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Microplastics Affect Anaerobic Oxidation of Methane and Sedimentary Prokaryotic Communities in Cold Seep Areas

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ABSTRACT

In this study, a microcosm experiment for 120 days exposure of microplastics (MPs) on cold seep sediment was conducted. The results showed that different types and doses of MPs addition negatively affected the anaerobic oxidation of CH_4. The CH_4 oxidation rates with 0.05 % PA and PET addition were only account for approximately 33% of that for the biotic control group. MPs addition did not significantly influence the archaeal community structures but caused a redistribution of bacterial community compositions. The addition of PA, PE, and PET could significantly increase the relative abundance of Desulfobacterota and made Caldatribacteriota almost undetectable. By the present study, we can have a preliminary understanding of the effects of microplastics on anaerobic oxidation of CH_4 and prokaryotic communities in the cold seep sediment.

Keywords: Cold seep, Anaerobic oxidation of methane, Microplastics, Prokaryotic communities

1. INTRODUCTION

Due to the widespread use of plastic products, plastic pollution has evolved as a global environmental concern [1]. At least 300 million tons of plastics are produced annually for their use in various industries and approximate 8 million tons were thrown into ocean as marine debris [2]. Plastic particles with a particle size of less than 5 mm are defined as microplastics (MPs) [3]. MPs are capable of posing a huge threat to marine algae [4], shellfish [5], fish [6] and microbial community [7], etc., which further adversely affect the biodiversity of marine ecosystems. It is estimated that 14 million tons of MPs have accumulated on marine sediment [8]. Consequently, it is commonly accepted that deep-sea sediment is the main sink for marine microplastic debris.

CH_4 is the second most important greenhouse gas in the world, contributes 30 % to current global warming [9]. Deep-sea sediments are the largest reservoirs of CH4, containing approximately 500–2500 Gt of dissolved and hydrated methane [10]. Cold seep areas are typical methane burying areas in deep sea, where hydrocarbon-rich fluids (mainly CH_4) emit from the ocean floor [11]. As continuous supply of methane fluids from sediment to seawater and atmosphere, about 88% methane would be consumed by anaerobic oxidation of methane (AOM) of methanotrophic archaea in the sediment [12]. Therefore, AOM is a key reaction controlling the release of methane from the oceans to the atmosphere, which plays an important role in governing the efficiency of methane filtering and carbon turnover in cold seep areas.

As important areas of storing up CH_4 hydrate resource, cold seep areas are also currently contaminated with microplastics. More than 16 types of MPs were detected in cold seep sediment and methane seeps contributed to MPs capture into sediment [13]. These plastic debris may affect the ecological function of cold seep ecosystem. Considering the conservation of methane hydrate resources and the abatement of greenhouse gas, it is meaningful to evaluate the impact of MPs on the AOM in cold seep areas. Therefore, this study aimed to investigate: (1) the effect of different MPs on the anaerobic oxidation rate of methane, (2) and the responses of prokaryotic communities to the present of MPs in cold seep sediment.

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2. MATERIAL AND METHODS

2.1 Experimental sediment, seawater and microplastic

Sampling of sediment and seawater was conducted in Haima cold seep (16° 43′ N, 110° 28′ E) located in the South China Sea during June 2022. The sediment samples were collected by pushcore controlled by manipulator on Remote Operated Vehicle (ROV). The seawater samples were obtained by using Niskin bottles attached to ROV. Then the sediment and seawater were stored in the dark at 4°C before incubation experiment. All experimental microplastics were purchased from Dongguan City Jie cheng Plastic Chemical Co., Ltd. and Polyamide (PA), polyethylene (PE), polypropylene (PP) and polyethylene terephthalate (PET) were selected for microscopic experiment.

2.2 Microscopic incubation experiment

The experimental design consisted of a no-MPs biotic control group (S-BC), a heat inactivated control group (S-IC), four low-dose MPs treatments (0.05% (w/w) PA, PE, PP and PET), and four high-dose MPs treatments (0.5% (w/w) PA, PE, PP and PET). In brief, approximately 1200 g wet sediment and 2.8 L seawater were mixed under sterile environment. Then the mixtures were thoroughly homogenized by stirring and large debris were removed. 100 mL aliquot mixtures were sequentially added to 200 mL high-pressure reactors. No other operation was done in S-BC, and the mixtures in S-IC were sterilized at 121°C for 20 min to kill microorganisms. For MPs treatment groups, 0.05 g or 0.5 g four types of MPs were added to the corresponding high-pressure reactors. Then headspace air of all reactors was extracted with a vacuum pump and were replaced with nitrogen to achieve anaerobic environment. Finally, 13C-labelled methane was injected into the reactors and all reactors were pressurized with N2 to 12 MPa to obtained a final 13CH4 concentration of 300 ppm in headspace. Each group was replicated three times (n=30). All reactors were operated with constant temperature at 4°C for 150 days and mixed upside down every 3 days. Headspace air was sampled on day 1, 15, 25, 35, 55, 85, 120 and 150 for the analysis of CH4 concentration. At the end of the exposure experiment, the mixtures for each microcosm were collected and centrifuged at 8,000 rpm for 5 min. Then the sediments were used for the measures of prokaryotic communities.

