

## RESEARCH ARTICLE

# Unveiling the effects of two distinct microplastics on the sediment microbial community and greenhouse gas emissions: a case study of the Nu River

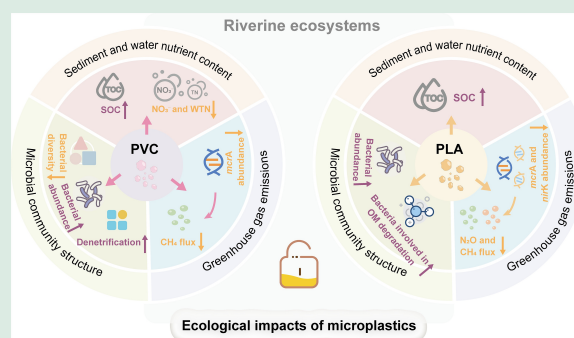
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## HIGHLIGHTS

- PVC and PLA both significantly increased the sediment organic carbon content.
- PVC had a more pronounced effect on the microbial community structure than PLA.
- PVC enhanced the intensities of the sediment nitrate reduction and denitrification.
- PLA enriched bacteria genera involved in complex organic matter degradation.
- PVC & PLA decreased CH<sub>4</sub> fluxes due to reduced abundances of methanotrophic bacteria.



**ABSTRACT:** Microplastics (MPs) in river ecosystems significantly affect sediment microbial communities and biogeochemical cycling. However, the specific microbial responses to distinct MPs and their subsequent effects on greenhouse gas (GHG) emissions remain poorly understood. In this study, the effects of conventional polyvinyl chloride (PVC) and biodegradable polylactic acid (PLA) on sediment microbial communities and GHG emissions were compared. Both PVC and PLA increased sediment total organic carbon (STOC) by 31.3% and 36.7%, respectively, leading to an increase in sediment bacterial abundance. Notably, compared with PLA, PVC reduced bacterial richness more significantly. Community composition and FAPROTAX function prediction analysis revealed that PVC-enriched taxa involved in nitrate reduction (e.g., *Desulfuromonas*, *Hydrogenophage*, *Azospira*) resulted in a significant decrease in NO<sub>3</sub><sup>-</sup> contents. In contrast, PLA increased the abundance of microorganisms associated with organic matter degradation (*Chitinophagaceae*, *Comamonadaceae*, and *Caulobacteraceae*). Both PVC and PLA significantly reduced the relative abundance of the *mcrA* gene, leading to decreased CH<sub>4</sub> fluxes, likely due to competition from enriched acetate-utilizing bacteria (*Desulfuromonas*, *Pseudomonas*, and *Azospira*). Additionally, PLA significantly reduced the abundance of the *nirK* gene. This study systematically elucidates the differential effects of PVC and PLA on microbial community structure and GHG emissions, providing new insights into the ecological impacts of MPs.

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**KEYWORDS:** Microplastics, Microbial community, Function predication, Greenhouse gas emissions

## 1 Introduction

Microplastic (plastic particles < 5 mm (Thompson et al., 2004)) pollution has become a global environmental concern due to the widespread use of plastics. Global plastic production reached 390.7 million tons in 2021, with microplastics (MPs) in ocean ecosystems estimated to exceed 51 trillion particles (Wang et al., 2024e). While the primary sources of MPs are terrestrial—stemming from the degradation of plastic waste, tire wear, and industrial processes (Sun et al., 2022b), they eventually enter water bodies through sewage discharge, surface runoff, and atmospheric deposition, thereby threatening aquatic ecosystems (Lebreton et al., 2017; Blettler et al., 2018; Brahney et al., 2020). Owing to their stability and resistance to degradation, MPs are transported and eventually accumulate in sediments and are influenced by both hydrodynamic and biological factors (Li et al., 2023). The concentration of MPs ranges from 14.44 to 18088.08 items/kg (dry weight) in lake sediments worldwide (Chen et al., 2024b) and reaches 9187.5 items/kg in marine protected areas worldwide (Nunes et al., 2023) and 32947 items/kg in riverine sediments (Kumar et al., 2021). MP contamination in riverine ecosystems is becoming increasingly severe due to the continuous growth of the human population and plastic production, thereby drawing global attention (Chen et al., 2022). However, the ecological impacts of MPs in riverine regions are not well understood, and their ecosystem-scale impacts warrant further investigation.

In recent years, the potential impact of MPs on microbial communities has garnered considerable attention, given that these communities are the key drivers of nutrient cycling and biogeochemical processes across diverse ecosystems. Recent research has focused predominantly on the effects of MPs on microbial ecology in soil and marine environments (Boots et al., 2019; Chen et al. 2023b; Rauscher et al., 2023). For example, polylactic acid (PLA) was found to increase the abundance of soil denitrifiers (*Noviherbaspirillum*) but decrease that of nitrifiers (*Nitrospira*) (Wang et al., 2024b). Both nondegradable polyethylene (PE) and degradable PLA were found to enrich microbial taxa with potential roles in nitrogen cycling and MP degradation in terrestrial settings, respectively (Yu et al., 2024). Similarly, Seeley et al. (2020) reported that the presence of PE, polyvinyl

chloride (PVC), and PLA could alter the composition of marine sediment microbial communities and significantly affect the relative abundance of functional genes related to nitrification and denitrification. A few works have investigated the impact of MPs on microbial communities in freshwater sediments (primarily lake ecosystems) and illustrated that MPs such as polyethylene glycol terephthalate (PET) and polystyrene (PS) could alter the physicochemical properties of sediments and influence microbial diversity (Zhang et al., 2022; Li et al., 2024b). Nevertheless, the specific impacts of these changes on microbial functions, particularly those related to nutrient cycling, still need further refinement and clarification.

River ecosystems are a major source of greenhouse gas (GHG) emissions, contributing substantially to the global GHG budget (Liu et al., 2022). Riverine GHGs such as CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O are produced primarily through biogeochemical cycling processes driven by sediment microorganisms (Chen et al., 2023c). For example, N<sub>2</sub>O is released during incomplete denitrification (as an intermediate product) and nitrification (as a byproduct) processes. River sediments, often considered hotspots for microbial nitrogen and carbon cycling, provide favorable conditions for GHG emissions because of their rich substrates and the anoxic environment in deeper layers (Gong et al., 2022; Wang et al., 2024e). Variations in microbial community structure, such as those induced by MP contamination, are likely to affect GHG emissions. For example, PE was reported to increase N<sub>2</sub>O emission from the paddy soils by regulating the abundance of the nitrous oxide reductase gene *nosZ* (Yu et al., 2023b). The presence of poly (butylene-adipate-co-terephthalate) (PBAT) and PE led to changes in CO<sub>2</sub> and CH<sub>4</sub> emissions from lake sediments, which was attributed to the altered sediment organic carbon content and the abundance of specific functional microbes (Fan et al., 2023). However, the dynamics of microbial community structure and function in river sediments, as well as their interactions with GHG emissions under distinct levels of MP contamination, have not been well demonstrated.

In this study, we selected PVC and PLA as representatives of the most common conventional petroleum-based and biodegradable MPs, respectively, to investigate their effects on the microbial ecology of

river sediments. Owing to its unique chemical structure, PVC may enrich microbes capable of tolerating or utilizing chlorinated compounds in PVC-contaminated sediments (Miao et al., 2021). However, its impact on microbial communities in freshwater sediments, particularly with respect to its role in element cycling, remains relatively underexplored. PLA, as a representative biodegradable MP (Lin et al., 2024), is relatively bioavailable and may serve as a carbon source because of its carbonyl structure, potentially stimulating microbial community growth (Dai et al., 2024). To explore these dynamics, we collected sediments and surface water from the Nu River, one of the largest rivers in South-east Asia, and constructed a microcosm experiment to address the following objectives: 1) to examine the alteration of nutrients in the sediment–water system in the presence of PVC and PLA; 2) to compare the effects of PVC and PLA on the diversity, composition, and function of microbial communities; and 3) to assess how PVC and PLA affect sedimentary GHG emissions.

