pots within each tank were amalgamated to represent tank-level means, thus avoiding inflation of degrees of freedom and ensuring valid inference on the true experimental unit (Oksanen, 2001; Quinn and Keough, 2002). This design allowed testing of both main effects (MPs, MHW) and their interaction, directly addressing predictions from multiple-stressor theory regarding additive versus synergistic impacts on seagrass performance.

2.2. Measurement of response variables in plants

At the end of each experimental phase, plants were carefully taken from pots to preserve the integrity of both above- and below-ground tissues, including rhizomes and roots. Each plant was gently rinsed with filtered seawater to remove residual sediment, and tissues were separated into aboveground (leaves) and belowground (rhizomes and roots) fractions. Fresh weight (FW) was recorded separately for each fraction, and morphometric measurements were conducted to evaluate changes in plant growth and structure. Rhizome elongation (rhizome length; cm) was calculated as the difference between the final and initial rhizome lengths divided by the duration of the experimental period. The total root length (TRL; cm shoot^{-1}) was quantified using a semi-automated image-base method widely applied in plant morphology studies (e.g., Ziegler et al., 2023). Root systems were scanned at 600 dpi using an Epson XP-2200 flatbed scanner, and the resulting high-resolution images were analyzed in Image-J software (NIH, USA) with the SmartRoot plugin (Lobet et al., 2011), a semi-automated tracing algorithm designed for root structure analysis. Each image was calibrated using a reference scale scanned concurrently with the sample to ensure accurate pixel-to-millimeter conversion. The leaf growth rate (LGR; cm shoot^{-1} d^{-1}) was determined using a modified Zieman punch method, a standard approach in seagrass studies (Jiménez-Ramos et al., 2024b). A small reference hole was made just above the ligule of the youngest leaf to track new growth, and leaf elongation was measured at the end of the experiment. This process was repeated on day 31 to facilitate LGR calculations for Phase II. Net production rate (NPR; mg FW shoot^{-1} d^{-1}) was calculated as the difference between final and initial shoot fresh biomass divided by the number of days in the experimental. All equations used to derive plant growth metrics are presented in Table S1 (Supplementary Material).

Non-structural carbohydrate (NSC) content, including sucrose and starch, was analyzed separately for above- and below-ground tissues following established seagrass protocols (Alcoverro et al., 1999). Dried leaves and rhizome samples were ground to a fine powder using a ball mill. Soluble sugars were extracted through four sequential washes in 96% (v/v) ethanol at 80 °C for 15 min each. The combined ethanol extracts were evaporated under a gentle air stream of 40 °C and redissolved in 10 mL of deionized water. Starch remaining in the ethanol-insoluble residue was extracted by incubation in 1 N NaOH for 24 h. Sucrose and starch concentrations were then determined spectrophotometrically using the resorcinol (for sucrose) and anthrone (for starch) assay at absorbances wavelengths of 486 and 640 nm, respectively (Yemm and Willis, 1954). This combination of morphometric and biochemical indicators provided an integrated assessment of plant performance, linking morphological responses (growth and biomass allocation) with physiological responses (carbon reserve dynamics) under the imposed stressor treatments.

2.3. Sampling collection and measurement for associated microbiome

Microbiome samples were collected and processed following procedures for seagrass-associated microbial communities (e.g., Wang et al., 2021; Egea and Jiménez-Ramos, 2025b). For each treatment and experimental phase, three replicate shoots were aseptically collected using sterile gloves to avoid cross-contamination. Roots and leaves were separated immediately in the laboratory and placed in sterile 50 mL vials. From each shoot, an 8-cm segment of the second youngest leaf from each plant was cut and gently rinsed three to five times with sterile seawater to dislodge loosely attached sediment while preserving the rhizoplane microbial community. Cleaned tissue samples were then divided by compartment: leaves representing the phyllosphere and roots representing the rhizosphere. Each sample was preserved in DNA/RNA Shield (Zymo Research, CA, USA) with sterile DNA LoBind tubes and stored at −80 °C until DNA extraction. This approach preserved both aerobic and anaerobic taxa and ensured consistent preservation across treatments, a critical step for reproducible microbial community analyses under the holobiont framework.

Sequencing and bioinformatic analyses were performed by Novogene GmbH (Munich, Germany). DNA concentration was standardized across samples. The V4-V5 region of the 16S rRNA gene was amplified using the primers 515F (GTGCCAGCMGCCGCGGTAA) and 907R (CCGTCAATTCCTTTGAGTTT) (Westermann et al., 2017), and library preparation was performed using the NEBNext® Ultra™ II DNA Library Prep Kit with index adapters synthesized in-house by Novogene. Qubit and real-time PCR were used to enumerate the resultant libraries, and a bioanalyzer was employed to assess the fragment size distribution. Libraries were then sequenced on the Illumina NovaSeq 6000 SP platform (Caporaso et al., 2012) with 150 bp paired-end reads, which yielded 5.84 million read-pairs. The chimeric sequences in Clean Tags were detected and removed, leaving the Effective Tags which were used for subsequent analysis. Reads were clustered into operational taxonomic units (OTUs) at 97% similarity using the default Opti clustering method implemented in mothur (Westcott and Schloss, 2017). Together, these analyses captured both compositional and structural aspects of the seagrass microbiome response to combined stressors.

2.4. Implementation of quality assurance and quality control QA/QC procedures

Quality assurance and quality control (QA/QC) procedures were implemented throughout the experiment and analytical workflows. Sediments were homogenized prior to pot filling to ensure consistent physical and chemical properties across treatments. Experimental units were randomly distributed among flumes to minimize positional and tank effects. Plant material was standardized before planting, and all samples were processed using identical protocols. For microbiome analyses, sterile techniques were applied during sampling, negative controls were included during DNA extraction and amplification, and chloroplast, mitochondrial, and low-quality sequences were removed during bioinformatic processing.

