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Temperature Dominates Microplastic Effects on Microbial and CO_2/CH_4 Responses in Seagrass Sediments

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Abstract: Microplastics and warming may jointly affect sediment microbial processes and carbon cycling in coastal ecosystems, yet their relative importance and interactions remain poorly understood. Here, we conducted a 28-day sediment–water microcosm experiment using seagrass-bed sediments to compare the effects of polyethylene (PE) and polypropylene (PP) under four temperature regimes (21, 28, 35, and fluctuating 28/35 °C). Temperature was the dominant driver of bacterial community structure and carbon-substrate utilization, whereas the overall effect of microplastic type was not significant based on PERMANOVA. Biolog profiles likewise showed stronger temperature-associated than polymer-associated variation. In contrast, PE and PP were associated with distinct and temporally variable responses in selected biogeochemical variables and CO_2 and CH_4 emissions. NH_4^+ declined across treatments, with a greater early decline under PE, whereas PP was associated with higher DOC under fluctuating temperature on day 7. CO_2 and CH_4 emissions also varied with temperature and polymer type, with CH_4 generally highest at 35 °C. However, these gas responses cannot be directly attributed to specific microbial pathways because archaeal communities, methanogenic functional genes, methane oxidation, and relevant process rates were not characterized. Overall, temperature exerted stronger and more consistent effects on community-level microbial responses than microplastics, while PE and PP produced comparatively weaker, polymer-specific, and context-dependent changes in sediment biogeochemistry and CO_2 and CH_4 dynamics. These findings highlight temperature as a key control on short-term microbial responses to microplastic exposure in seagrass sediments.

Keywords: microplastic; bacterial community; gas emission; sediment; seagrass bed

1. Introduction

Wetlands occupy only 4–6% of the global land surface, yet they represent one of the largest terrestrial carbon (C) reservoirs and play a disproportionate role in regulating the global carbon cycle [1,2]. Permanently or seasonally anoxic conditions in wetland soils favor methanogenesis, making wetlands the largest and most uncertain natural source of methane (CH_4), contributing approximately 20% of global CH_4 emissions while storing 20–30% of the world's soil organic carbon [3]. Microbial regulation of carbon and nitrogen (N) turnover strongly influences greenhouse-gas (GHG) production, particularly CH_4 and carbon dioxide (CO_2) [4,5]. Consequently, wetland soils play a critical role in balancing carbon storage and GHG production. However, whether wetlands will function predominantly as carbon sinks or GHG sources under future warming remains uncertain. Global

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warming of 1.5–2.0 °C has been estimated to increase the 100-year global warming potential of wetlands by 57%, highlighting the sensitivity of wetland carbon cycling to climate change [2,5].

Microplastic pollution represents an additional and increasingly pervasive environmental stressor. By 2015, approximately 6300 Mt of plastic waste had been generated globally, with nearly 79% accumulating in landfills or the natural environment, and this amount is projected to reach 12,000 Mt by 2050 [6]. Research on microplastics has expanded from terrestrial soils [7,8] to aquatic sediments [9–11], where microplastics have been associated with changes in sediment physicochemical properties, microbial processes, and biogeochemical cycling [12–14]. Increasing evidence indicates that microplastics can also influence carbon cycling and GHG dynamics in wetland sediments. For example, PE microplastics altered microbial communities and GHG emissions during litter decomposition in coastal wetland sediments [15], whereas PP microplastics affected carbon and nitrogen cycling in coastal wetlands [16] and CO_2 dynamics in intertidal mangrove sediments [17]. In terrestrial soils, PE microplastics have likewise been shown to alter bacterial community composition and metabolic functions [18,19]. These findings suggest that microplastics can modify microbial and biogeochemical processes, but that the magnitude and direction of responses may vary with polymer type and environmental context.

Blue-carbon ecosystems may be particularly susceptible to microplastic accumulation because dense vegetation reduces hydrodynamic energy, enhances particle retention, and promotes sediment deposition [20]. Microplastics can also modify the concentration and molecular composition of dissolved organic matter (DOM) [21–23]. Through selective adsorption of high-molecular-weight DOM components and the release of additives and polymer-derived products, microplastics may alter the composition and availability of organic substrates in surrounding environments [24]. Such changes could influence microbial carbon and nitrogen processing and, consequently, GHG dynamics. However, the extent to which these effects occur in wetland sediments, and whether they are comparable to those imposed by temperature, remains poorly understood.

Seagrass meadows are globally important blue-carbon ecosystems that support biodiversity, nutrient cycling, and other key ecosystem functions while contributing substantially to long-term carbon sequestration [25–27]. Their dense canopies reduce water-flow velocity, enhance particle retention, and promote sediment deposition [28,29], potentially increasing the accumulation and persistence of microplastics in sediments. Seagrass meadows also provide structurally heterogeneous habitats that can shape microbial and biogeochemical processes [30]. Recent evidence further suggests that microplastics may interact with thermal stress in seagrass ecosystems, as microplastic exposure can reduce eelgrass tolerance to heat stress with potential implications for blue-carbon functioning [31]. Because both temperature and microplastic exposure can influence sediment microbial processes and carbon cycling, their relative effects may be particularly important in seagrass sediments. Importantly, microbial community shifts do not necessarily translate directly into changes in biogeochemical process rates or GHG emissions, and responses may depend on polymer type and environmental context. Resolving these responses is therefore necessary to determine the relative influence of microplastic exposure and temperature on microbial and GHG dynamics in seagrass sediments.

2. Materials and Methods

2.1. Sediment Collection and Microplastic Materials

Surface sediments (0–5 cm) were collected from a dense seagrass meadow in Li'an Bay (18.43° N, 110.04° E), Hainan Island, China. Sediments were collected from multiple locations within the seagrass meadow and pooled to obtain three composite samples (>10 kg in total). The composite sediments were transported to the laboratory at 4 °C within 24 h. Visible debris, including roots, shells, and plant residues, was removed under sterile conditions. A portion of the sediment was used for physicochemical characterization, whereas subsamples designated for microbial analyses were stored at −80 °C until DNA extraction. Seawater collected from the same site was filtered through 0.45 μm glass-fiber filters and used as the overlying water during the incubation experiment.