## 2 Materials and methods

### 2.1 Experimental microplastics and microcosm construction

The MPs used in this study were PVC and PLA, which were purchased from Tesulang Chemical Materials Co., Ltd., Dongguan, China. The diameters of the MPs were from 50 to 300  $\mu\text{m}$ , a size range that is known to constitute a significant proportion of the MPs in natural sediment environments (Chen et al., 2024b) and exert notable ecological impacts (Wang et al., 2024a). All the reagents were of analytical grade.

Both sediment and surface water samples for microcosm construction were collected from the Nu River (25.55°N 98.49°E, altitude: 882 m), one of Asia's largest rivers. The detailed sampling procedures and the physicochemical properties of these samples are provided in Supplementary Information S1. Prior to culturing, the sediment samples were precultured at 22 °C for three days. With respect to the incubation period, previous studies have investigated soil ecosystems over extended periods, with experimental durations ranging from 14 d to 365 d (Yu et al., 2021; Chen et al., 2023a; Yu et al., 2023b; Zhou et al., 2023). In freshwater sediments, Zhang et al. (2022) conducted a 90-d incubation experiment to investigate the effects of MPs on GHG emissions in lake sediments, revealing significant alterations in GHG emissions after just 7 d of cultivation. To balance time efficiency with the

ability to capture significant effects, we selected a 19-d incubation period for our study, which allowed us to assess the impact of MPs on sediment microbial communities and subsequent GHG emissions. This duration aligns with those of similar studies, such as the 20-d experiment conducted by Seeley et al. (2020). The sediment–water microcosms were constructed in 1-L glass jars. Each microcosm was filled with approximately 400 g of wet sediment, into which the MPs were thoroughly mixed to achieve a concentration of 0.5% (w/w), corresponding to 2.0 g of MPs per microcosm. This concentration is within the typical environmental levels of MP loading (Seeley et al., 2020). Following the addition of MPs, in each microcosm, 50 mL of filtered river water sample was introduced, permitting the sediments to settle properly. An additional 300 mL of surface water was carefully added to minimize disturbance to the sediment layer. To prevent evaporation, these microcosms were covered with aluminum foil containing small holes, providing aeration while reducing moisture loss. These microcosms were then incubated at a constant temperature of 22 °C in the dark. The experimental design included two microplastic treatments (PVC and PLA) alongside a control group without plastic (CON), with three replicates for each treatment ( $n = 9$ ).

### 2.2 Sediment and water nutrient measurements

Nutrient concentrations were analyzed before incubation (initial, Table S1) and after 19 d of incubation. For nitrogen analysis, the concentrations of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and total nitrogen (TN) were measured in both the overlying water and the sediments.  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  were extracted from the sediments by adding 2 mol/L KCl solution (sediment: water ratio of 1:5, weight: volume) and shaking the mixture at 150 r/min for 30 min. The resulting leachate was then filtered through 0.45  $\mu\text{m}$  aqueous filter membranes (Millipore, USA). The concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  in both the overlying water and the sediment leachate were measured using a continuous flow analyzer (AACE, Seal, Germany), while TN content was analyzed using an element analyzer (Vario EL cube, Elementar, Germany).

For carbon analysis, sediment samples were used to determine total carbon (TC), organic carbon (OC), and dissolved organic carbon (DOC) concentrations. To analyze sediment TOC (STOC), the samples were sieved through a 0.15 mm mesh and carbonates were removed by treatment with 1 mol/L HCl, followed by drying the sediments to a constant weight at 60 °C. The

STOC content was then determined using total organic carbon analyzer (TOC-L, Shimadzu, Japan). DOC was extracted by shaking the sediment samples in deionized water (1:4 ratio, weight: volume) at 250 r/min for 30 min. The DOC concentrations in the extracts and overlying water were measured using the same TOC analyzer (TOC-L, Shimadzu, Japan). Finally, sediment TC concentrations were measured using an element analyzer (Vario EL cube, Elementar, Germany).

### 2.3 Sediment DNA extraction and microbial community analysis

Sediment samples were collected both before and after 19 d of incubation for microbial community analysis. Total genomic DNA was extracted from the sediments using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's introductions. The quality of the extracted DNA was assayed with N60 Touch (Implen, Germany) and agarose gel electrophoresis, and the A260/280 ratios were within the range of 1.8 to 2.0. The primers 515F (5'-GTGY CAGCMGCCGCGGTAA-3') and 806R (5'-GGACTA CHVGGGTWTCTAAT-3'), which target the V4 region, were used to amplify the bacterial 16S rRNA gene. Following amplification, the PCR products were purified and cleaned using the AxyPrep DNA Gel Extraction Kit, and their quality was evaluated with a QuantiFluor™ -ST Quantification System (Promega). The purified amplicons were pooled in equimolar amounts and sequenced via high-throughput sequencing, using the Illumina NovaSeq 6000 platform, by Majorbio Biopharm Technology Co., Ltd., Shanghai, China. The raw reads were first spliced on the basis of overlap relationships, followed by quality control and filtering to ensure high sequence quality. The samples were subsequently differentiated for operational taxonomic unit (OTU) clustering and species taxonomic analysis using the QIIME 2 platform and Usearch tools. To mitigate potential sequencing depth biases, the OTUs were rarefied to the lowest sequence count observed across all these samples. Following the quality filtering process, a total of 74672 16S rRNA high-quality sequences were retrieved. Bacterial taxonomic classification was conducted utilizing OTU feature sequences and bacterial communities were annotated through the Silva database (version 132) at a confidence level of 0.7.

### 2.4 Functional gene quantification using Q-PCR analysis

To quantify changes in the relative abundance of

functional genes, we employed quantitative PCR analysis using Q-PCR (ABI 7300, Applied Biosystems, USA). The targeted functional genes included the bacterial population marker 16S rRNA; denitrification-related genes *nirS* and *nirK*; and genes associated with CH<sub>4</sub> production (*mcrA*) and oxidation (*pmoA*). Each 20 μL reaction mixture included 10 μL of 2X IQ SYBR Green Supermix (Bio-Rad, USA), 8 μL of sterile water, 0.8 μL of each primer (0.8 μmol/L), and 2.0 μL of template DNA. Details regarding the primers and thermal cycling conditions are provided in Table S2.

### 2.5 Greenhouse gas collection and measurement

Greenhouse gases, specifically CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O, were sampled from the microcosm headspace at intervals of 0, 2, 4, 6, 9, 12, and 19 d after culture. The initial sealing films on the bottle necks were removed, replaced with rubber caps fitted with hoses, and then sealed with Vaseline. A syringe was used to extract 13 mL of each gas sample from the microcosm. An equal volume of argon (Ar) was then injected for correction to ensure constant pressure. The gas collection procedure was conducted four times at intervals of 30, 60, 90, and 120 min. To measure the concentrations of the greenhouse gases, a gas chromatograph (GC, Agilent 7890B, USA) with three detectors was used. Specifically, CO<sub>2</sub> was analyzed with a thermal conductivity detector (TCD), CH<sub>4</sub> with a flame ionization detector (FID), and N<sub>2</sub>O with an electron capture detector (ECD). The emission fluxes for these gases were calculated using the Eq. (1):

$$F = \frac{V}{A} \times \frac{\Delta C}{\Delta t} \times \frac{273}{273 + T}, \quad (1)$$

where  $F$  is the flux of gas (mg/m<sup>2</sup>/h);  $V$  is the volume of the chamber above the water–air interface (m<sup>3</sup>);  $A$  is the cross-sectional area of the water–air interface (m<sup>2</sup>);  $\Delta C$  is the concentration difference between two sampling times (mg/m<sup>3</sup>);  $\Delta t$  is the time interval between two sampling periods (h);  $T$  is the temperature of the incubation system (°C).