2.5. Data and statistical analyses

All statistical analyses and plots were conducted in R 4.4.1 (R Core Team, 2024). Statistical procedures were designed to evaluate both the main and interactive effects of microplastics (MPs) and marine heatwaves (MHWs) on plant and microbiome response variables, consistent with the multiple-stressor framework (Crain et al., 2008; Piggott et al., 2015). Generalized linear models (GLMs) were used to evaluate the effect of stressors on response variables in each experimental phase. Assumptions of normality and homoscedasticity were tested through Shapiro-Wilk and Levene's tests. All response variables were initially modeled with a Gaussian distribution and identity link. However, when the residuals of the model did not meet normality, GLMs with a Gamma error distribution and reciprocal link function were used instead.Post hoc comparisons between treatments were completed using estimated marginal means with Bonferroni correction (“emmeans” R package; Lenth, 2024). The significance level (α) set for all tests was 0.05.

For microbiome analysis, OTUs grouped into phyla were plotted (chloroplast, mitochondria and others rated as low-quality OTUs were prior removed). Changes in bacterial diversity (i.e., the number of different OTUs observed) and abundance (i.e., the number of reads per OTU) was assessed by bacterial families. Rarefaction curves were deployed to evaluate differences among treatments (including Phase I and II simultaneously) through rarefaction and extrapolation implemented in the ‘iNEXT’ R package (Hsieh et al., 2016). Microbiome dissimilarity between seagrass compartments (i.e., phyllosphere and rhizosphere) and among treatments were tested using non-metric multidimensional scaling (NMDS) according to the Bray-Curtis distance (Oksanen et al., 2024). The significance of differences among obtained clusters were further tested by permutational multivariate analysis of variance (PERMANOVA) with 999 permutations implemented using the adonis function from the ‘vegan’ package. The taxa which most contributed to the observed dissimilarity among treatments were reported by using similarity percentage analysis (SIMPER) with 999 permutations. The top-ranked OTUs in this analysis were overlaid on the NMDS graph with the ‘envfit’ function (a list of all OTUs obtained from ‘envfit’ results is provided as Table S2 in the Supplementary Material). Moreover, to better analyze the differences in bacterial assemblage among treatments, the top OTUs with higher (|>0.5%|) average difference in relative abundances between treatments and its control counterpart were plotted.

3. Results

3.1. Effects on Z. marina morphometry and production

Microplastic (MP) exposure significantly impacted the structural and growth-related traits in *Z. marina*, particularly in the belowground compartment (Fig. 2; Table S3). Rhizome elongation decreased by an average of −34.9% in Phase I and −15.2% in Phase II, while the total roots length (TRL) declined sharply by −65.4% and −58.6%, respectively. These reductions led to a pronounced decrease in below-ground biomass, significant in Phase I (−34.9%) (Fig. 2 and Table S3 in Supplementary Material). Similarly, the simulated marine heatwave reduced belowground traits, with declines of −22.2%, −29.7% and −18.6% in rhizome length, TRL and belowground biomass, respectively.

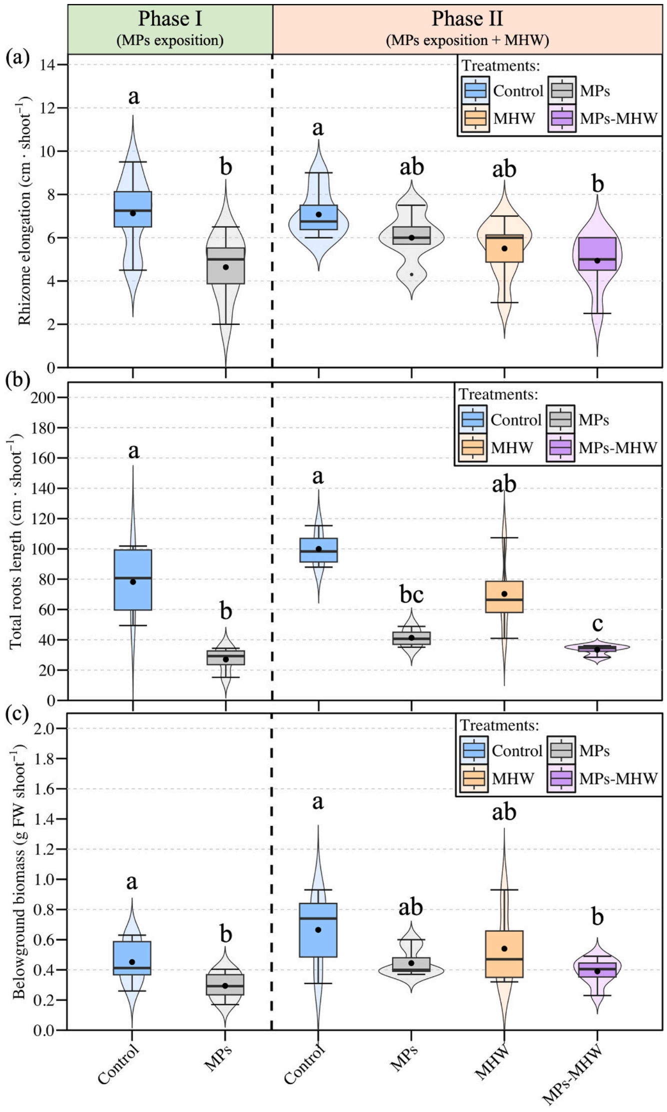

Fig. 2. Changes in *Zostera marina* below-ground traits during the experimental period. (a) Rhizome elongation, (b) total root length (TRL), and (c) belowground biomass during the experimental period. Each violin plot includes an internal boxplot in black showing the mean (back point), interquartile range (box), median (black line) and minimum and maximum values (whiskers). The width of the violin reflects the kernel density of the data distribution. Vertical dotted lines separate experimental phases (Phase I and Phase II). Different letters denote statistically significant differences among treatments that are independent of the phases ($p < 0.05$).

When both stressors co-occurred, their effects were synergistic, producing the lowest values observed across all metrics. In the combined MPs-MHW treatment, in rhizome elongation fell by −30.2%, TRL by −66%, and belowground biomass by −41%. This interactive reduction supports predictions from multiple-stressor theory (Crain et al., 2008; Piggott et al., 2015), where co-occurring physical and thermal constraints jointly limit resource acquisition and storage.