Commercial polyethylene (PE) and polypropylene (PP) microplastic particles with an average particle size of approximately 30 μm were used in this study. The available characterization information was limited to polymer identity and particle size. Surface area, surface functional groups, additive composition, crystallinity, and aging status were not independently characterized. Therefore, the experiment was designed to compare short-term sediment responses associated with PE and PP exposure under identical experimental conditions rather than to attribute potential differences to specific physicochemical properties of the particles.

2.2. Experimental Design

A 28-day sediment–water microcosm experiment was conducted to investigate short-term microbial and biogeochemical responses to PE and PP exposure under contrasting temperature regimes. Approximately 200 g of

wet sediment was transferred into acid-washed 250-mL glass bottles. Three microplastic treatments were established: a control without added microplastics (CON), PE-amended sediment, and PP-amended sediment.

PE and PP microplastics were added at 0.1% (*w*/*w*, wet sediment basis). This concentration was selected as a controlled exposure level to facilitate comparisons among treatments. Because only one microplastic concentration was tested, the present experiment does not allow assessment of concentration-dependent responses or exposure thresholds. Accordingly, the results should be interpreted within the tested exposure conditions. Four temperature regimes were established: 21 °C, representing a lower-temperature reference condition; 28 °C, representing summer seawater temperature; 35 °C, representing a heat-stress scenario; and a fluctuating-temperature regime (28/35 °C). The fluctuating regime consisted of 3 d at 28 °C, followed by 2 d at 35 °C and 2 d at 28 °C within each weekly cycle. Each treatment was conducted in triplicate. After microplastic addition, 50 mL of filtered seawater was mixed with the sediment, followed by the careful addition of 100 mL of filtered seawater as overlying water to minimize sediment disturbance. The microcosms were pre-equilibrated for 24 h at 25 °C, covered with aluminum foil to minimize evaporation and light exposure, and subsequently incubated in the dark for 28 d. The experiment was designed to characterize short-term treatment responses under controlled laboratory conditions and was not intended to simulate long-term environmental exposure or determine persistent ecosystem-scale effects.

2.3. Sampling Procedures and Physicochemical Analyses

Samples were collected on days 0, 7, 14, 21, and 28 to characterize temporal changes during incubation. At each sampling time, headspace gas, sediment, and overlying water were collected for subsequent analyses. For CO_2 and CH_4 measurements, incubation bottles were sealed with butyl rubber stoppers and incubated undisturbed for 12 h. The headspace volume of each incubation bottle was approximately 100 mL. Before gas sampling, the headspace was homogenized for at least 10 s using a gas-tight syringe. Subsequently, 15 mL of headspace gas was collected and transferred to gas-sampling bags for analysis within 24 h using a cavity ring-down spectroscopy analyzer (Picarro G2508, Picarro, Inc., Santa Clara, CA, USA). CO_2 and CH_4 concentrations were determined from headspace gas measurements, and gas emission rates were calculated based on the measured concentration change during the incubation period, incubation time, and microcosm configuration. Because this study quantified only CO_2 and CH_4, we refer to these measurements specifically as CO_2 and CH_4 emissions, rather than total greenhouse-gas emissions or overall greenhouse-warming potential.

Sediment subsamples were collected for physicochemical analyses, DOM characterization, Biolog-based carbon-substrate utilization assays, and DNA extraction. Samples designated for microbial community analysis were immediately frozen and stored at −80 °C until further processing. Overlying water samples were filtered through 0.45 μm membranes and preserved for nutrient analyses. Sediments pH and salinity were measured in a 1:5 sediment-to-water suspension using a calibrated pH meter and conductivity probe, respectively. Concentrations of NH_4^+ and NO_3^- were quantified using a continuous-flow analyzer (CleverChem Anna, DeChem-Tech, Hamburg, Germany). Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were quantified using a total organic carbon analyzer (TOC-L CPH, Shimadzu, Kyoto, Japan).

2.4. Dissolved Organic Matter Characterization

Sediment DOM was extracted with ultrapure water at a sediment-to-water ratio of 1:10 (*w*/*v*). Samples were shaken for 24 h at 25 °C, centrifuged at 10,000 rpm for 10 min, and filtered through 0.45 μm membranes before analysis. Fluorescence excitation-emission matrix (EEM) spectra were acquired using a fluorescence spectrophotometer equipped with a 1-cm quartz cuvette. Samples were diluted to maintain an absorbance below 0.05 at 254 nm to minimize inner-filter effects. Parallel factor analysis (PARAFAC) was applied to identify major fluorescent components and estimate their relative contributions. The fluorescence index (FI), humification index (HIX), and biological index (BIX) were calculated to characterize relative differences in DOM optical properties, potential source characteristics, and transformation-related patterns. Because DOM released directly from microplastic particles was not isolated or traced in the present experiment, observed differences in DOM characteristics were interpreted as microplastic-associated changes in sediment DOM rather than direct evidence of microplastic-derived DOM.

2.5. Bacterial Community Composition and Predicted Functional Profiles

Bacterial community composition was characterized using 16S rRNA gene amplicon sequencing. Total DNA was extracted from sediment samples using the PowerSoil DNA Isolation Kit according to the manufacturer's instructions. The V3–V4 region of the 16S rRNA gene was amplified using primers 341F and 806R. Amplicons were sequenced on an Illumina MiSeq platform using paired-end 2 × 300 bp sequencing. Raw sequences were processed

using QIIME2 and DADA2 to generate amplicon sequence variants (ASVs). Taxonomic assignment was conducted against the SILVA database (v138). Alpha-diversity indices, including Chao1, Shannon, and Ace indices, were calculated to characterize bacterial community diversity. Bray–Curtis dissimilarity was used for principal coordinate analysis (PCoA) to visualize differences in bacterial community composition among treatments.