### 2.6 Statistical analysis

One-way analysis of variance (ANOVA) followed by post hoc Tukey's test was employed to evaluate the differences among treatments, with significance set at  $p < 0.05$ . The alpha diversity indices, including the *Sobs*, *Chao1*, and Shannon indices, were calculated using the QIIME 2 platform. To examine the dissimilarities in the microbial communities, principal component analysis (PCA) was conducted based on the Bray-Curtis dissimilarity metric. The linear

discriminant analysis effect size (LEfSe) method, executed on the Galaxy platform, was applied to identify biomarker profiles for both the control (CON) and microplastic (MP) treatments. Bacterial functions were annotated using the FAPROTAX database on the Majorbio Cloud Platform. To conduct Mantel's test, the "linkET" package in R 4.2.1 was used, while all other visualizations were created in R 4.2.1 using the "ggplot2" package and GraphPad Prism 9.5.

## 3 Results and discussion

### 3.1 Effects of microplastics on nutrient contents

#### 3.1.1 Changes in carbon contents in the sediment and overlying water

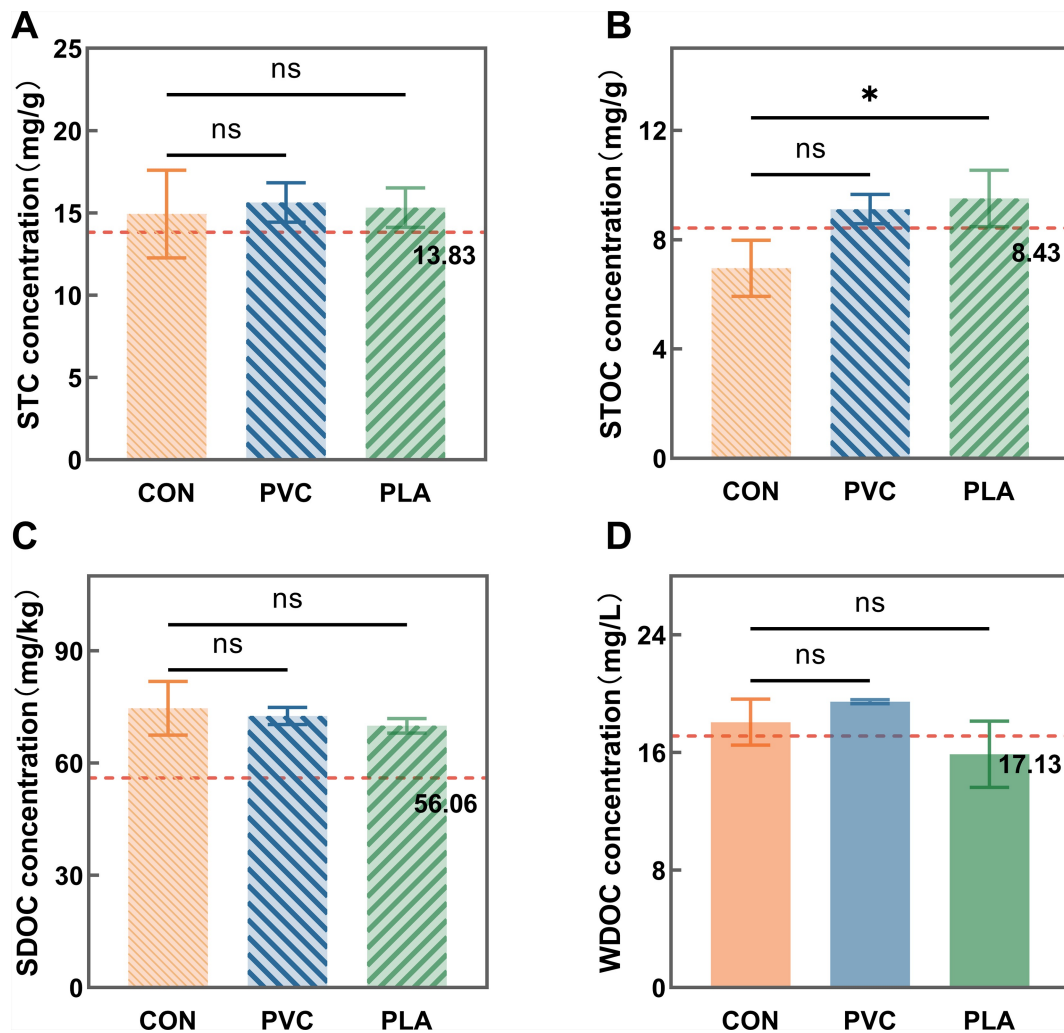
After 19 d of exposure, the addition of PVC and PLA did not significantly affect the contents of sediment total carbon (STC), sediment dissolved organic carbon (SDOC), or water dissolved organic carbon (WDOC) ( $p < 0.5$ ). However, compared with the control, both PVC and PLA increased the concentration of sediment total organic carbon (STOC) by 31.3% and 36.7%, respectively (Fig. 1). Carbon is a major component of both PVC and PLA, and the addition of MPs can influence sediment carbon storage and conversion (Jiang et al., 2019). MPs can be incorporated into sediment aggregates in combination with minerals and organic matter, disguised as a component of sediment carbon storage (Rillig and Bonkowski, 2018; Yu et al., 2023a). This study demonstrated that both PVC and PLA enriched the sediment carbon substrate (STOC) for microorganisms, a finding that is consistent with previous studies (Zhang et al., 2022; Su et al., 2024). The increased TOC in sediments can alter their physiochemical properties, such as porosity and aeration, which, in turn, affects microbial communities (Chen et al., 2024c; Chen et al., 2024d).

The PVC and PLA treatments significantly increased the STOC content, whereas no notable increase was observed in the STC concentration. This can be attributed to two possible reasons. First, the addition of these MPs likely altered the microbial community structure, indirectly influencing sediment inorganic carbon levels. For example, studies have shown that PLA can promote denitrification, potentially leading to the consumption of inorganic carbon sources (Yin et al., 2023). Second, the proportions of PVC and PLA in the TC content were relatively low, and the changes in TC were not significant within the experimental error margin. DOC is known to be a byproduct during the

fragmentation and oxidation of MPs in freshwater and marine sediments (Romera-Castillo et al., 2018). Previous studies have demonstrated that adding 1% MPs to sediment resulted in a twofold increase in DOC content compared with adding 0.1% MPs (Huang et al., 2021). However, in this study, neither PLA nor PVC significantly affected the DOC levels, suggesting limited degradation and oxidation of the MPs during the short-term incubation period. Additionally, variations in carbon utilization patterns and microbial responses to MP exposure may result in fluctuations in carbon content (Feng et al., 2024). The organic matter generated during MP exposure may have been utilized by sediment microorganisms, leading to nonsignificant changes in WDOC content (Seeley et al., 2020).

#### 3.1.2 Changes in nitrogen contents in the sediment and overlying water

$\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  are two primary forms of inorganic nitrogen in sediments, and their fluctuations can provide insights into the nitrification and denitrification processes occurring under different treatments (Huang et al., 2023). After 19 d of incubation, the concentrations of water  $\text{NH}_4^+$  ( $\text{WNH}_4^+$ ) and sediment  $\text{NH}_4^+$  ( $\text{SNH}_4^+$ ) remained largely unchanged with the addition of MPs (Fig. 2A). In contrast, the concentrations of water  $\text{NO}_3^-$  ( $\text{WNO}_3^-$ ) and water TN (WTN) were significantly decreased by 94.1% and 71.3%, respectively, in PVC treatment compared to the control ( $p < 0.05$ , Figs. 2B and 2C). This substantial decrease in  $\text{NO}_3^-$  may disrupt nutrient balances, potentially suppressing organisms with high nitrogen requirements and altering the composition and functionality of aquatic ecosystems.  $\text{NO}_3^-$  storage relies on a delicate balance between its production (e.g., mineralization and nitrification) and consumption (e.g., immobilization and denitrification) processes (Wang et al., 2024b). A reduction in  $\text{NO}_3^-$  concentration has been documented in previous studies (Shi et al., 2022; Wang et al., 2023), particularly in terrestrial ecosystems. The increase in OC content observed in the PVC treatment stimulated microbial N assimilation and immobilization (Boots et al., 2019). Additionally, enhanced denitrification contributed to the decrease in  $\text{NO}_3^-$  by transforming  $\text{NO}_3^-$  to  $\text{NO}_2^-$ . PVC has been shown to enrich nitrate-reducing bacteria in the soil microbiota, potentially through the release of phthalate plasticizers (Zhu et al., 2022a). Dai et al. (2024) reported that PVC stimulated dissimilatory nitrate reduction to ammonium (DNRA) rates to facilitate  $\text{NO}_3^-$  consumption. Nevertheless, the inhibitory effect of PVC on denitrification has also been reported



**Fig. 1** Changes in carbon contents in the microcosms under MP exposure. The concentrations of TC, TOC, and DOC in the sediment (A, B, and C), along with the DOC in the overlying water (D) are shown for each MP treatment as well as for the CON microcosms. The initial concentrations on Day 0 are indicated by red dotted lines. STC, STOC, and SDOC represent sediment TC, TOC, and DOC, respectively. WDOC represents water DOC (ns: not significant; \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ ).