Although MPs were introduced only into the sediment, their influence extended to the aboveground tissues. Leaf growth rate (LGR) decreased in MP-treated plants by −38.8% and −22.7% in Phase I and II, respectively (Fig. 3a). In contrast, exposure to the MHW alone led to a relative but not significant 30.9% increase in LGR, corresponding with a 41.5% rise in above-ground biomass (Fig. 3b). Consequently, the above-ground to below-ground (AG:BG) biomass increased significantly under elevated temperature, from 1.38 in the control to 2.17 in the MHW

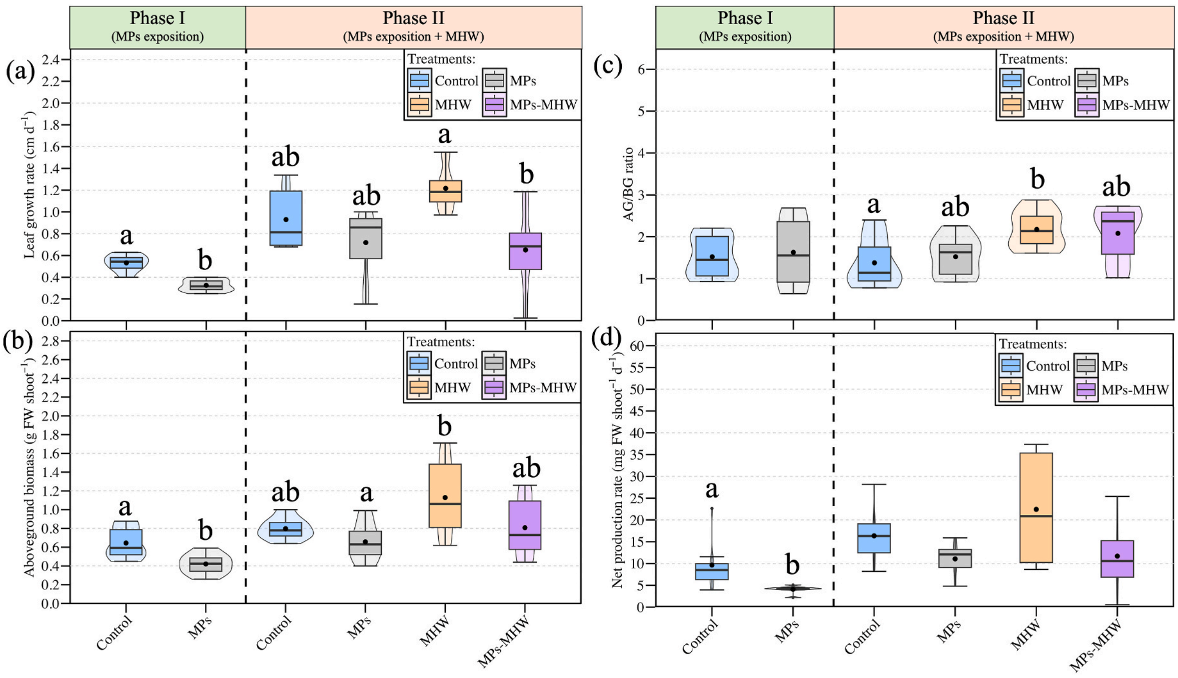

Fig. 3. Changes in *Zostera marina* above-ground traits during the experimental period. (a) Leaf growth rate (LGR) and (b) above-ground biomass, (c) above-to below-ground (AG:BG) biomass ratio, and (d) net production rate (NPR) under the treatments. Each violin plot and boxplot element (mean, interquartile range, median, and whiskers) are presented as described in Fig. 2. Vertical dotted lines separate experimental phases (Phase I and Phase II). Different letters denote statistically significant differences among treatments ($p < 0.05$).

treatment (Fig. 3c), indicating a shift in carbon allocation toward photosynthetic tissues under thermal stress. However, this apparent compensation did not translate into higher overall productivity. The net production rate (NPR), which integrates both growth and metabolic cost, showed consistent declines under MP exposure (−57.5% in Phase I and −32.3% in Phase II), with statistical significance reached in Phase I (Fig. 3d; Table S4). This reduction in NPR, alongside depleted below-ground biomass, is consistent with carbon-balance and trait-based theories predicting that stress-induced increases in maintenance costs and reduced resource acquisition deplete non-structural carbohydrate (NSC) reserves, ultimately constraining growth and resilience.

3.2. Physiological responses of *Z. marina*

Non-structural carbohydrates (NSC) declined under both stressors, with the largest reductions recorded in the warming treatments (Fig. 4

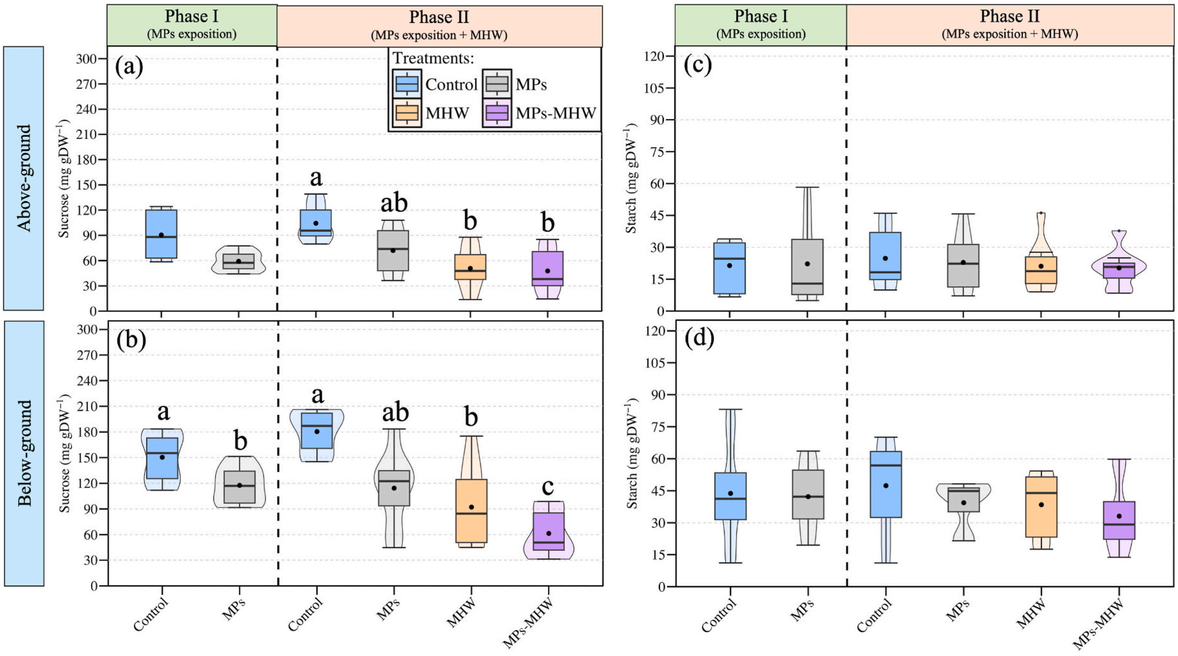

Fig. 4. Changes in non-structural carbohydrates (NSC) content of *Zostera marina* during the experiment. Panels a, c) represent above-ground tissues and (b, d) below-ground tissues. Violin and boxplot details follow Fig. 2; vertical dotted lines indicate Phase I and Phase II, and different letters denote significant differences among treatments ($p < 0.05$).

