Energy Proceedings
Vol 49, 2024
ISSN 2004-2965

Impact of Microplastics on the Gene Abundance of ANME-1 Methane Metabolism#

Xiaonan Wu[1, 2, 3, 4], Jing-chun Feng[1, 2, 3, 4*], Xiao Chen[1, 2 ,3, 4], Si Zhang[1, 2 ,3, 4], Zhifeng Yang[1, 2 ,3, 4]

1 School of Ecology, Environment and Resources, Guangdong University of Technology, Guangzhou, 510006, China

2 Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou, 511458, China

3 Research Centre of Ecology & Environment for Coastal Area and Deep Sea, Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou, 510006, China

4 Guangdong Basic Research Center of Excellence for Ecological Security and Green Development, Guangdong University of Technology, Guangzhou, 510006, China

(* Corresponding Author: fengjc@gdut.edu.cn)

ABSTRACT

Microplastics (MPs), produced in large quantities due to their affordability and durability, have raised concerns about their environmental impact. In 2021, global plastic production reached 390.7 million tons. Cold seeps harbor significant populations of ANME-1 archaea, which are involved in methane production and oxidation. However, the effect of MPs on the gene abundance related to methane metabolism in ANME-1 remains unclear. To address this gap, the present study conducted laboratory cultivation experiments using sediment and seawater samples from the Haima cold seep. The main findings are: (1) The addition of MPs did not significantly affect the gene abundance of the ANME-1 community ($p > 0.05$). (2) Methane metabolism gene coverage in sediment was higher than in seawater ($p < 0.001$). (3) Environmental factors significantly impacted the gene abundance of nitrogen and methane metabolisms in seawater compared to sediment. This work provides a better perspective on the impact of MPs on methane production and oxidation in the cold seep.

Keywords: Microplastic, ANME-1, Methane metabolism, Function gene, Environmental factor.

NONMENCLATURE

Abbreviations	
MP	Microplastic
ANME	Anaerobic methanotrophic archaea
con	Control
PE	Polyethylene
PET	Polyethylene Terephthalate
PLA	Polylactic Acid
PVC	Polyvinyl Chloride
TOC	Total organic carbon
TIC	Total inorganic carbon
STOC	TOC in the sediment
STIC	TIC in the sediment
NH_4^+	Ammonium
NO_3^-	Nitrate
NO_2^-	Nitrite

1. INTRODUCTION

Microplastics (MPs) are plastic debris smaller than 5 mm1 that have become one of the most severe global environmental pollution issues. Due to their affordability and durability, MPs are produced in large quantities, with global plastic production reaching 390.7 million tons in 20212. However, poor management and their resistance to degradation have led to their widespread presence in the environment. MPs affect organisms in the surface ocean and microbial communities in classical deep-sea ecosystems, especially cold seeps3.

Cold seeps are unique ecosystems where methane-rich fluids gush from the seabed into the seawater column, supporting complex microbial and macrobiotic communities. The metabolic activities of microorganisms in cold seep environments play a crucial role in the global carbon cycle, particularly methanotrophic archaea. Currently, three types of anaerobic methanotrophic archaea have been identified: ANME-1, ANME-2, and ANME-34. Previous studies have indicated that ANME-2 can not only oxidize methane but also has the potential to fix nitrogen. Although ANME-1 cannot fix nitrogen, it can produce and oxidize methane4,5. Methanotrophic archaea, mainly ANME-1 and ANME-2, are vital in

This is a paper for the 16th International Conference on Applied Energy (ICAE2024), Sep. 1-5, 2024, Niigata, Japan.

methane-oxidization. These microorganisms, through their symbiotic relationship with sulfate-reducing bacteria, effectively convert methane into carbon dioxide6, reducing the release of this potent greenhouse gas by 90% in the seabed7. Therefore, they are referred to as the seabed methane barriers. ANME-1 alternates between oxidizing methane and producing methane, depending on the energy production process5, further complicating its ecological functions.

Previous studies have confirmed that MPs can affect microbial community structure and function in marine environments8,9. However, research on the impact of MPs on key microbial communities, such as ANME-1, in cold seep environments is limited. This research gap limits our comprehensive understanding of the environmental impact of MPs. Therefore, studying the effects of MPs on the metabolic activities of ANME-1 in cold seep environments is of significant importance.