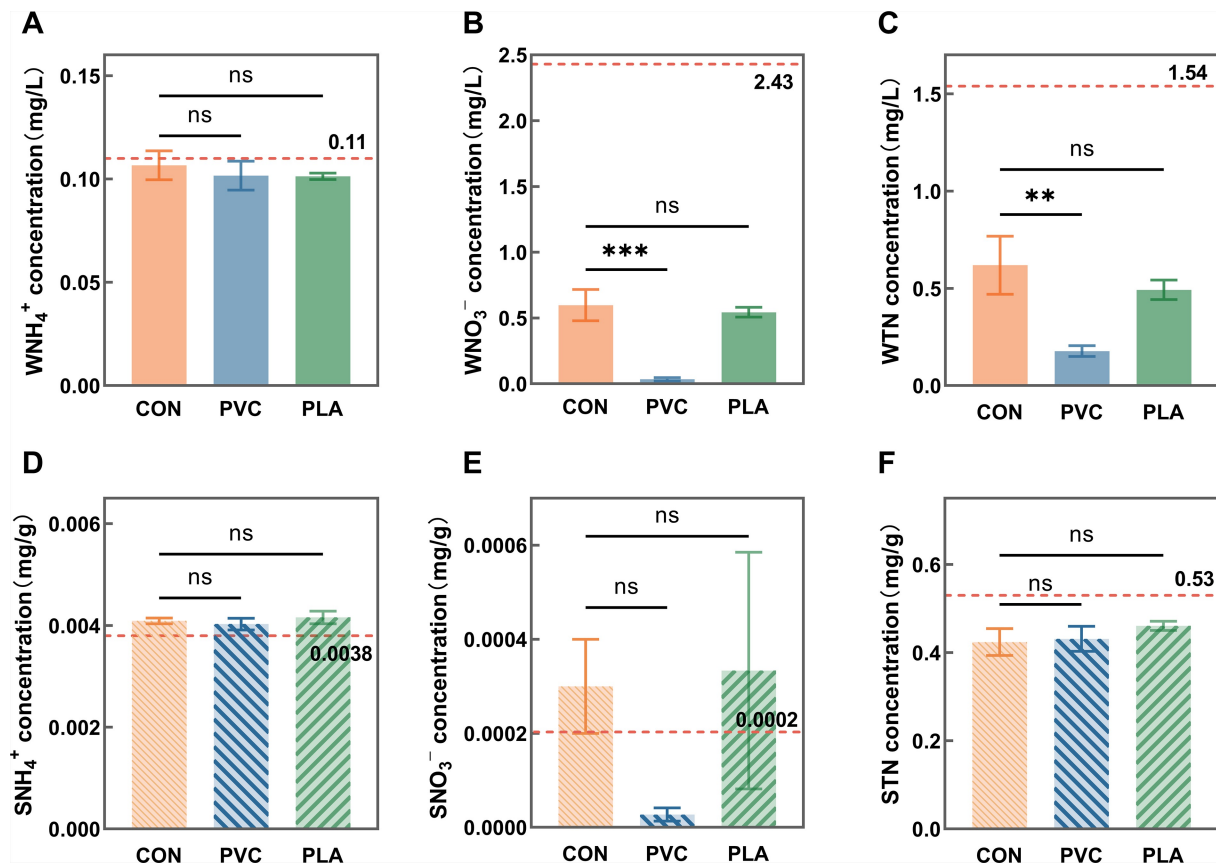
(Seeley et al., 2020), primarily due to the enrichment of sulfate-reducing bacteria in the incubation system, which further inhibited the denitrification process.

Previous studies have reported better adaptation of microorganisms to the degradable PLA, resulting in a minimal effect on the microbial community structure and assembly (Zhu et al., 2022b). Additionally, PLA has been observed to promote both nitrification and denitrification, ultimately leading to nonsignificant changes in  $\text{NO}_3^-$  content (Sun et al., 2022a). Thus, a comprehensive analysis of microbial communities is needed to fully elucidate the underlying mechanisms through which these two distinct MPs exert their effects. In the CON group, we observed an increase in  $\text{SNH}_4^+$  and  $\text{SNO}_3^-$  compared with the initial (Day 0) state, whereas STN decreased. This is likely due to

enhanced microbial mineralization within the incubation system, which resulted in elevated  $\text{NH}_4^+$  levels (Dai et al., 2024). The increase in  $\text{NO}_3^-$  content may be attributed to stronger production than consumption. However, nitrogen loss still occurred in the system because of denitrification processes in the hypoxic microenvironment, where nitrogen was converted into  $\text{N}_2$  or  $\text{N}_2\text{O}$ , leading to a reduction in TN.

### 3.2 Effects of microplastics on the microbial community structure

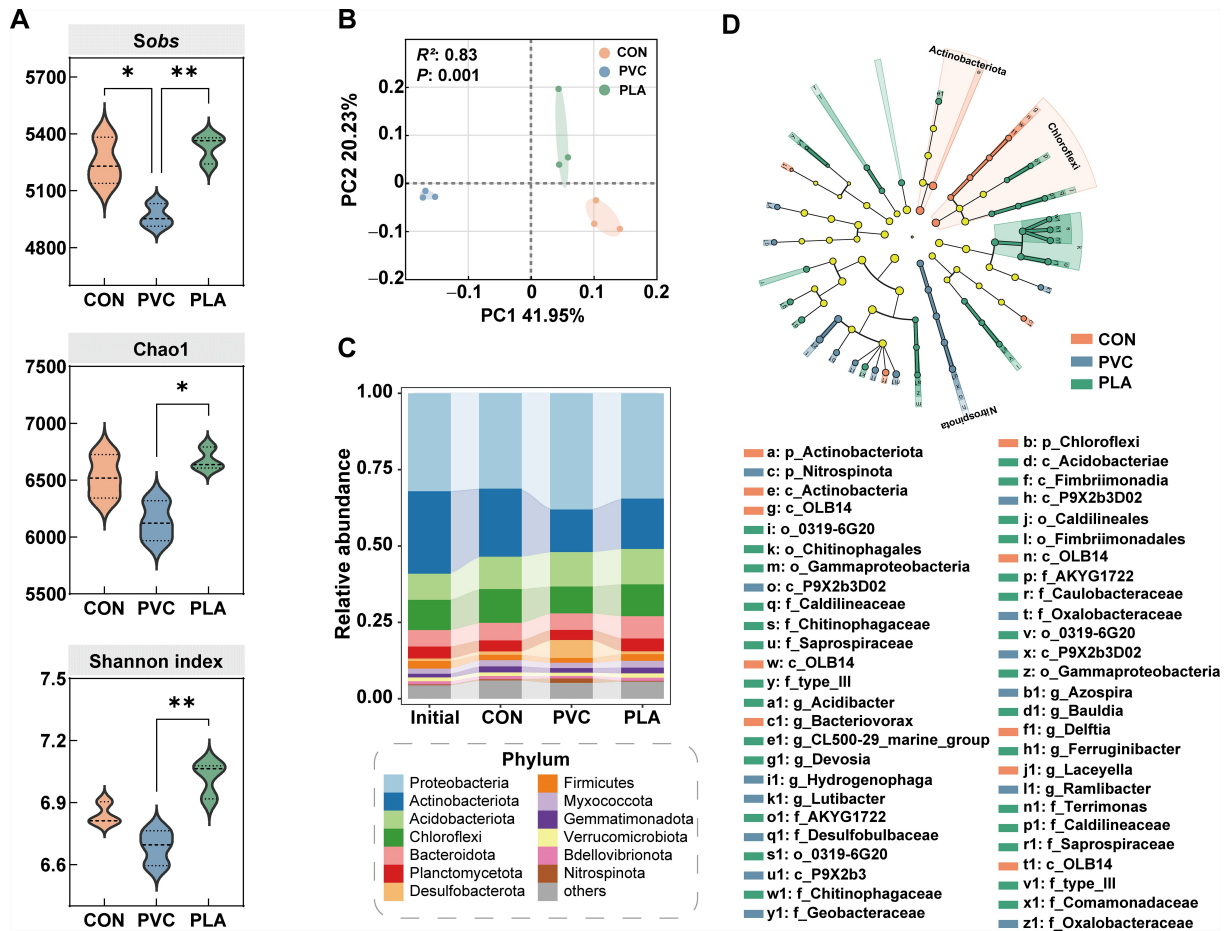
Sediment microorganisms are key drivers of various biogeochemical processes, and investigating shifts in their diversity and composition is essential for understanding the mechanisms through which MPs