and Table S5). Microplastic exposure led to lower soluble carbohydrates (sucrose) content in both above-ground and below-ground tissues, averaging −34.6% and −31.0% in Phase I and II, respectively for leaves and −21.8% and −36.7% for rhizomes. These declines suggest that plants had fewer useable carbon reserves because maintenance demands increased while resource uptake decreased.

Warming alone caused an even sharper decline in sucrose, with mean losses of −51.4% in leaves and −48.9% in rhizomes. When combined with MPs, the effects were exacerbated, where sucrose content dropped by −54.3% and −66% in the MPs + MHW treatment compared with the control. This synergistic decrease suggests that elevated temperature amplified the carbon imbalance already induced by MPs, further reducing the plant's capacity to replenish reserves.

Starch concentrations were consistently lower than sucrose values, averaging about one-third of total NSC (0.31-fold in leaves and 0.34-fold in rhizomes). Although differences among treatments were not statistically significant, a general declining trend −15% to −30.1% was observed under warming conditions, irrespective of MP exposure. Overall, these results indicate that both stressors constrained carbon storage, with combined exposure producing the strongest depletion of metabolic reserves in *Z. marina*.

3.3. *Effects on the associated microbiome*

A total of 3,330,325 raw reads were generated from bacterial 16S rRNA sequences across all samples. After quality filtering to remove low-quality, chloroplast, mitochondria and unclassified reads, 1,490,785 operational taxonomic units (OTUs) were retained for analysis. OTUs were classified into 447 bacterial families, with 314 families detected in the phyllosphere and 402 families in the rhizosphere (see repository

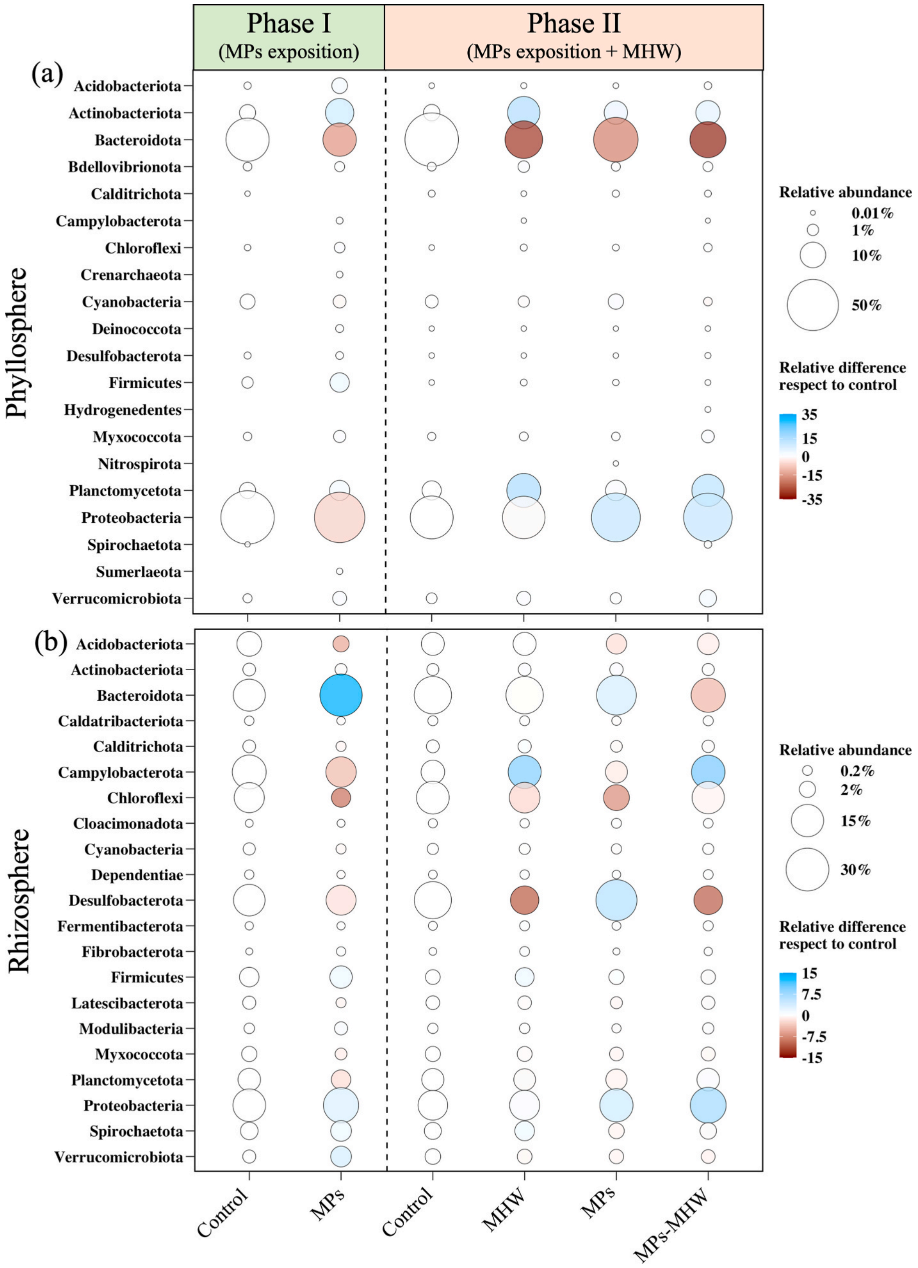

Fig. 5. Relative abundance of dominant bacterial phyla associated with *Zostera marina*. Bubble plots shows the main phyla detected in the (a) phyllosphere and (b) rhizosphere (relative abundance >0.1%). Bubble size indicates the mean relative abundance of operational taxonomic units (OTUs) in each treatment (%), while bubble color represents the direction of change compared with the control (blue = increase, red = decrease). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

dataset at 10.5281/zenodo.17603933). The dominant phyla were Proteobacteria and Bacteroidota in the phyllosphere, while the rhizosphere was dominated by Bacteroidota, Desulfobacterota, Proteobacteria, Campylobacterota and Chloroflexi (Fig. 5).