The study focuses on the impact of MPs on the methane production and oxidation processes of ANME-1, aiming to study the balance of methane release and sequestration, which has profound implications for global climate change. We collected sediment and seawater samples from the Haima cold seep site to conduct laboratory cultivation experiments, simulating in situ environmental conditions. The study was conducted in three parts: (1) Analyzing the richness of the abundance of function-predicted genes. (2) Using PICRUSt2 to predict the ANME-1 community and assess the impact of MPs on their nitrogen and carbon cycle pathways. (3) Investigating the impact of environmental factors on the genes involved in carbon and nitrogen cycling.

2. MATERIAL AND METHODS

2.1 Geological information and sampling location

The Haima cold seep is located in the western part of the Pearl River Estuary Basin in the South China Sea. ROV1 is the middle cold seep development stage with strong methane seepage. We collected all samples in December 2022.

2.2 Laboratory cultivation experiments

Before the formal experiments, the high-pressure reactors were sterilized. MPs were soaked in anhydrous ethanol for 1 hour and cleaned three times using ultrapure water. 60 g of sediment with 8 g of cleaned MPs and 100 mL of seawater with 8 g of cleaned MPs were successively added to the reactor. The experiment was conducted for two months.

2.3 DNA extraction and 16s rRNA gene sequencing

DNA in sediment samples was extracted using the manufacturer's instructions for A Magnetic Soil and Stool DNA Kit (TIANGEN, China). 1.5 μL of qualified genomic DNA was used for PCR amplification targeting the V3-V4 region of the archaea 16s rRNA gene with primer pairs 349 F and 806 R[10]. The PCR amplification conditions were conducted using the method[9]. The experimental conditions were optimized based on the characteristics of the samples. DNA libraries were tested using Agilent 2100 Bioanalyzer (Agilent, USA). Eligible libraries were sequenced based on insert size using the Illumina HiSeq2500 platform (Illumina, USA).

A seawater sample was passed three times through a 0.22 μm sterile filter membrane, which was considered to have filtered all microorganisms from the seawater onto the filter membrane. Other steps of DNA library construction were carried out using the sediment method.

2.4 Detection of environmental factors

2.4.1. TOC and TIC

Before seawater samples were filtered, seawater sample and synthetic seawater were added to TOC bottles at a ratio of 1:9. TOC and TIC were measured by autosampler for TOC Analyzers (Xylem Analytics, USA), combined with the standard curve. The standard solution prepared with potassium hydrogen phthalate created the total organic carbon standard curve. The standard solution prepared with sodium carbonate anhydrous created the total inorganic carbon standard curve.

2.4.2 STOC and STIC

Sediment samples were freeze-dried for 48 hours. The dried samples were ground and sieved through a 200-mech stainless steel sieve. STOC was measured by high-temperature external heat potassium dichromate oxidation-volumetric method, and STIC was measured by titration.

2.4.3 NH_4^+, NO_3^-, and NO_2^-

The concentration of three nutrients in the seawater, including NH_4^+, NO_3^-, and NO_2^-, was determined by measuring their absorbance and comparing them with the standard curves. The method was based on the national standard [GB/T12763.4(2007)].

2.4.4 SO_4^{2-}

Seawater samples and synthetic seawater were added to specific bottles at a ratio of 1:9. The concentration of SO_4^{2-} was measured using an ion chromatograph (Thermo Scientific, USA).

2.5 Bioinformatic analysis

The high-quality sequences were obtained using QIIME2 software package with DATA2[11]. The taxonomy of each ASV was assigned according to the Silva 138 database. The specific analysis methods can be referred to the previous article[12].

The archaea community function from 16s gene sequences was predicted using PICRUSt2 software installed on a Linux system.

2.6 Statistical analysis

Mantel's test was conducted to evaluate the relevance between community compositions and environmental variables, using the Bray-Curtis distance for ASV data and Euclidean distance for ecological data, followed by correction for multiple testing and visualization of correlations with Pearson's r and significance levels. The correlation between function matrix and environmental factors was also carried out using the above method. Network analysis was performed to explore the interactions between environmental factors, and gene abundances. The analysis method calculated the correlation matrix using Pearson correlation coefficients and tested the significance of these correlations using t-tests.