**Fig. 2** Changes in nitrogen concentrations in the microcosms under MP exposure. The concentrations of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and TN in the overlying water (A, B, and C) and in the sediments (D, E, and F) are presented for each microplastic treatment as well as the CON microcosms. The concentrations on Day 0 are indicated by red dotted lines.  $\text{WNH}_4^+$ ,  $\text{WNO}_3^-$ , and WTN refer to water  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and TN, respectively.  $\text{SNH}_4^+$ ,  $\text{SNO}_3^-$ , and STN denote sediment  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and TN, respectively (ns: not significant; \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ ).

affect microbial ecology (Wu et al., 2024). In terms of bacterial diversity, compared with the control, PVC exposure significantly decreased the *Sobs* index (Fig. 3A,  $p < 0.05$ ). In contrast, compared with the control, the PLA treatment did not significantly affect any of the three diversity indices. When the two MPs were compared, the *Sobs*, Chao1, and Shannon indices were lower in the PVC treatment than in the PLA treatment ( $p < 0.05$ ). Principal coordinate analysis (PCoA) yielded similar results, revealing a significant separation between the PVC treatment and the control, whereas the microbial communities in the PLA treatment exhibited only minor disparities compared with those in the control (Fig. 3B). Discrepant changes in microbial diversity induced by MPs have been previously documented and are likely due to the distinct properties of MPs (biodegradability, polymer components, etc.) and the colonization of diverse microbial communities (Sun et al., 2022c; Yin et al., 2023).

As shown in Fig. 3C, the dominant bacterial phyla across all sediment samples were Proteobacteria, Actinobacteriota, Acidobacteriota, and Chloroflexi, collectively accounting for more than 75% of the total sequences. The predominance of these phyla in freshwater sediments is well documented (Fan et al., 2023; Zhang et al., 2024). After 19 d of incubation, the relative abundance of Proteobacteria decreased in the CON group, whereas the relative abundances of Actinobacteriota and Acidobacteriota slightly increased compared with those in the initial bacterial community. Compared with those in the control, the sediment in the PVC treatment presented greater abundances of Proteobacteria (CON: 31.2%; PVC: 38.0%) and Desulfobacterota (CON: 1.1%; PVC: 5.9%) but lower abundances of Actinobacteriota (CON: 22.3%; PVC: 14.0%) and Chloroflexi (CON: 11.0%; PVC: 8.8%). The effect of PLA on the composition of the bacterial phyla was similar to that of PVC, albeit less pronounced.



**Fig. 3** Effects of microplastics on sediment bacterial community diversity and structure. Alpha diversity indices (A) and PCA analysis of the microbial communities (B). The composition of the bacterial phyla is shown for the initial sediment (Day 0) as well as the microplastic and control treatments on Day 19 (C). Cladogram of bacterial biomarkers in the microplastic and control treatments based on the linear discriminant analysis effect size analysis (LEfSe) (D). Inside-out cladogram circles represent phylogenetic taxa from phylum to genus (\*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ ).

At the genus level (Fig. S1), PVC exposure increased the relative abundances of *Desulfuromonas*, *Pseudomonas*, *Sulfuritalea*, *Methylotenera*, and *unclassified\_f\_Oxalobacteraceae*, but decreased the abundances of *Bauldia*, *Hyphomicrobium*, *Pedomicrobium*, and *Terrimonas* relative to the control. PLA treatment reduced the relative abundances of *Pseudomonas* and *Delftia*, but increased those of *Massilia*, *unclassified\_f\_Comamonadaceae*, and *Bauldia*. Both *Desulfuromonas* and *Pseudomonas* are known denitrifying bacteria with the potential for MP degradation (Liao et al., 2024), and their enrichment in PVC-supplemented microcosms has been observed in previous studies (Li et al., 2024a; Panthi et al., 2024). *Sulfuritalea* is capable of coupling the reduction of  $\text{NO}_3^-$  to  $\text{N}_2$  with the conversion of thiosulfate to sulfate (Guan et al., 2022), contributing to nitrogen elimination, which may explain the reduced  $\text{NO}_3^-$

content in the PVC treatment. Among the bacteria inhibited by PVC, *Hyphomicrobium* and *Pedomicrobium* are associated with hydrocarbon degradation (Coll et al., 2020), and *Terrimonas* has been reported as a denitrifying genus (Li et al., 2024c). Conversely, PLA negatively affects heterotrophic nitrifying-aerobic denitrifying bacteria such as *Pseudomonas* and *Delftia* (Zhao et al., 2023). *Massilia* and *Bauldia*, which are enriched in degradable PLA treatment, are involved in organic matter degradation (Ge et al., 2023; Zheng et al., 2024).

LEfSe analysis was conducted to identify bacterial responders under distinct MP stresses (Fig. 3D). A total of 52 bacterial clades exhibited significant differences ( $\text{LDA} > 2.5$ , Fig. S2). LEfSe analysis confirmed the greater abundance of the classes *Actinobacteria* and *OLB14*, which belong to the Actinobacteriota and Chloroflexi phyla, respectively, in the CON group.



*Actinobacteria* are known to be sensitive to MP addition, and their decreased abundance in PVC-amended soil has been previously reported (Sun et al., 2022c). The PVC treatment harbored a higher abundance of *Rhodoferrax*, *Ramlibacter*, *Azospira*, and *Hydrogenophage*. The enrichment of MPs on *Rhodoferrax* and *Ramlibacter* has been reported in water and sediment matrices (Hu et al., 2021). *Azospira* and *Hydrogenophage* were characterized as nitrogen-removal bacteria (Tan et al., 2020; Lalinská-Voleková et al., 2022), possibly explaining the lower  $\text{WNO}_3^-$  and WTN levels in the PVC treatment.

More biomarkers, including bacterial families such as *Chitinophagaceae*, *Saprospiraceae*, *Comamonadaceae*, *Caldilineaceae*, and *Caulobacteraceae*, were identified in the PLA treatment. Genera including *Acidibacter*, *Ferruginibacter*, *Terrimonas*, *Devosia*, and *Bauldia* were identified as PLA-treated biomarkers. Most of them (*Chitinophagaceae*, *Comamonadaceae*, *Caulobacteraceae*, and *Bauldia*) displayed superior abilities in degrading complex organic matter (e.g., polyesters), and their enrichment under biodegradable MP treatment has been reported in previous studies (Ge et al., 2023; Rauscher et al., 2023; Zheng et al., 2024). Other biomarkers included those involved in nitrogen cycling (*Saprospiraceae*, *Acidibacter*, and *Terrimonas*) (Zhou et al., 2020; Jia et al., 2024) and *Ferruginibacter*, which is sensitive to organic contaminants (e.g., MPs and bisphenol S) (Cui et al., 2021).