Rarefaction analysis indicated consistently higher diversity in the rhizosphere than in the phyllosphere, averaging, 1.9-fold greater richness, whereas the total abundance remained comparable between plant compartments and treatments (Fig. 6a and b). Although overall alpha-diversity and abundance did not differ significantly among treatments, revealed clear compositional changes associated with MP exposure.

In Phase I, microbial communities from MP-treated plants, both in phyllosphere and rhizosphere, were clustered separately from the controls, forming distinct branches in ordination space (PERMANOVA; Phyllosphere $F = 6.12$, p-value $= 0.0012$, and $R^2 = 0.33$; Rhizosphere $F = 3.43$, p-value $= 0.0029$, and $R^2 = 0.22$) (Fig. 6c and d; Fig. S2 in Supplementary Material). In Phase II, community differentiation among treatments was weaker, yet MP-exposed rhizosphere samples still grouped closely within their respective clusters, suggesting a partial persistence of the MP effect regardless of temperature condition.

Analysis of relative abundances showed that several bacterial families shifted noticeably under the different treatments (Fig. 7). MP exposure produced higher proportions of Flavobacteriaceae, Sulfurimonadaceae and Desulfocapsaceae in the rhizosphere, while Saprospiraceae and Rhodobacteraceae declined in the phyllosphere. The MHW treatment alone resulted in increased Sulfurovaceae but reduced Desulfobulbaceae in rhizosphere and a decrease in Rhodobacteraceae and especially in Saprospiraceae in the phyllosphere. When MPs and MHW were combined, community shifts more closely resembled those caused by MHW alone than those produced by MPs. However, the combined treatment also showed notable increases in Rhizobiaceae within rhizosphere and Rhodobacteraceae within phyllosphere.

Overall, the results indicate that MP exposure altered microbial community structure, most strongly during Phase I, while warming primarily modified specific sulfur- and nitrogen-cycling families. Combined stressors produced additive to moderately synergistic changes, with the strongest effects detected in the rhizosphere, where bacterial diversity and functional guild shifts were most pronounced.

4. Discussion

This study provides experimental evidence that microplastic (MP) contamination and marine heatwaves (MHWs) together reduce the growth, carbon storage, and microbiome stability of *Zostera marina*. MPs primarily impaired below-ground traits, including rhizome elongation, root length, and biomass, while also depleting non-structural carbohydrates (NSC). Warming alone stimulated short-term above-ground growth but further depleted carbohydrate reserves, particularly in rhizomes. When both stressors co-occurred, their effects were synergistic, resulting in the lowest growth, NSC, microbiome stability observed. Together, these patterns suggest that MPs restrict resource acquisition and oxygen supply, while MHWs amplify metabolic demand, producing an energetic imbalance that limits eelgrass resilience and ecosystem functions.

4.1. *Eelgrass responses to individual and combined stressors*

The decline in *Z. marina* growth under microplastic (MP) exposure indicates that MPs negatively affect processes at the sediment-root interface. In other systems, MPs have been shown to alter sediment physical properties such as permeability and porewater exchange (Kalčíková et al., 2017; Ge et al., 2021), potentially limiting oxygen and nutrient availability to roots. However, this mechanism is unlikely to explain our results, as the sediment used here was already fine-grained and muddy, conditions that inherently restrict porewater movement. Therefore, the strong reductions in rhizome elongation, root length, and below-ground biomass observed in this study are more plausibly associated with chemical and microbial alterations rather than bulk physical effects.

MPs can release dissolved organic compounds and additives that stimulate microbial activity and shift sediment redox dynamics (Yu et al., 2022; Egea et al., 2026b). The enrichment of sulfur-cycling bacterial taxa, such as Sulfurimonadaceae and Desulfocapsaceae in MPs-exposed rhizospheres suggests a tendency towards more reducing microenvironments. Such conditions are typically linked to sulfide accumulation, which can impair seagrass root metabolism and meristematic growth (Liu et al., 2018; Holmer and Hasler-Sheetal, 2014;