The 16S rRNA sequencing analysis was primarily used to characterize bacterial communities. Archaeal sequences represented only a small proportion of the sequencing dataset and were not analyzed in sufficient depth to resolve archaeal community composition or methanogenic responses. Therefore, bacterial community data were not used to infer the microbial mechanisms underlying the observed CH_4 responses. FAPROTAX was used to generate taxon-based predictions of potential ecological functions from bacterial community composition. These results represent predicted functional profiles based on taxonomic assignments and do not constitute direct measurements of functional gene abundance, gene expression, enzyme activity, or biogeochemical process rates. Accordingly, FAPROTAX results were interpreted as predicted functional patterns rather than direct evidence of changes in microbial metabolic activity.

2.6. Biolog-Based Carbon-Substrate Utilization

Microbial carbon-substrate utilization patterns were assessed using Biolog EcoPlates containing 31 carbon substrates representing six substrate categories. Fresh sediment was suspended in sterile phosphate-buffered saline, serially diluted, and inoculated into the EcoPlates. The plates were incubated at 25 °C for 7 d, and absorbance was measured every 24 h at 590 and 750 nm. Average well-color development (AWCD), Shannon diversity, Simpson dominance, and principal component analysis (PCA) were used to characterize differences in carbon-substrate utilization patterns among treatments. The EcoPlate-derived indices represent assay-dependent responses to the supplied carbon substrates under the incubation conditions. Therefore, they were interpreted as Biolog-based carbon-substrate utilization diversity and patterns rather than comprehensive measurements of whole-community metabolic diversity or in situ microbial metabolic activity.

2.7. Statistical Analysis

All statistical analyses were performed using SPSS 26.0, R version 4.3.0, and Origin 2023b. The effects of microplastic treatment and temperature on physicochemical variables, nutrient concentrations, DOM properties, bacterial diversity, Biolog-based carbon-substrate utilization indices, and CO_2 and CH_4 emissions were evaluated using factorial analysis of variance (ANOVA), followed by Tukey's HSD test when appropriate. Because the incubation experiment included repeated measurements across multiple sampling times, temporal variation was explicitly considered when evaluating treatment responses. For variables measured repeatedly during incubation, microplastic treatment, temperature regime, and sampling time were considered in the statistical evaluation, and the interaction among these factors was examined where supported by the experimental design. When assumptions of normality or homogeneity of variance were not satisfied, data transformation or appropriate non-parametric alternatives were applied. Differences in bacterial community composition were evaluated using Bray–Curtis-based PCoA and permutational multivariate analysis of variance (PERMANOVA). ANOSIM was used as a complementary assessment of group separation. The relative contributions of temperature and microplastic treatment to bacterial community variation were evaluated using multivariate analyses based on the experimental design.

PCA was used to visualize differences in Biolog-based carbon-substrate utilization profiles among treatments. Redundancy analysis (RDA) was applied to examine associations between bacterial community composition and measured environmental variables. Spearman correlation analysis was used to explore statistical associations among CO_2 and CH_4 emissions, microbial parameters, and sediment biogeochemical variables. These associations were interpreted as patterns of co-variation and were not considered evidence of direct causal relationships. A significance level of $p < 0.05$ was adopted unless otherwise specified.

3. Results

3.1. Temporal Dynamics of Nutrients and Dissolved Organic Matter

Temporal changes in nutrient and dissolved organic matter concentrations are shown in Table 1. Initial concentrations of NO_3^-, NH_4^+, and DON in the overlying water were 78.40, 98.84, and 0.30 mg/L, respectively. NO_3^- concentrations generally increased during the 28-day incubation, particularly after day 14, whereas NH_4^+ concentrations declined across all treatments, with PE-amended sediments showing a modestly greater decrease during the first two weeks; differences among treatments became less evident by day 28. In contrast, sediment NO_3^- and NH_4^+ concentrations remained relatively stable and did not differ significantly among treatments ($p > 0.05$).

DOC concentrations generally declined during incubation (Figure 1). However, PP-amended sediments showed higher DOC concentrations on day 7 under the fluctuating-temperature regime. This difference was not consistently observed across temperatures or sampling times, indicating a transient and context-dependent response. Because microplastic-derived DOM was not isolated or traced, these changes are interpreted as treatment-associated differences in sediment DOM dynamics. DON showed clear temporal variation, increasing during the first half of the incubation and declining thereafter. The highest DON concentration occurred in the control on day 14 (19.19 ± 4.68 mg/L). Under elevated and fluctuating temperatures, DON concentrations decreased to very low levels after day 14. PE treatments also showed lower DON concentrations than PP during the later incubation stage. Overall, nutrient and DOM dynamics were characterized primarily by temporal and temperature-associated variation, whereas PE and PP produced comparatively smaller and transient differences in NH_4^+, DOC, and DON.