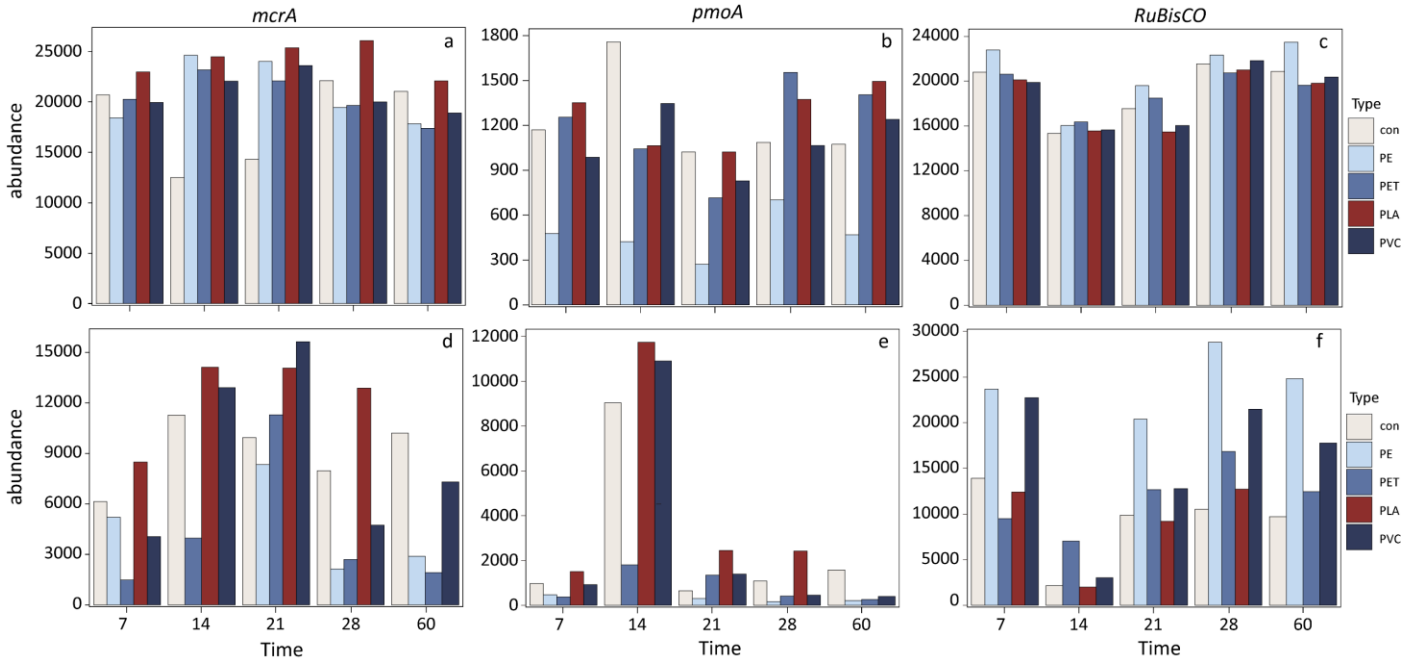

Fig. 1. The gene abundance of *mcrA*, *pmoA* and *RuBisCO* in sediment (a)-(c) and in seawater (d)-(f)

3. RESULTS

3.1 Gene abundance of carbon cycling

To explore the impact of MPs on the gene involved in carbon cycling as predicted by PICRUSt2, the gene abundance was calculated, including *mcrA*, *pmoA*, and *RuBisCO* (Fig. 1). Previous studies indicate ANME-1 can oxidize and produce methane[4,5]. ANME-1 community is predicted and indeed confirmed to contain genes for both methane-producing and methane-oxidizing in the present study. *mcrA* is an essential gene responsible for the methane production process in ANME-1[4]. Whether in the sediment or the seawater, adding PLA contributes to the growth of *mcrA* abundance. There is a trend of first increasing and then decreasing over time. However, adding non-biodegradable MPs has another trend of first inhibiting, then promoting, and then inhibiting again over time. The result is consistent with the previous study in cold seep sediment[13]. *pmoA* is a critical gene responsible for the methane oxidation process in ANME-1. In sediment, the *pmoA* abundance in most non-biodegradable MPs groups decreases compared to the control group. PLA still promotes an increase of the *pmoA* gene. In seawater, the variation pattern of the abundance of the *mcrA* and *pmoA* genes is consistent. *RubisCO* is a vital carbon dioxide-fixation gene. In sediment, the *RuBisCO* abundance in the PE group increases compared to that in the control group, whereas that in the PLA and PVC groups decreases compared to that in the control group.

ANME-1 community plays a more significant role in methane metabolism compared to nitrogen metabolism. Methane metabolism in sediment capability of ANME-1 in sediment is higher than in seawater ($p < 0.001$) (Fig. 2).