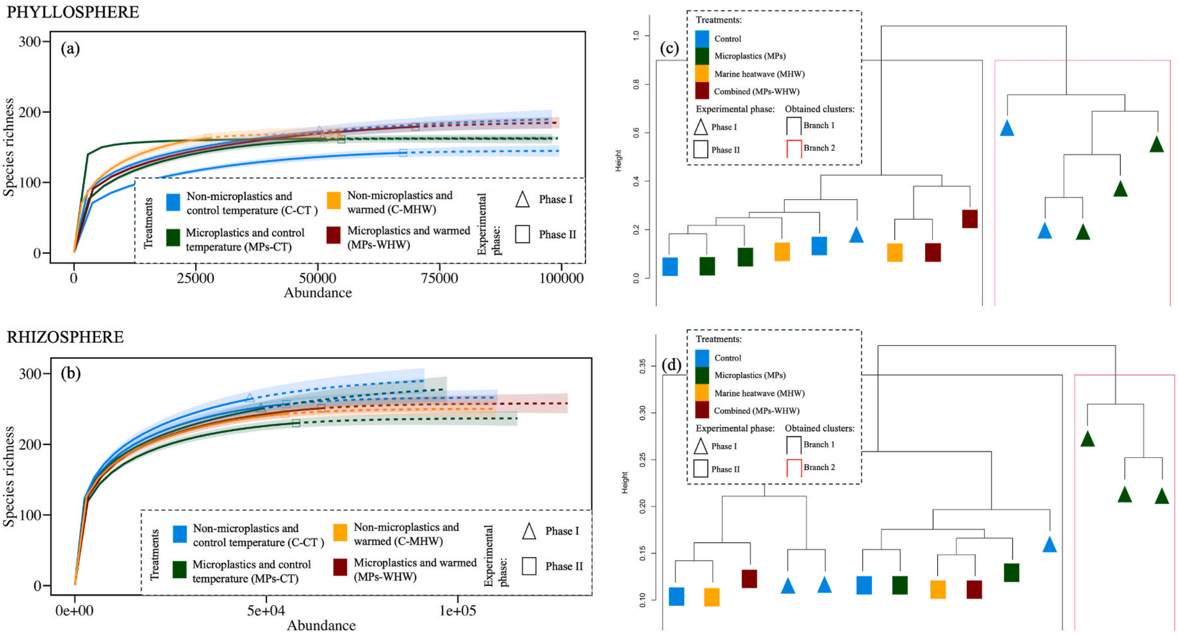

Fig. 6. Diversity and community structure of the *Zostera marina*-associated microbiome. Left panels: Rarefaction (interpolation; solid lines) and extrapolation (prediction; dotted lines) curves showing mean bacterial abundance and family-level richness for each treatment in (a) phyllosphere and (c) rhizosphere. Right panels: Dendrogram based on Bray-Curtis dissimilarity matrices illustrating clustering of microbial communities in (b) the phyllosphere and (d) the rhizosphere.

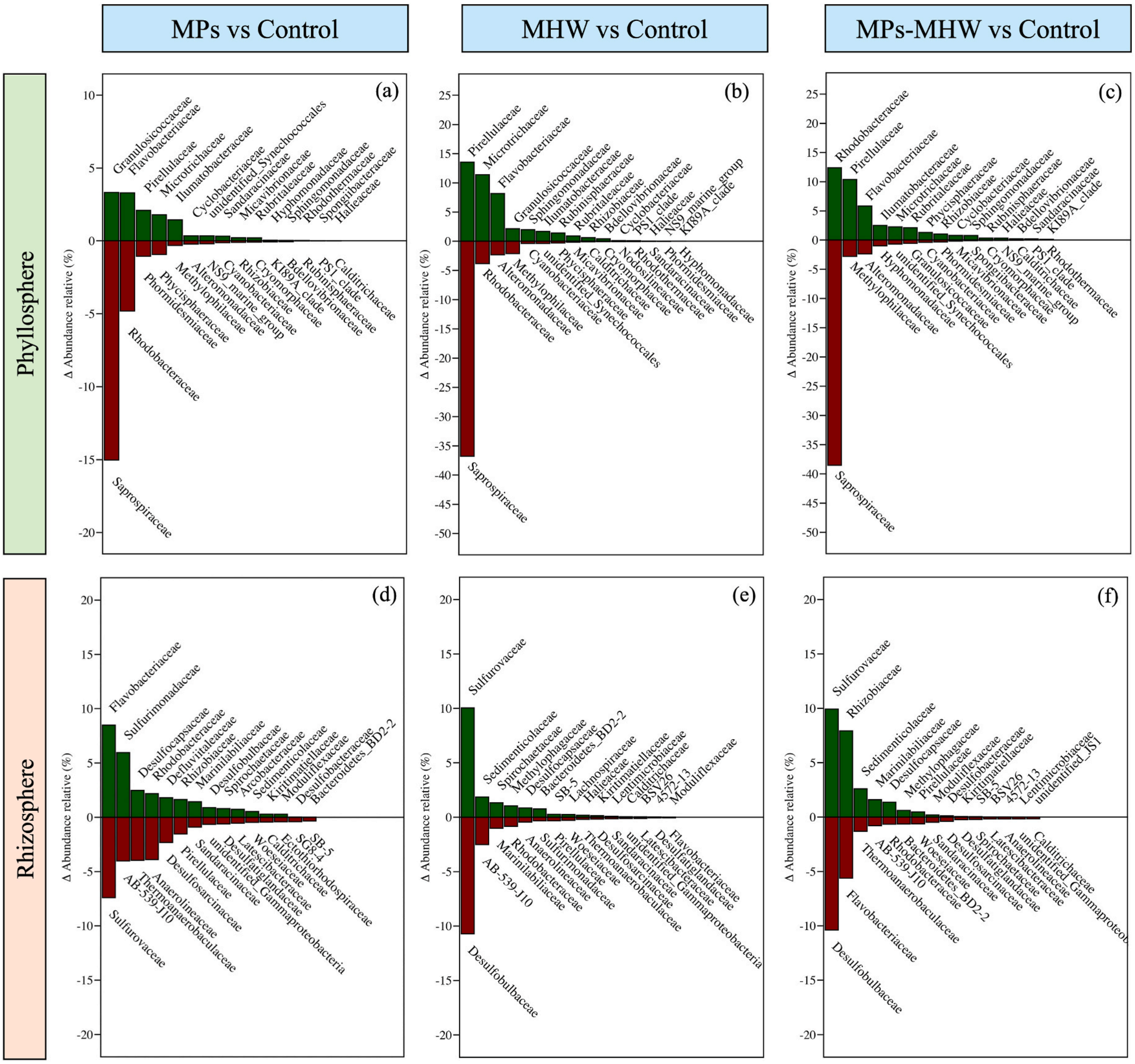

Fig. 7. Differentially abundant operational taxonomic units (OTUs) in *Zostera marina* microbiomes. Bar plots show the top 30 OTUs with the greatest average differences in relative abundance between each treatment and the control for both the phyllosphere (a, b and c) and rhizosphere (d, e and f). Positive values indicate OTUs enriched under the treatment, whereas negative values represent OTUs reduced relative to the control.

Vilaplana et al., 2025). While we did not measure porewater chemistry directly, the enrichment of sulfur-cycling taxa in MP-exposed rhizospheres and the concurrent reductions in below-ground growth are consistent with a shift toward more reducing microenvironments. However, these patterns should be interpreted as inferential, and future studies integrating direct measurements of redox conditions, sulfide concentrations, and oxygen availability are needed to confirm this causal pathway.