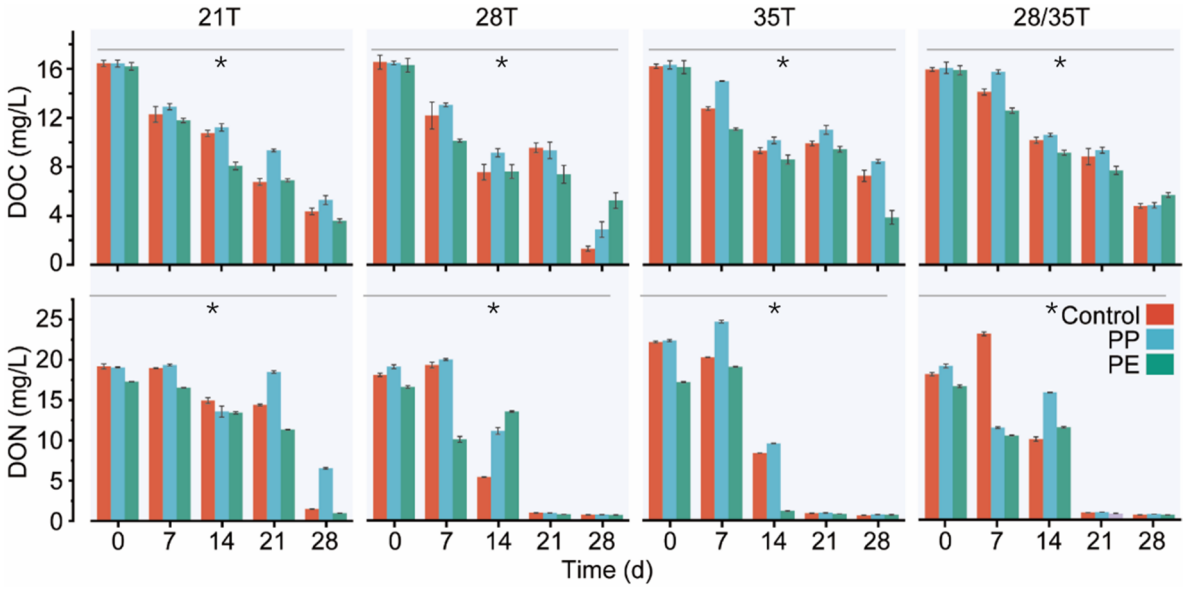


Figure 1. Dynamics of DOC and DON in the overlying water. * $p < 0.05$.

Table 1. Dissolved organic and inorganic nitrogen concentrations in water and sediments across different plastic-type treatments.

Treatments	Time	NO_3^- (mg/L)	NH_4^+ (mg/L)	NO_3^- (mg/kg)	NH_4^+ (mg/kg)	DON (mg/L)
		Water	Water	Sediment	Sediment	Water
Initial	0 d	78.4 ± 0.8	98.8 ± 1.2	0.24 ± 0.05	1.16 ± 0.14	0.30
Control	14 d	85.6 ± 6.8	82.6 ± 13.8	0.30 ± 0.03	1.08 ± 0.08	19.2 ± 4.7
	28 d	113.9 ± 4.5	53.9 ± 5.5	0.26 ± 0.03	0.96 ± 0.06	0.8 ± 0.3
PE	14 d	89.2 ± 4.2	73.9 ± 15.2	0.38 ± 0.09	1.12 ± 0.25	16.8 ± 3.3
	28 d	113.3 ± 9.8	53.6 ± 1.6	0.25 ± 0.03	0.99 ± 0.21	0.7 ± 0.1
PP	14 d	88.6 ± 2.1	91.4 ± 14.2	0.34 ± 0.05	1.15 ± 0.25	14.3 ± 2.9
	28 d	112.6 ± 4.5	56.3 ± 4.2	0.31 ± 0.05	0.94 ± 0.06	2.5 ± 1.1

3.2. Temperature Dominated Bacterial Community Responses

After quality filtering, 1,379,639 high-quality 16S rRNA gene sequences were obtained, averaging 44,504 reads per sample. Bacterial sequences accounted for >97.79% of total reads, whereas archaeal sequences accounted for <2.21% and were not analyzed further. Bacterial α-diversity, including Shannon, Chao1, and Ace indices, did not differ significantly among treatments ($p > 0.05$; Table 2). Thus, short-term PE and PP exposure did not produce detectable changes in bacterial richness or diversity under the tested conditions. Several dominant taxa varied among treatments (Figure 2a). *Firmicutes*, *Proteobacteria*, *Bacteroidota*, *Desulfobacterota*, and *Fusobacteriota* dominated the bacterial communities. *Fusobacteriota* increased at 35 °C, whereas *Campilobacterota* and *Cyanobacteria* were relatively more abundant at 21 °C. *Calditrichota*, *Cloacimonadota*, and *Marinimicrobia* showed higher relative abundances under elevated or fluctuating temperatures. PE and PP were associated with changes in several individual taxa, but these shifts did not translate into significant changes in overall community structure. Temperature was the dominant factor associated with bacterial community variation. PCoA showed

clear separation along the temperature gradient, whereas substantial overlap occurred among control, PE, and PP treatments within individual temperature groups (Figure 2c,d). The first two axes explained 32.7% of the total variation. Similar patterns were observed in NMDS (Stress = 0.132; Figure 2e,f). PERMANOVA confirmed a significant temperature effect ($p = 0.001$), whereas the overall effect of microplastic treatment was not significant ($p = 0.76$, Figure 2). The 21 and 35 °C treatments showed the greatest separation, while communities under 28 °C and fluctuating temperatures were comparatively similar.