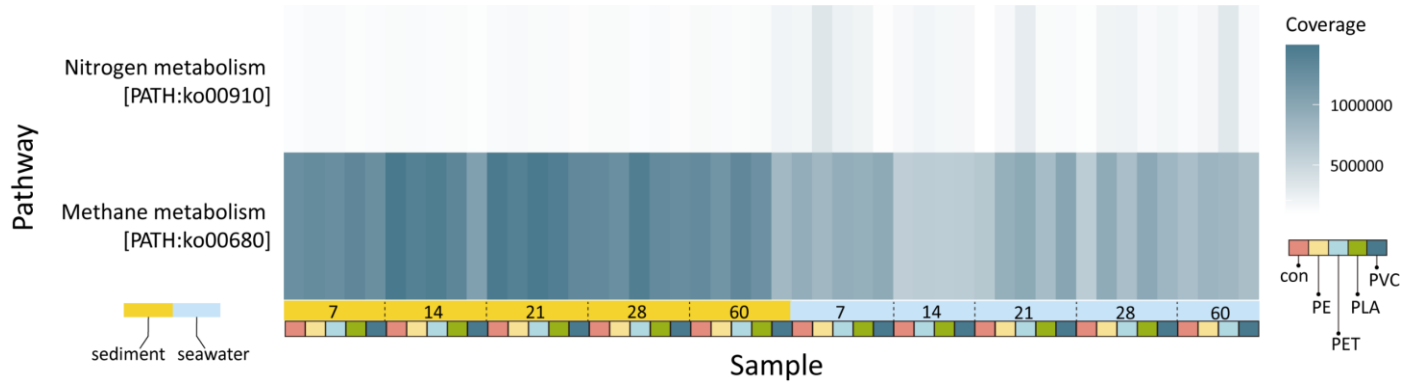

Fig. 2. The nitrogen and methane metabolism pathway coverage.

3.2 The influence of environmental factors

ANME-1 community is significantly affected by TOC, TIC, and NO_2^-. In sediment, STIC is also an important environmental factor (Fig. 3a). PICRUSt2-based predicted nitrogen cycling function in sediment is affected by TIC, NH_4^+, and NO_2^-, and that in seawater is affected by TOC and SO_4^{2-}. The main environmental factors affecting carbon cycling function in sediment are STIC. TOC, TIC, SO_4^{2-}, and NH_4^+ are the main environmental factors affecting carbon cycling function in seawater (Fig. 3b).

In sediment, only the *mcrA* gene is significantly affected by the MPs type. The correlations in the sediment network are primarily reflected between environmental factors and between functional genes. In contrast, the correlations between environmental factors and functional genes are weak and insignificant (Fig. 4a).

In seawater, the type of MPs has no effect on function genes or community composition. The connection between environmental factors and function genes is closer (Fig. 4b).

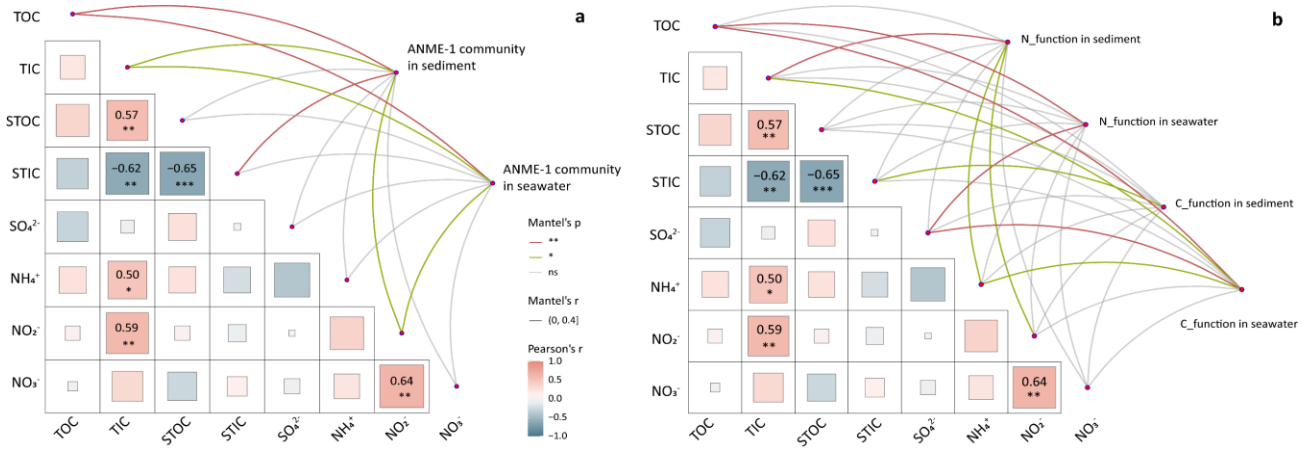

Fig. 3. (a) Correlations of ANME-1 community in sediment and seawater (Bray-Curtis distance) with the environmental variables; (B) Correlations of PICRUSt2-based predicted function in the sediment and the sediment environment (Bray-Curtis distance) with the environmental variables.