### 3.3 Effects of microplastics on microbial function

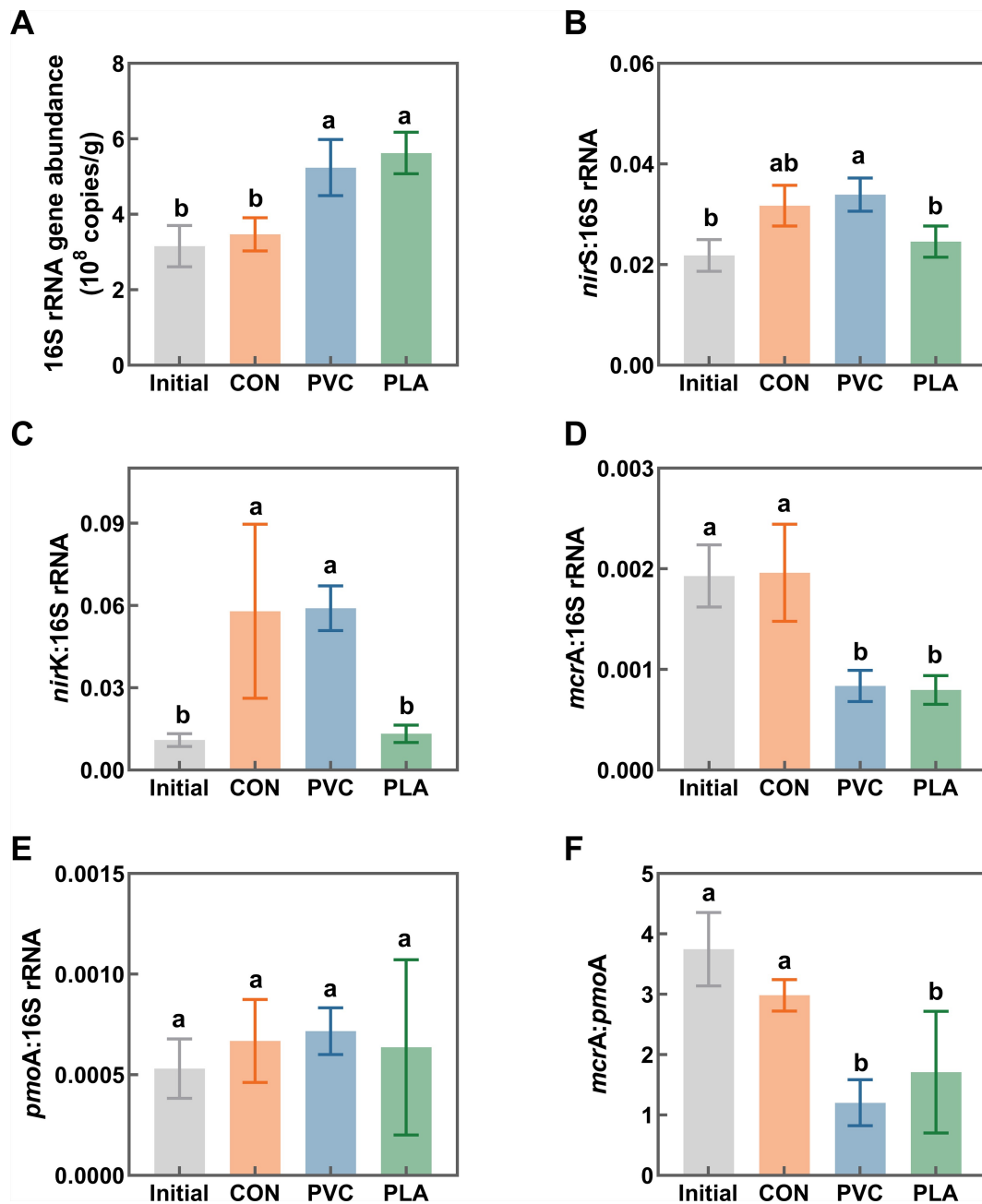
The effects of MPs on microbial function were investigated by combining qPCR analysis of targeted functional genes with FAPROTAX function prediction (Fig. S3). As shown in Fig. 4A, the abundance of the 16S rRNA gene in the PVC and PLA treatments was significantly higher than that in the initial and CON groups, indicating that the bacterial population was stimulated by MP addition. The increase in the STOC content in the MP treatments likely provided a carbon source for the bacterial community, promoting its growth. This is supported by the significant positive correlation between the STOC content and 16S rRNA abundance ( $p < 0.05$ , Fig. S4). Compared with the control, PLA treatment significantly reduced the relative abundance of the *nirK* gene, which encodes nitrite reductase (Figs. 4B and 4C), in line with the results of the microbial community analysis. The functional genes *mcrA* and *pmoA* encode methyl coenzyme M reductase and particulate methane monooxygenase, respectively, and the ratio of copy numbers of *mcrA* to *pmoA* could represent the  $\text{CH}_4$

release potential (Qi et al., 2020). Both the PVC and PLA treatments significantly reduced the relative abundance of methanogenic bacteria ( $p < 0.05$ ), suggesting a potential impact on  $\text{CH}_4$  emissions. Many of the bacterial species enriched under the PVC and PLA treatments are acetate or  $\text{H}_2$ -utilizing microorganisms (*Desulfuromonas*, *Pseudomonas*, *Azospira*, and *Terrimonas*) that can compete for substrates with methanogenic bacteria (Vikromvarasiri et al., 2023; Zhang et al., 2023; Chang et al., 2024). Their enrichment may occupy the ecological niche of methanogens, contributing to the observed reduction in methanogenic abundance.

FAPROTAX is a tool based on functional annotations from a prokaryotic microbial taxonomic database that is primarily employed to predict biogeochemical cycling processes (Jiang et al., 2021). Compared with the control, PVC addition enriched bacterial groups involved in nitrogen respiration, nitrate reduction, and nitrate respiration (Fig. S3), without significantly altering the abundance of bacteria associated with nitrite denitrification and respiration. Furthermore, both the PVC and PLA treatments increased the intensities of aerobic chemoheterotrophy and chitinolysis, while reducing those related to hydrocarbon degradation and ligninolysis. The increased abundance of chemoheterotrophic functions in the plastisphere has been reported in previous studies (Wang et al., 2024d). The decreased intensities of hydrocarbon degradation and ligninolysis may indicate the suppression of microbial degradation of some complex carbon compounds (Chen et al., 2024a), which aligns with the findings that PVC decreased the relative abundance of hydrocarbon-degrading microorganisms.

### 3.4 Effects of microplastics on greenhouse gas emissions

The preceding analyses of microbial community structure and function suggest that the addition of PVC and PLA influenced C/N cycling processes in the sediment. The subsequent changes in GHG emissions mediated by microorganisms are discussed below. Fig. 5 shows the emission fluxes ( $\text{mg}/(\text{m}^2 \cdot \text{h})$ ) and cumulative emissions ( $\text{mg}/\text{m}^2$ ) of  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$  in the sediment–water microcosms. The  $\text{CO}_2$  flux in both the MP and control groups initially increased but then decreased (Fig. 5A). On the 6th day, the  $\text{CO}_2$  fluxes in the PVC and PLA treatments were lower than those in the control. However, subsequently, the  $\text{CO}_2$  flux in the PVC treatment increased and exceeded that in the control group. After 19 d of incubation, the  $\text{CO}_2$  cumulative emission ranged from 3448.2 to 3814.7