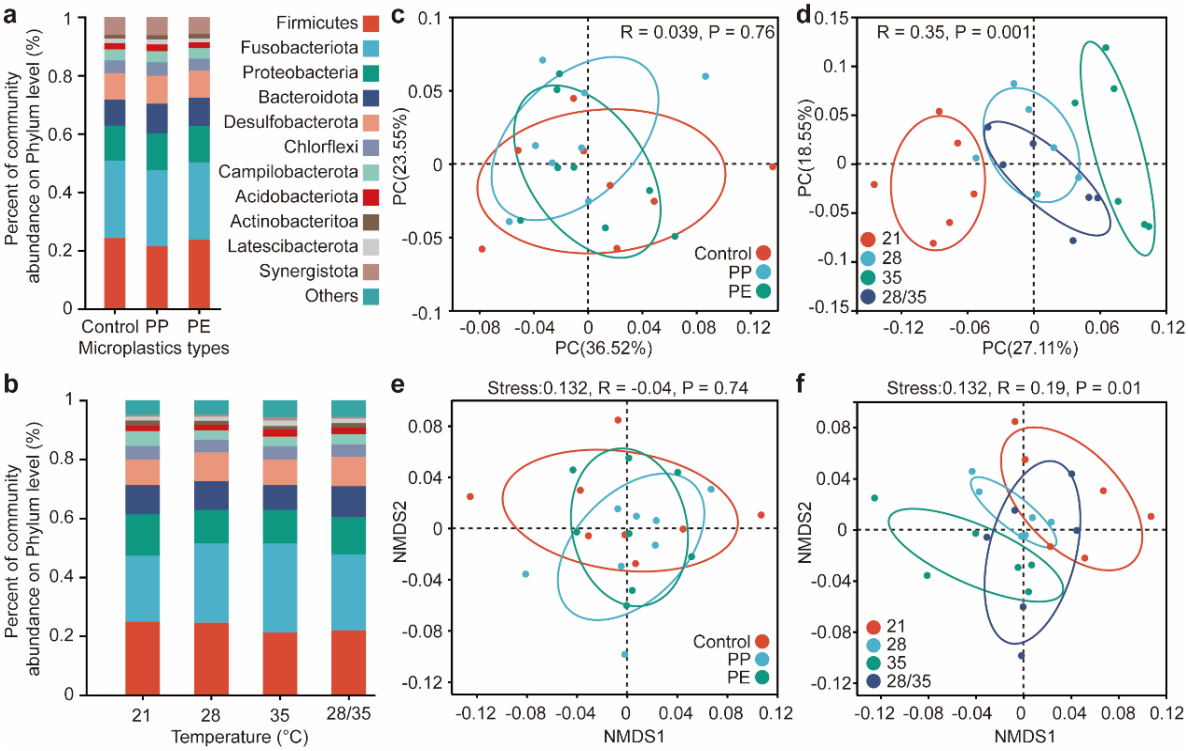


Figure 2. Differences in bacterial communities at the phylum level ((**a**,**b**) community composition and relative abundance; (**c**,**d**) PCoA plots; (**e**,**f**) NMDS plots).

Table 2. Alpha diversity indices of sediment bacterial communities in response to different plastic types.

Treatments	Time	Shannon	Chao 1	Ace
Initial	0 d	5.06 ± 0.11	3140.70 ± 143.25	3135.36 ± 66.71
Control	14 d	4.74 ± 0.23	3841.60 ± 194.40	3211.58 ± 104.09
	28 d	5.12 ± 0.13	3450.43 ± 212.14	3316.88 ± 30.82
PE	14 d	4.75 ± 0.06	3394.28 ± 166.56	2988.57 ± 86.07
	28 d	5.12 ± 0.06	3372.79 ± 85.19	3318.83 ± 94.20
PP	14 d	4.93 ± 0.18	3378.28 ± 307.07	3098.32 ± 81.76
	28 d	5.05 ± 0.11	3300.03 ± 143.18	3258.82 ± 116.52

PLS-DA provided a complementary visualization of community patterns grouped by microplastic treatment and temperature regime (Figure 3). FAPROTAX analysis revealed treatment-associated differences in predicted functional profiles (Figure 4). PP treatments showed higher relative abundances of several FAPROTAX-predicted sulfur-related functional profiles than the control and PE treatments. However, these results are taxon-based predictions and do not demonstrate changes in functional gene abundance, microbial activity, or actual biogeochemical process rates. Together, the microbial data indicate that temperature was the dominant factor structuring bacterial community-level variation, whereas PE and PP were associated primarily with shifts in selected taxa and predicted functional profiles.

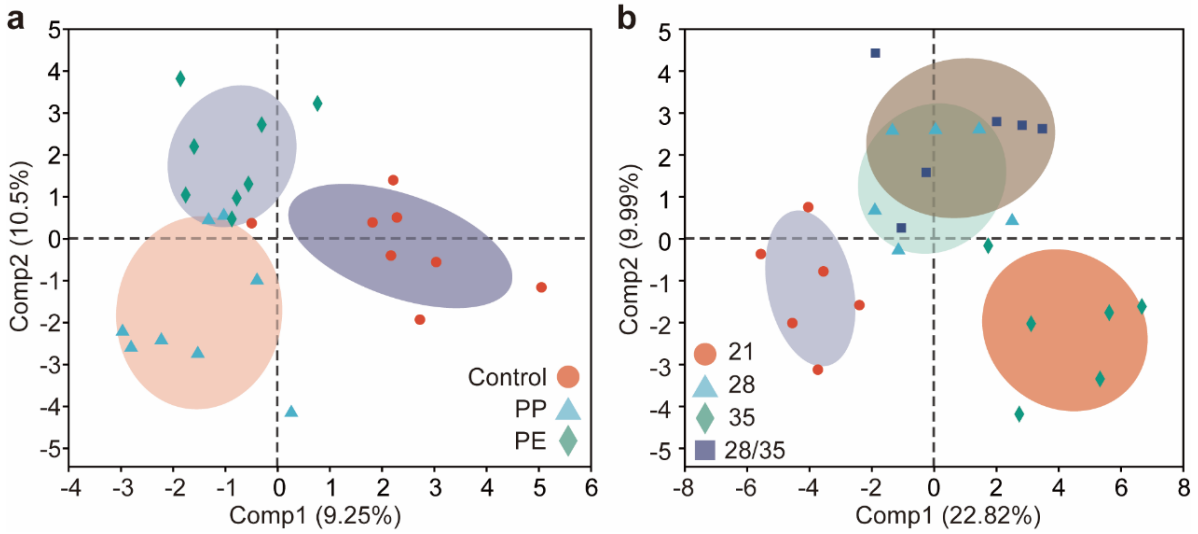


Figure 3. PLS-DA of sediment bacterial communities grouped by (**a**) microplastic treatment and (**b**) temperature regime.