4. DISCUSSION

Our study indicates that MPs can affect the composition and functional genes of ANME-1 communities, although these effects were not statistically significant ($P > 0.05$). The adsorption and desorption behaviors of MPs introduce complexity into the surrounding environment. The rough surface of MPs provides numerous attachment sites for various additives, organic matter, and metals[14], forming complex and diverse habitats. This complexity can support the growth of different ANME-1 strains, thereby increasing the richness and diversity of the community.

When MPs enter the aquatic environment, they rapidly release organic matter and additives[15], potentially causing microbial discomfort or stress. This stress can lead to a decrease in microbial richness and evenness. However, some ANME-1 strains may tolerate the more complex environment created by MPs, resulting in higher community richness.

Environmental factors, such as the presence of MPs, can serve as nutrient sources for ANME-1. The impact of these factors on the structure and function of microbial communities can vary[16]. For example, in this study, SO_4^{2-} did not significantly impact community structure but showed a significant positive correlation with function in seawater. Environmental factors can directly or indirectly affect ANME-1 or synthetic microorganisms, such as sulfate-reducing bacteria, influencing methane oxidation and production processes.

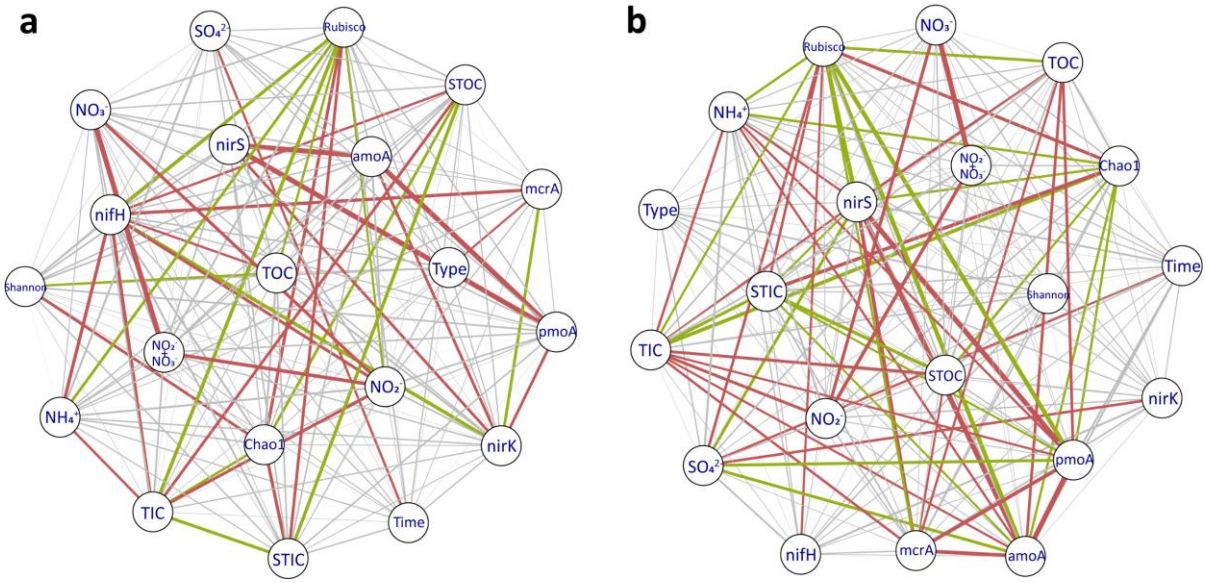

Fig. 4. The network of environmental factors and functional genes in ANME-1 communities in sediment (a) and seawater (b). The thickness of the edges represents the strength of the correlation. Red edges represent significant positive correlations ($p < 0.05$), green edges represent significant negative correlations ($p < 0.05$), and gray edges represent non-significant correlations ($p > 0.05$).

Certain gene abundances significantly correlate with specific enzyme activities[17]. For instance, the abundance of the *mcrA* gene is significantly correlated with CH_4 production rates using H_2/CO_2[17]. In our study, PLA showed higher *mcrA* and *pmoA* gene abundances compared to the control group, suggesting that MPs can promote gene abundance, thereby enhancing the enzyme activities involved in methane production and oxidation.