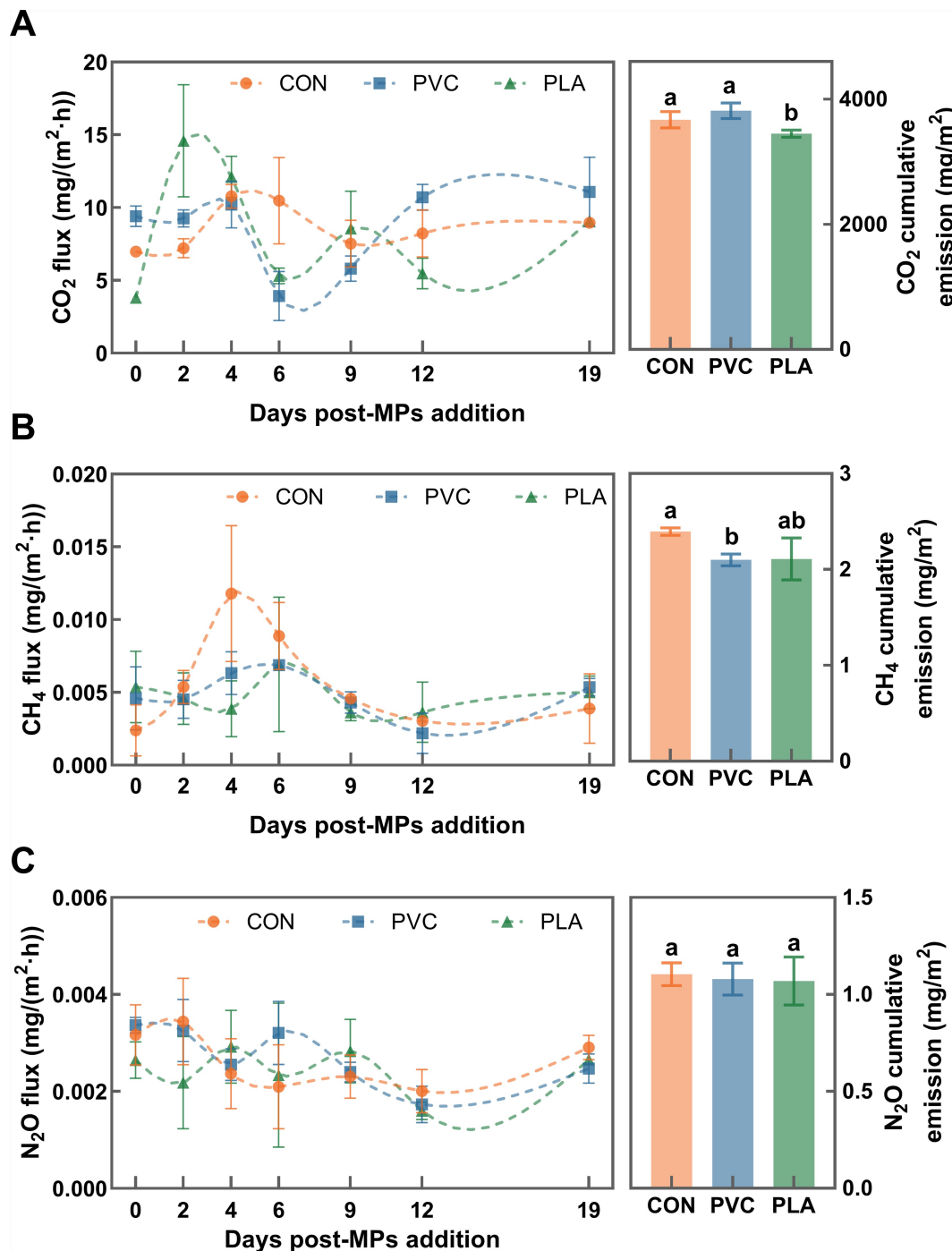


**Fig. 4** Functional gene abundance changes under MP exposure. Abundances of the 16S rRNA gene (A); and the relative abundances of genes encoding nitrite reductases (*nirS*, B; *nirK*, C). Genes encoding methyl-coenzyme M reductase (*mcrA*, D) and methane monooxygenase (*pmoA*, E). Ratio of *mcrA* to *pmoA* (F). The letters located above the bars denote significant differences ( $p < 0.05$ , ANOVA).

mg/m<sup>2</sup> across these treatments. PLA exhibited a negative effect on CO<sub>2</sub> cumulative emissions compared with those of the control ( $p < 0.05$ ). As shown in Fig. 5B, PVC and PLA decreased the CH<sub>4</sub> flux on Days 4 and 6 of incubation, with the CH<sub>4</sub> cumulative emission in the PVC treatment being lower than that in the CON group ( $p < 0.05$ ). For N<sub>2</sub>O flux, PLA had inhibitory

effects on Days 2, 12, and 19 of incubation (Fig. 5C) but had nonsignificant effects on N<sub>2</sub>O cumulative emissions.

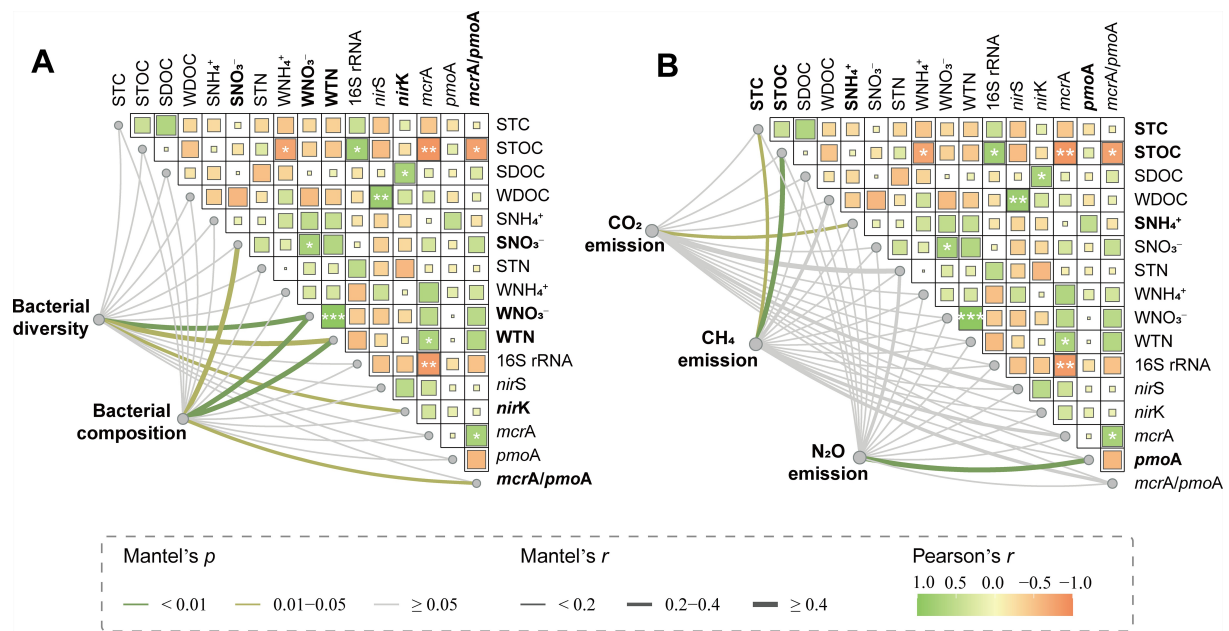
We further performed the Mantel's test to identify potential drivers of bacterial community alterations and changes in GHG emissions (Fig. 6). Both bacterial diversity and composition were strongly correlated with



**Fig. 5** Changes in sedimentary greenhouse gas fluxes and emissions under MP exposure. The release fluxes (mg/(m<sup>2</sup>·h)) and cumulative emissions (mg/m<sup>2</sup>) of CO<sub>2</sub> (A), CH<sub>4</sub> (B), and N<sub>2</sub>O (C) are shown for the different microplastics treatments and the control. The error bars indicate the standard errors of the means ( $n = 3$ ). Different letters above the bars indicate significant differences ( $p < 0.05$ , ANOVA).

WNO<sub>3</sub><sup>-</sup> and WTN levels (Fig. 6A), supporting our previous hypothesis that the enrichment of nitrogen-reducing bacteria in the PVC treatment led to a significant reduction in WNO<sub>3</sub><sup>-</sup> and WTN levels.