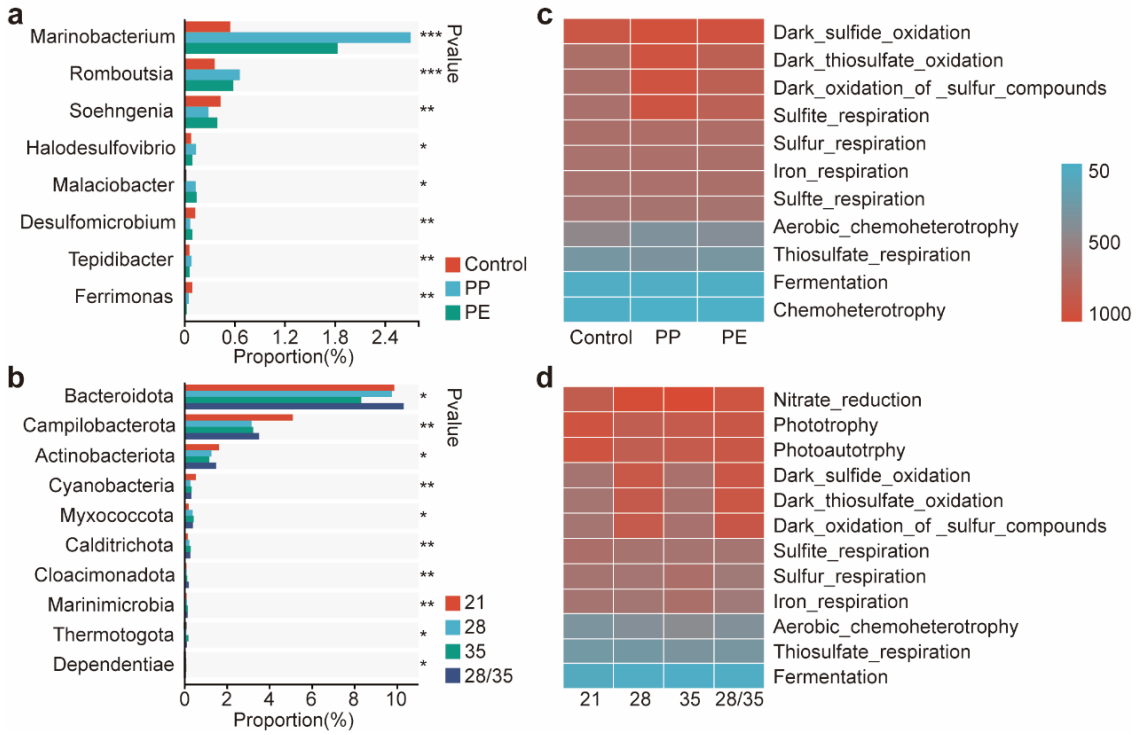


Figure 4. Differential responses and functional profiles of bacterial communities ((**a**,**b**) significantly different taxa among treatments; (**c**,**d**) bacterial functional profiles).

3.3. PE and PP Were Associated with Distinct CO_2 and CH_4 Responses

CO_2 and CH_4 emissions showed distinct temporal patterns among temperature and microplastic treatments (Figure 5). CH_4 emissions generally increased over time in the control and PE treatments, whereas PP treatments showed an initial decline followed by a gradual increase. Across all treatments, CH_4 emissions were generally highest at 35 °C. Relative to the control, PE was generally associated with higher CH_4 emissions, whereas PP tended to be associated with lower emissions. CO_2 emissions also varied among treatments. Microplastic-amended sediments generally exhibited higher CO_2 emissions than the control, with PE showing a stronger response in several treatment combinations. The highest CO_2 emissions were observed under fluctuating temperature conditions. These results indicate that both temperature and polymer type were associated with short-term variation in CO_2 and CH_4 emissions. However, the mechanisms underlying these responses cannot be resolved because archaeal community composition, methanogenic functional genes, methane oxidation, and direct process rates were not measured.