Previous studies on terrestrial and marine plants indicate that microplastics can be taken up by roots, inducing oxidative stress, reducing cell viability and disrupting nutrient transport (Kalčíková et al., 2017; Yu et al., 2021; Menicagli et al., 2022). Once in plants, MPs may be translocated to leaves, where they can cause structural and biochemical damage, including reduced chlorophyll *a* content, membrane disruption, and cell lysis (Thiagarajan et al., 2021). These processes can reduce photosynthetic efficiency and increase reliance on internal carbon reserves, potentially leading to mobilization of non-structural carbohydrates (NSC). Accordingly, the NSC depletion observed here, particularly in rhizomes, may reflect direct MP tissue interactions that limit carbon acquisition and/or increase metabolic costs. Similar NSC mobilization has been reported in seagrass exposed to other stressors, such as light limitation (Griffiths et al., 2020; Jiménez-Ramos et al., 2023), herbivory (Egea and Jiménez-Ramos, 2025a), and warming (Moreno-Marín et al., 2018; Kaldy et al., 2022). Further work directly assessing MP uptake and tissue-level physiological responses (e.g., cell viability and oxidative stress markers) would help to confirm these mechanisms.

The negative impact of MPs on *Z. marina* performance was further aggravated by marine heatwave (MHW) stress, indicating a synergistic interaction between both factors. Warming alone produced a short-term increase in leaf growth and above-ground biomass, indicating temporary acclimation through enhanced photosynthetic activity under the temperature rise (Ralph et al., 2007). This response was accompanied by a rise in the above-ground-to-below-ground biomass ratio, from 1.38 to 2.17, suggesting carbon reallocation towards photosynthetic tissues to offset higher respiratory costs in roots and rhizomes. Similar short-term positive responses to mild warming have been reported for northern eelgrass populations (Beca-Carretero et al., 2018; Breiter et al., 2024). However, when warming coincided with MP exposure, this compensatory effect disappeared. The combined stress of elevated temperature and MPs treatments caused the greatest decline in below-ground traits and sucrose reserves. These results suggest that MPs may lower the capacity of eelgrass to tolerate moderate heat stress, possibly by intensifying the imbalance between oxygen supply and metabolic demand, a metabolic squeeze predicted by multiple-stressor and carbon-balance frameworks (Crain et al., 2008; Piggott et al., 2015; Kooijman, 2010). The resulting depletion of NSC and collapse of below-ground growth demonstrate that the thermal tolerance of northern eelgrass populations may be exceeded under simultaneous pollution stress. Such combined effects highlight how multiple stressors can interact synergistically, amplifying physiological constraints predicted by ecological theory.

4.2. *Effects on eelgrass microbiome*

Microplastic (MP) exposure clearly altered the composition of the *Z. marina* microbiome, with more pronounced effects in the rhizosphere than in the phyllosphere (Fig. 6). This pattern was expected, as MPs were present only in sediments, and the difference was most evident during in Phase I. By Phase II, microbial communities in MP-exposed plants became more similar to those in controls, suggesting that the initial disturbance weakened over time. This temporal convergence coincided with the partial recovery of plant performance (LGR and NPR), and indicates that the microbiome and host physiology may have co-adjusted as stress conditions evolved. Several mechanisms could explain this transient effect. MPs release toxic chemical such as stabilizers and antioxidants which are incorporated during manufacturing (Hermabessiere et al., 2017; Luo et al., 2022). The leaching of these compounds can be enhanced by UV exposure, temperature and salinity (Do et al., 2022; Cai et al., 2025). Additive release typically peaks early during exposure and then declines as the polymer surface stabilizes (Egea et al., 2024a; Park et al., 2025). Because the MPs used here were virgin, leachate release was likely greatest at the start of the experiment, and diminished later, potentially reducing toxicity to the sediment microbiome. In parallel, microbial communities can adapt through successional reorganization and functional redundancy (Li et al., 2022). Moreover, MPs provide a colonization surface for biofilm development, the plastisphere, (Amaral-Zettler et al., 2020), which is initially dominated by Proteobacteria and Bacteroidota, and later by Desulfobacterota as it matures (Hou et al., 2025). The establishment of such a mature plastisphere towards the end of the experiment could have mitigated further impacts on both the rhizosphere and the plant host. This interpretation aligns with reports that epiphytic algae and biofilms can reduce MP-induced stress by stabilizing local microenvironments around seagrass tissues (Menicagli et al., 2022; Molin et al., 2023; Hou et al., 2024).

Functionally, MP exposure increased the relative abundance of Flavobacteriaceae, a group capable of degrading complex polymeric organic matter such as lignin and plant-derived polysaccharides (Randell et al., 2023). Similar enrichment has been observed in MP-contaminated sediments (Yan et al., 2024; Cholewińska et al., 2025), suggesting that these taxa may utilize MP-derived carbon or colonize plastic surfaces. Under heat stress, the microbiome responded differently, showing an increase in Sulfurovaceae, sulfur-oxidizing bacteria that can mitigate sulfide toxicity and support seagrass root function (Martin et al., 2018). When MPs and MHW were combined, community composition more closely resembled those of the warming treatment alone, but also included enrichment of Rhodobacteraceae in phyllosphere and Rhizobiaceae in the rhizosphere. Rhodobacteraceae are commonly found on seagrass leaves, contributing to CO_2 fixation (Korlević et al., 2021) and antioxidant protection (Miyamoto et al., 2023), whereas Rhizobiaceae are important in nitrogen fixation, potentially enhancing plant growth during recovery (Randell et al., 2023). Their enrichment under combined stress suggests that *Z. marina* may recruit or favor beneficial microbial partners as a microbiome-mediated resilience strategy (Ruan et al., 2024; Egea et al., 2026a). Overall, these findings support the concept that seagrass-associated microbial assemblages are dynamic and responsive to environmental stressors. The observed community shifts illustrate how the *Z. marina* holobiont reorganizes its microbiome in response to changing conditions, first exhibiting stress-related dysbiosis and later favoring beneficial taxa that enhance sulfur detoxification, nitrogen supply, and antioxidant defense. Similar adaptive restructuring has been described in terrestrial plants, where stress drives host-mediated selection for microbial taxa aligned with plant physiological needs (Chaparro et al., 2014; Edwards et al., 2018). This plasticity may represent an important mechanism through which seagrasses buffer the combined effects of pollution and warming.

4.3. *Ecological implications and future directions*

Seagrass meadows are effective sinks for microplastics (MPs), trapping and accumulating them within their canopy and sediments (Huang et al., 2020; de los Santos et al., 2021). This study experimentally demonstrates that MPs, as contaminants of emerging concern, can impair both seagrass performance and the composition of associated microbiome, with significant implication for blue-carbon processes. By reducing shoot density and below-ground biomass, MPs are likely to decrease the meadow capacity to fix and store organic carbon (Champenois and Borges, 2021; Kindeberg et al., 2024). Because most seagrass organic carbon is stored in rhizomes and roots (Fourqurean et al., 2012), the observed declines in these structures suggest a direct loss of sequestration potential.