Assuming that a specific concentration of MPs can significantly affect the functional genes involved in methane production and oxidation in ANME-1, this could have substantial implications for global climate change. Enhanced methane metabolism in ANME-1 due to MP presence could alter methane dynamics in the cold seep environments, potentially impacting greenhouse gas emissions and climate regulation.

5. CONCLUSIONS

Whether in the sediment or the seawater, adding PLA benefits the growth of *mcrA* abundance. PLA still promotes an increase of the *pmoA* gene in sediment. Whether in the sediment or the seawater, the *RuBisCO* abundance in the PE group increases compared to that in the control group.

ACKNOWLEDGEMENT

Financial support received from the Guangdong Natural Resources Foundation (GDNRC[2023]30), the National Natural Science Foundation of China (42325603, 42227803, 42376215, 42206223), the National Key Research and Development Program of China (2021YFF0502300), the PI project of Southern Marine Science and Engineering Guangdong Laboratory

(Guangzhou) (GML20190609, GML2022009, and GML20230921).

REFERENCE

[1] Thompson RC, Olsen Y, Mitchell RP, et al. Lost at sea: where is all the plastic?. *Science* 2004;304(5672):838.

[2] Wang S, Zhou Q, Hu X, Tao Z. Polyethylene microplastic-induced microbial shifts affected greenhouse gas emissions during litter decomposition in coastal wetland sediments. *Water Res* 2024;251:121167.

[3] Feng JC, Li CR, Tang L, et al. Tracing the Century-Long Evolution of Microplastics Deposition in a Cold Seep. *Adv Sci (Weinh)* 2023;10(10):e2206120.

[4] Lloyd KG, Alperin MJ, Teske A. Environmental evidence for net methane production and oxidation in putative ANaerobic MEthanotrophic (ANME) archaea. *Environ Microbiol* 2011;13(9):2548-2564.

[5] Kevorkian RT, Callahan S, Winstead R, Lloyd KG. ANME-1 archaea may drive methane accumulation and removal in estuarine sediments. *Environ Microbiol Rep* 2021;13(2):185-194.

[6] Dekas AE, Poretsky RS, Orphan VJ. Deep-sea archaea fix and share nitrogen in methane-consuming microbial consortia. *Science* 2009;326(5951):422-426.

[7] Yang S, Lv Y, Liu X, et al. Genomic and enzymatic evidence of acetogenesis by anaerobic methanotrophic archaea. Nat Commun 2020;11(1):3941.

[8] Oberbeckmann S, Labrenz M. Marine Microbial Assemblages on Microplastics: Diversity, Adaptation, and Role in Degradation. *Ann Rev Mar Sci* 2020;12:209-232.

[9] Seeley ME, Song B, Passie R, Hale RC. Microplastics affect sedimentary microbial communities and nitrogen cycling. *Nat Commun* 2020;11(1):2372.

[10] Takahashi S, Tomita J, Nishioka K, Hisada T, Nishijima M. Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. *PLoS One* 2014;9(8):e105592.

[11] Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2 [published correction appears in Nat Biotechnol 2019 Sep;37(9):1091.

[12] Zhong S, Feng J, Kong J, Huang Y, Chen X, Zhang S. Differences in Bacterial Co-Occurrence Networks and Ecological Niches at the Surface Sediments and Bottom Seawater in the Haima Cold Seep. *Microorganisms* 2023;11(12):3001.

[13] Chen C, Deng Y, Zhou H, et al. Revealing the response of microbial communities to polyethylene micro(nano)plastics exposure in cold seep sediment. *Sci Total Environ* 2023;881:163366.

[14] Rafa N, Ahmed B, Zohora F, et al. Microplastics as carriers of toxic pollutants: Source, transport, and toxicological effects. *Environ Pollut* 2024;343:123190.

[15] Fauvelle V, Garel M, Tamburini C, et al. Organic additive release from plastic to seawater is lower under deep-sea conditions. *Nat Commun* 2021;12(1):4426.

[16] Louca S, Parfrey LW, Doebeli M. Decoupling function and taxonomy in the global ocean microbiome. Science 2016;353(6305):1272-1277.

[17] Morris R, Schauer-Gimenez A, Bhattad U, et al. Methyl coenzyme M reductase (mcrA) gene abundance correlates with activity measurements of methanogenic H_2 /CO_2 -enriched anaerobic biomass. *Microb Biotechnol* 2014;7(1):77-84.