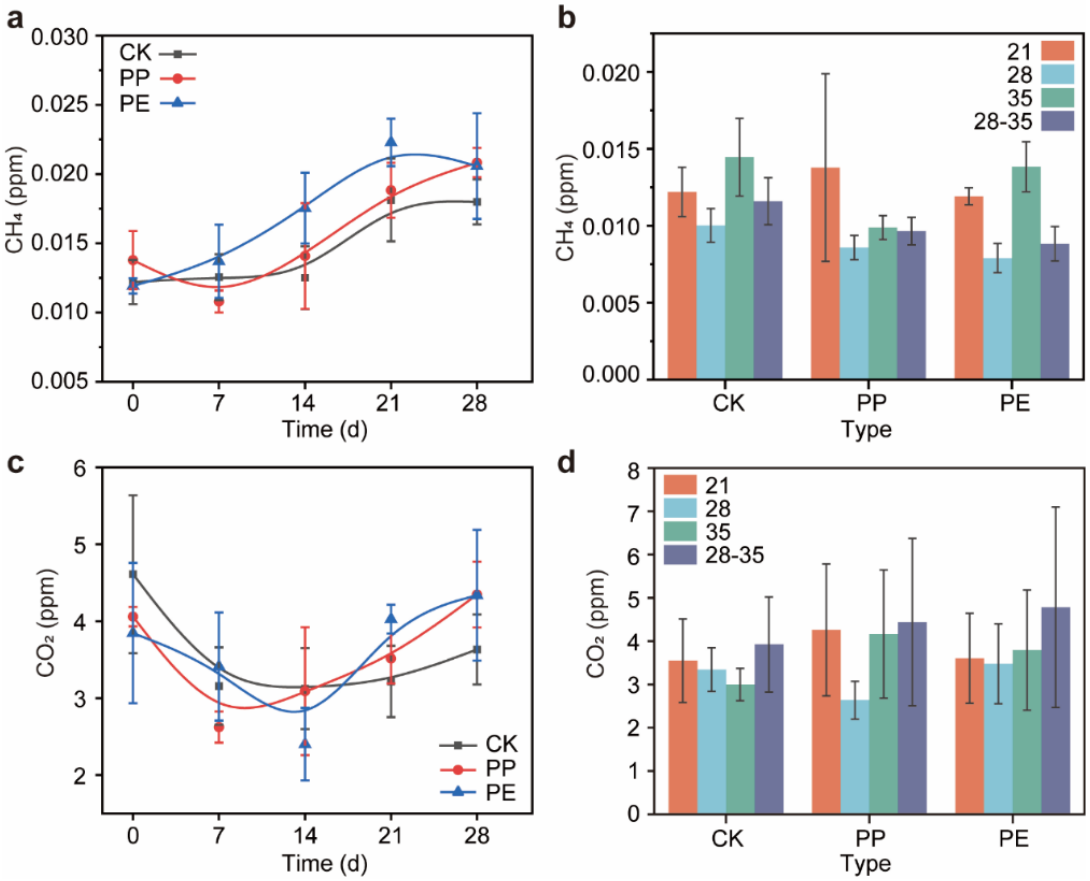


Figure 5. CO_2 and CH_4 emissions under different treatments ($p > 0.05$). (**a**,**c**) Temporal dynamics; (**b**,**d**) responses across microplastic and temperature treatments.

3.4. Temperature Structured Carbon-Substrate Utilization Patterns

Biolog EcoPlate analysis showed clear differences in carbon-substrate utilization patterns among temperature treatments (Figure 6). All treatments exhibited an initial lag phase followed by increasing AWCD values, which generally stabilized after approximately 120 h. AWCD values were generally highest at 21 °C and lowest at 35 °C, particularly in PE- and PP-amended treatments. The Shannon index differed significantly among treatments ($p < 0.05$), whereas the Simpson index showed no significant differences (Table 3). PCA based on substrate utilization profiles at 96 h showed that PC1 and PC2 explained 45.29% and 15.28% of the total variation, respectively (Figure 7). Samples separated primarily along the temperature gradient, with 21 and 35 °C treatments occupying distinct regions of the ordination space. In contrast, microplastic treatments largely overlapped within individual temperature groups. Overall, temperature exerted a stronger influence than PE and PP on Biolog-based carbon-substrate utilization patterns. Microplastic treatments were associated with comparatively smaller differences in substrate-utilization diversity.

Table 3. Biolog EcoPlate-derived carbon-substrate utilization diversity indices in seagrass sediment samples.

Sample	T (°C)	Shannon Diversity Index	Simpson Diversity Index
Control	21	1.38 ± 0.01	0.91 ± 0.01
	28	2.85 ± 0.02	0.99 ± 0.01
	35	2.83 ± 0.01	0.99 ± 0.01
	28/35	2.49 ± 0.03	0.98 ± 0.01
PE	21	2.75 ± 0.02	0.99 ± 0.01
	28	2.91 ± 0.04	0.99 ± 0.01
	35	2.88 ± 0.02	0.99 ± 0.01
	28/35	2.83 ± 0.04	0.99 ± 0.01
PP	21	1.68 ± 0.01	0.93 ± 0.01
	28	2.82 ± 0.01	0.99 ± 0.01
	35	2.83 ± 0.02	0.99 ± 0.01
	28/35	2.81 ± 0.01	0.99 ± 0.01

Data in the table are mean ± SE, n = 3.

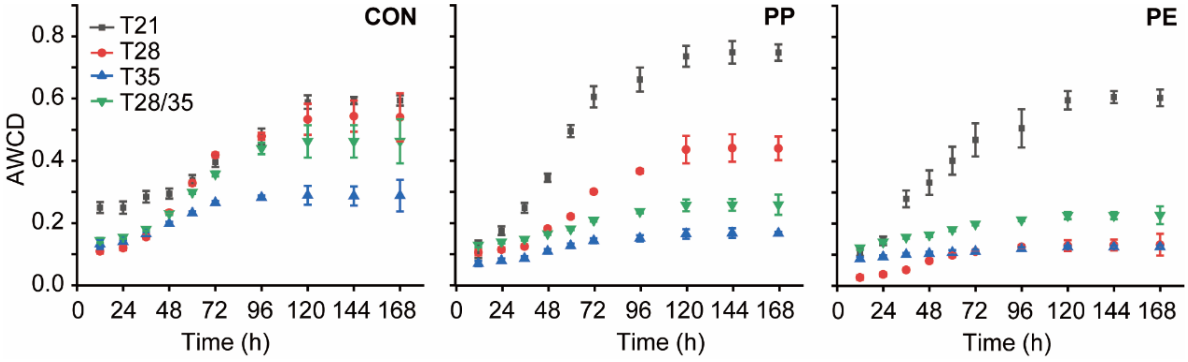


Figure 6. AWCD of sediment microbial communities measured using Biolog EcoPlates under different microplastic and temperature treatments.

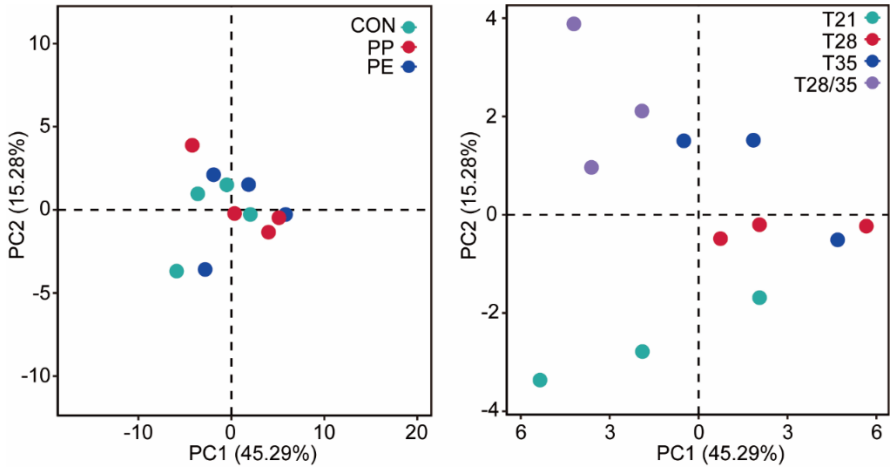


Figure 7. PCA of Biolog EcoPlate carbon-substrate utilization profiles at 96 h under different microplastic and temperature treatments.