Additionally, bacterial composition was found to be correlated with the SNO<sub>3</sub><sup>-</sup> concentration and the *mcrA/pmoA* ratio. In terms of GHG emissions, CO<sub>2</sub> emissions were significantly associated with SNH<sub>4</sub><sup>+</sup>.



**Fig. 6** Biotic and abiotic factors driving changes in the microbial community and GHG emissions. Mantel's test revealing the potential driving forces of changes in bacterial diversity and composition (A). Mantel's test revealing the potential driving forces of alterations in CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O emissions (B) (\*: Pearson's  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ ).

CO<sub>2</sub> release and NH<sub>4</sub><sup>+</sup> production typically occur simultaneously during the microbial metabolism of organic matter, both of which are closely linked to the intensity of microbial activity (Yang et al., 2022). Despite the enrichment of organic matter-degrading microorganisms in the PLA treatment, which have been reported to exacerbate GHG emissions (Chen et al., 2023a), no significant increase in CO<sub>2</sub> emissions was observed during the short-term 19-d incubation in this study. Zhang et al. (2024) reported that PLA suppressed soil CO<sub>2</sub> emission, which was attributed to this effect to the reduction in soil porosity and the inhibition of soil respiration caused by MP addition. Another study indicated that while MPs promoted CO<sub>2</sub> release from macroaggregates, they inhibited CO<sub>2</sub> emission from macroaggregates, silt, and clay (Wang et al., 2024c). The underlying mechanism for this inhibition remains to be explored, particularly regarding the identification of the sources of CO<sub>2</sub> emissions.

As shown in Fig. 6B, the changes in STOC and STC contents induced by MPs also contributed to alterations in CH<sub>4</sub> emissions. Previous studies have demonstrated that water bodies with higher STOC tend to release more CH<sub>4</sub> (Wang et al., 2025). However, although PVC and PLA increased the STOC in this study, they exhibited inhibitory effects on methanogenic microorganisms, resulting in a slight reduction in CH<sub>4</sub> emissions. This effect may be due to microorganisms preferentially utilizing the organic carbon provided by

MPs. When they focus on metabolizing PVC and PLA-derived carbon sources, the availability of CO<sub>2</sub> as an electron acceptor for methanogenesis is reduced, thereby inhibiting CH<sub>4</sub> release (Chen et al., 2024d). Additionally, changes in the microbial community structure may play a significant role. As discussed earlier, PVC and PLA enrich acetate- or H<sub>2</sub>-utilizing microorganisms, which compete with methanogenic bacteria for substrates, thus inhibiting their growth and metabolism (Zhang et al., 2023; Chang et al., 2024). Previous studies have demonstrated that other plastics, such as PE and PBAT, can aggravate CH<sub>4</sub> emission (Fan et al., 2023), whereas our previous research also revealed that PVC and PLA simultaneously inhibited the abundance of both methanogenic and methane-oxidizing bacteria, resulting in no significant change in CH<sub>4</sub> emission (Yi et al., 2024). These discrepancies are largely dependent on the MP type, sediment nutrient substrate, and variation in the microbial response (Zhang et al., 2022; Chen et al., 2023b).

With respect to N<sub>2</sub>O, no significant effect of PVC was observed. Although PVC was found to promote denitrifying microorganisms in microbial community structure analyses, functional gene analyses revealed that nitrate denitrification processes were primarily affected, with no significant changes in nitrite reduction. Similar nonsignificant effects of PVC on sediment N<sub>2</sub>O emissions and denitrification rates have been reported by Seeley et al. (2020) and Chen et al.

(2022). In contrast, functional gene analyses revealed that PLA inhibited the abundance of nitrite-reducing microorganisms, resulting in lower  $\text{N}_2\text{O}$  flux in the PLA treatment than in the control. The inhibitory effect of MPs on  $\text{N}_2\text{O}$  emissions was previously reported in terrestrial ecosystems (Yu et al., 2021).

### 3.5 Environmental implications

The ecological impacts of MPs on microbial communities, which play a central role in biogeochemical cycling (Yang et al., 2022), merit considerable attention. This study compared the impacts of PVC and degradable PLA on sediment microbial community structure, function, and subsequent GHG emissions. The addition of PVC and PLA induced complex and extensive changes in sediment microecology. Initially, the addition of MPs may affect sediment porosity, altering oxygen conditions and thereby influencing microbial activities. Additionally, MPs provide carbon sources for sediment microbial communities, stimulating bacterial growth, as evidenced by changes in 16S rRNA gene abundance in this study. Furthermore, additives or degradation products of MPs can modify the microbial community structure and nutrient cycling processes through the selective inhibition or promotion of microorganisms. Specifically, PVC selectively enriched nitrate-reducing and nitrogen-respiring microorganisms, accelerating the depletion of TN and  $\text{NO}_3^-$  and further disrupting nitrogen cycling. The significant reduction in  $\text{NO}_3^-$  content could limit the uptake and utilization of nitrogen by aquatic plants, potentially hindering their growth and negatively affecting ecosystem productivity (Wang et al., 2024b). In contrast, biodegradable PLA primarily influences carbon cycling. It significantly increased the STOC content and, compared to PVC, exerted a more moderate effect on the microbial community structure. PLA primarily promoted the growth of microorganisms involved in organic matter degradation while reducing the abundance of denitrifying microorganisms, as confirmed by functional gene analysis. Both PVC and PLA enriched acetate-reducing microorganisms, which may compete with methanogenic bacteria for substrates, leading to decreased methanogenic bacteria abundance and consequently inhibiting  $\text{CH}_4$  emission.

Although MP addition did not promote GHG emissions in this study, the alterations in sediment nutrient content and microbial community structure induced by both PVC and PLA are noteworthy. The distinct effects of PVC on nitrogen cycling and of PLA on carbon cycling, coupled with the inhibition of  $\text{CH}_4$

emission by both types of MP, contribute to changes in the nutrient cycles of the sediment–water system. To a certain extent, these changes may have positive effects, such as mitigating GHG emissions and optimizing local nutrient cycling. However, if these changes exceed the ecosystem threshold, they could disrupt the ecological balance, potentially leading to ecological risks. Future research is needed to investigate the mechanisms of MP-induced selective inhibition/enrichment of microbial communities, examine comprehensive impact pathways, and conduct large-scale field studies to fully elucidate the ecological risks associated with MP pollution.

## 4 Conclusions

Overall, following short-term 19-d exposure, the addition of PVC and PLA increased the STOC content, which subsequently stimulated an overall increase in bacterial abundance. In terms of microbial community diversity, PVC exhibited a stronger effect than PLA, with a significant negative impact. Both PVC and PLA notably altered the sediment microbial community structure, enriching different bacterial biomarkers. Specifically, PVC increased the abundance of genera with nitrogen removal capacity (e.g., *Pseudomonas*, *Azospira*, and *Hydrogenophaga*), leading to a significant decrease in  $\text{WNO}_3^-$  and WTN contents. In contrast, PLA primarily enriched species involved in the degradation of complex organic matter (e.g., *Chitinophagaceae*, *Comamonadaceae*, *Caulobacteraceae*, and *Bauldia*). A combination of functional gene analyses and FAPROTAX predictions revealed that PVC enhanced sediment nitrate denitrification and respiration, whereas PLA suppressed the relative abundance of nitrite-reducing bacteria. Both PVC and PLA reduced the abundance of methanotrophic bacteria, possibly because of the competition from certain acetate-utilizing bacteria that were selectively enriched in the MP treatments. The reduced abundance of methanotrophic bacteria led to a lower  $\text{CH}_4$  flux than that in the control. This study provides a comprehensive investigation into the effects of distinct MPs on sediment microbial community structure and GHG emissions, offering valuable insights into the ecological risk associated with MP pollution in riverine ecosystems.

**Conflict of interests** The author Yang Li is a member of youth editorial board of *Frontiers of Environmental Science & Engineering*. 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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