Weakened below-ground structure may also weaken sediment stabilization and promote sediment erosion, resuspension and organic matter remineralization (Infantes et al., 2022). Reduced canopy density increases hydrodynamic exposure promoting dissolved organic carbon (DOC) release and microbial degradation (Egea et al., 2023a; Chipman et al., 2010; Yamuza-Magdaleno et al., 2024). These feedbacks could shift meadows from long-term carbon sinks toward net carbon sources, particularly under warming events. Because seagrass productivity (Barrón and Duarte, 2009), carbohydrate accumulation (Alcoverro et al., 1999), and thermal sensitivity (Breiter et al., 2024) vary seasonally, future field research should test whether the effects observed here persist across different seasons, sediment types and latitudes.

The presence of MPs in sediments may also impact seagrass restoration success. Restoration programs aim to reverse seagrass loss caused by eutrophication and coastal development (Infantes et al., 2016; Evans et al., 2025) and their success depends on minimizing anthropogenic stressors and selecting suitable habitats (van Katwijk et al., 2016). While sediment type alone may not limit transplantation success (van Katwijk et al., 2009), positive feedbacks between seagrass and sediments are essential for meadow establishment (Bos et al., 2007). MP contamination could disrupt these feedbacks by impairing root development and anchoring strength, thereby increasing erosion risk during extreme weather events (Calumpong and Fonseca, 2001). Monitoring MPs and other contaminants of emerging concern (CECs) should therefore be integrated into restoration planning and blue-carbon policy frameworks.

Mesocosm systems, as used in this research, provide valuable experimental control over key variables such as light, temperature, and nutrient supply. However, they necessarily simplify natural complexity. Processes such as sediment heterogeneity, hydrodynamics, biotic interactions, grazing pressure, and algae competition were not represented in this study and may modulate responses in situ (Jiménez-Ramos et al., 2017; de la Hoz et al., 2025). Moreover, the 43-day duration captured short-term responses, while longer-term processes, such as microbial succession (Sfriso et al., 2025) or community-level shifts in carbon metabolism (Egea et al., 2023c), remain untested. Although the mesocosm systems used here follows similar design of previous studies (e.g., Infantes et al., 2021; Egea et al., 2026a,b), it is not without methodological limitations. First, because microplastics are pervasive in marine environments (Kushwaha et al., 2024), we cannot exclude baseline MP contamination in the field-collected sediments. Surveys from the Baltic and North Seas report minimum concentrations of ~103 particles kg^{-1} DW sediment (Chubarenko et al., 2022), while seagrass sediments show minima of ~68-80 particles kg−1 DW sediment (Huang et al., 2020; Dahl et al., 2021). Logistical and budgetary constraints prevented direct quantification of this baseline-contamination. However, because sediments were collected from an area with low population density and limited industrial activity, baseline MP levels were likely closer to these lower ranges and therefore substantially below the experimental exposure concentration (~870 particles kg^{-1}). Importantly, any baseline contamination would have been consistent across all treatments and thus would not affect treatment contrasts. Second, we cannot fully exclude minor contributions from PVC pot material. Nevertheless, fragmentation from macroplastics into microplastics typically requires prolonged weathering and stressors such as UV radiation and mechanical abrasion (Weinstein et al., 2016) and microplastics release from PVC is more relevant for aged materials or conditions that enhance degradation and leaching (Temam and Mortula, 2023). Given the short experimental duration and indoor conditions, any PVC-derived contribution is expected to be negligible and unlikely to alter the main trends observed in this study. Finally, our findings are based on experiments exposing individual plants to a relatively high MPs concentration (~870 particles kg^{-1} dry sediment), which lies within the upper range reported for seagrass meadows sediment (Huang et al., 2020; Dahl et al., 2021). However, substantially higher concentrations have been documented in some highly impacted areas, with seagrass sediments reaching up to 3819 particles kg^{-1} sediment, particularly in regions influenced by intensive greenhouse horticulture (Dahl et al., 2021). Consequently, future research should examine the effects of even higher MPs loads, not only on individual plants but also at the meadow scale, where clonal integration and microbiome-mediated processes may partially buffer stress responses (Cúcio et al., 2016;Zribi et al., 2023). In addition, future research should combine long-term and field-base approaches, integrating plant physiology, microbial ecology, and sediment biogeochemistry to assess the persistence and ecological significance of MP-MHW interactions.

5. Conclusions

This study demonstrates that microplastic pollution reduces the growth and productivity of *Z. marina* by altering sediment conditions, impairing root function and restructuring the associated microbiome. MPs and marine heatwaves (MHWs) act synergistically, resulting in the strongest declines in below-ground biomass and non-structural carbohydrate (NSC) reserves. These results indicate that microplastics can exacerbate thermal stress by intensifying the imbalance between oxygen supply and metabolic demand, leading to a depletion of carbon reserves essential for plant resilience and recovery.

The combined effects of MPs and MHWs suggest that even moderate warming may exceed the physiological tolerance of northern eelgrass populations when MP pollution is present. Shifts in the microbiome composition, including the enrichment of sulfur-oxidizing and nitrogen-fixing bacterial taxa, indicate potential microbiome-mediated resilience, where certain symbionts may temporarily support nutrient cycling and root function under stress. Together, these findings underscore the vulnerability of seagrass meadows to the interacting pressures of pollution and climate change. The demonstrated reductions in growth, below-ground traits, and carbon storage capacity have direct implications for blue-carbon sequestration and restoration success. Integrating microplastic pollution monitoring, seasonal dynamics, and microbial interactions into conservation management and restoration frameworks will be essential to maintain seagrass resilience and sustain the ecosystem services these habitats provide in a warming, increasingly contaminated ocean.

CRediT authorship contribution statement

Luis G. Egea: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rocío Jiménez-Ramos:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lucía Rodríguez-Arias:** Writing – review & editing, Investigation. **Eduardo Infantes:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2026.123980.

Data availability

Supplementary data to this article can be found online at 10.5281/zenodo.17603933

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