4. Discussion

Microplastics can provide novel niches for microbial colonization and alter sediment microbial assembly, but their effects depend on polymer type, exposure duration, and environmental context. Previous studies showed that PE, PVC, PUF, and PLA altered bacterial communities in salt-marsh sediments, with responses varying among polymers [13]. In contrast, short-term exposure to environmentally relevant concentrations (0.1%) of PE and PP had negligible effects on bacterial α-diversity, consistent with evidence that microplastics do not necessarily alter microbial diversity in soils and sediments [21,22]. This discrepancy likely reflects differences in exposure intensity, incubation duration, and ecosystem properties, suggesting relatively high resistance of seagrass sediment microbial communities to short-term microplastic disturbance.

Temperature emerged as a stronger environmental filter than microplastic type, regulating microbial assembly and carbon utilization. Bacterial communities were clearly separated along the temperature gradient, accompanied by shifts in taxa associated with organic matter degradation and sulfur cycling, consistent with evidence that warming can reshape microbial composition and functional potential in sediments [32]. AWCD increased rapidly during the first 4–5 d and subsequently stabilized, indicating the establishment of relatively stable substrate utilization patterns [33,34]. Elevated temperatures, however, reduced AWCD and altered carbon-substrate utilization profiles, suggesting that thermal stress may constrain microbial metabolic efficiency despite accelerating biogeochemical turnover. Thus, temperature exerted stronger control than microplastic exposure on microbial community assembly and carbon utilization.

Despite limited effects on overall community structure, PE and PP elicited distinct functional responses. PE increased microbial metabolic diversity and was associated with greater NH_4^+ turnover and DON depletion, whereas PP preferentially enriched sulfur-cycling microorganisms and transiently increased DOC concentrations. These contrasting responses suggest that polymer properties may selectively influence microbial functions without substantially altering community diversity. Although PE and PP are relatively recalcitrant, microplastics may affect microbial metabolism by modifying DOM availability and providing surfaces for microbial attachment.

Plastic particles can adsorb DOM while releasing additives and oxidation products, potentially altering substrate availability [35,36].

These responses were also reflected in the dynamics of CO_2 and CH_4 emissions. Both microplastic treatments generally increased CO_2 emissions relative to the control, whereas PE slightly promoted CH_4 production and PP tended to suppress CH_4 emissions. Similar increases in CO_2 emissions following microplastic exposure have been reported in terrestrial soils [37]. Plastic surfaces may provide additional sites for microbial colonization and biofilm development [38], potentially influencing microbial activity and CO_2 and CH_4 emissions production [39]. However, because plastic-associated microbial colonization was not directly quantified, this mechanism remains speculative.

Temperature exerted a stronger influence on the dynamics of CO_2 and CH_4 emissions than polymer type. CH_4 emissions were generally highest at 35 °C, whereas CO_2 emissions peaked under fluctuating temperatures (28/35 °C), highlighting the sensitivity of sediment gas dynamics to thermal conditions. Elevated temperatures may accelerate organic matter decomposition and microbial metabolism [40], potentially contributing to CO_2 and CH_4 production. Nevertheless, because carbon turnover rates and specific microbial processes were not directly measured, CO_2 responses should not be interpreted as direct evidence of enhanced carbon mineralization. The mechanisms underlying contrasting CH_4 responses also remain unresolved because archaeal communities, methanogenic genes, methane oxidation, and process rates were not characterized.

Overall, temperature exerted a stronger influence on bacterial community composition and Biolog-based carbon-substrate utilization patterns than short-term PE and PP exposure. Microplastic-associated differences were comparatively modest and varied with polymer type, temperature regime, and sampling time. Although CO_2 and CH_4 emissions also differed among treatment combinations, the microbial processes underlying these responses remain unresolved because archaeal communities, methanogenic functional genes, methane oxidation, and direct process rates were not characterized. Further studies incorporating concentration gradients, longer incubation periods, functional-gene analyses, multi-gas measurements, and field validation are needed to determine whether the short-term responses observed here persist under environmentally relevant conditions.

5. Conclusions

This study examined short-term responses of seagrass-bed sediment microbial and biogeochemical processes to PE and PP under contrasting temperature regimes. Under the tested 28-day experimental conditions, temperature exerted a stronger influence than microplastic exposure on bacterial community structure and Biolog-based carbon-substrate utilization patterns. In contrast, PE and PP were associated with distinct but comparatively weaker and temporally variable responses in selected carbon and nitrogen variables, indicating polymer-specific and context-dependent effects. CO_2 and CH_4 emissions also varied with polymer type and temperature, although the microbial mechanisms underlying these responses remain unresolved because archaeal communities, methanogenic functional genes, methane oxidation, and direct process rates were not characterized. Overall, temperature was the dominant driver of community-level responses, whereas PE and PP were associated with more selective responses in sediment biogeochemistry and CO_2/CH_4 emissions. Longer-term, concentration-gradient, multi-gas, functional, and field-based studies are needed to determine the persistence and ecological significance of these responses.

Author Contributions

X.L.: investigation, methodology, software, visualization, writing—original draft, funding acquisition; F.S.: data curation, formal analysis, investigation; D.L.: investigation, methodology, software; J.J.: investigation, methodology, software; M.L.: writing—original draft; J.L.: funding acquisition, conceptualization, supervision, writing—review & editing. All authors have read and agreed to the published version of the manuscript.

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Not applicable.

Data Availability Statement

The data generated and/or analyzed during this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no competing financial interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper

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