3. RESULTS AND DISCUSSION

3.1 Experimental sediment, seawater and microplastic

Microscopic experiment was designed to investigate the effects of different microplastics (PA, PP, PE, PET) on the anaerobic oxidation of methane in cold seep sediment. As shown in Fig. 1, no significant change was observed in the CH_4 concentration of the S-IC. While the CH_4 concentration of the S-BC gradually decreased from 300 ppm to 242.74 ppm during 150-day operation, which indicated that CH_4 removal was a biologically-dependent process. All the addition of various MPs could significantly inhibit the CH_4 utilization capacity of the sediment and the CH_4 concentrations of the all MPs-treated groups were higher than that of S-BC.

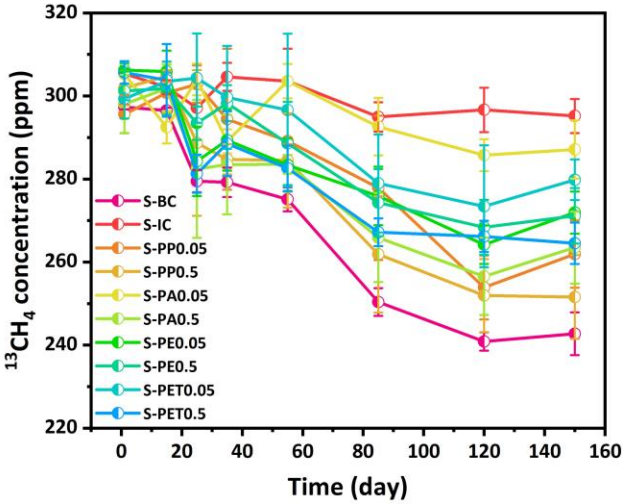

Fig. 1 Changes in CH_4 concentration in headspace for all experimental groups

The average methane oxidation rates were then calculated and the results are shown in Fig. 2. The methane oxidation rate of S-BC was 1.62 μmoL/L/day. PA and PET had the most significant negative effects on CH_4 oxidation rates. The corresponding CH_4 oxidation rates for PA-0.05 and PET-0.05 were 0.54 and 0.58 μmoL/L/day, respectively, which was only about 33% of that of S-BC.

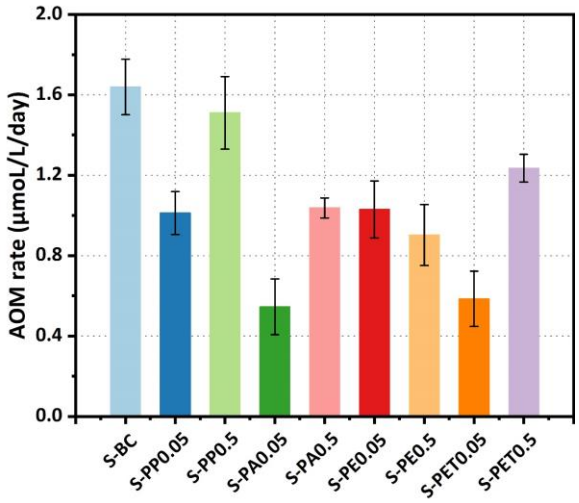

Fig. 2 Effects of MPs on the anaerobic oxidation rates of methane

3.2 MPs affect sedimentary prokaryotic communities

A total of 2,434,559 bacterial sequences and 2,773,489 archaeal sequences were obtained after filtering and merging raw data of 16S reads. The alpha diversities of bacterial and archaeal communities are shown in Table 1 and Table 2. Overall bacterial α-diversities of initial community were significantly greater than archaeal α-diversities, which is reflected at the observed species, Chao1, Ace, and Shannon indices. After the microscopic experiment, increased alpha-diversities were observed in both bacterial and archaeal communities. The types and exposure concentrations of MPs have different effects on microbial α-diversity. For bacterial community, the addition of PP had no significant influence on α-diversity whereas PA, PE, and PET significantly decreased the α-diversity compare to that of S-BC. Moreover, high-dose exposure of MPs led to the lower α-diversity. But for archaeal community, the α-diversity was lowest under PP treatment and the addition of 0.05% PA, PE, and PET could increase the archaeal α-diversity.

Table 1 Bacterial community alpha diversities

Samples	Observed species	ACE	Chao1	Shannon
S-0	936±0	1018.62±0	997.53±0	5.71±0
S-BC	1006.33±48.13	1087.23±63.87	1076.08±60.38	6.6±0.19
S-PA0.05	794.33±11.73	836.95±8.98	836.88±12.01	6.33±0.31
S-PA0.5	716.67±78.95	741.68±86.93	739.58±88.87	6.25±0.21
S-PE0.05	617±19.44	637.05±24.91	629.72±24.18	5.79±0.07
S-PE0.5	557.67±25.22	568.82±28.4	563.79±27.69	5.83±0.14
S-PET0.05	544.33±42.63	554.99±50.98	550.14±48.27	5.58±0.06
S-PET0.5	512±32.34	529.27±37.64	522.09±36.99	5.44±0.03
S-PP0.05	1064±29.13	1188.4±19.86	1192.82±16.71	6.62±0.05
S-PP0.5	943.33±41.33	1034.5±49.74	1034.2±48.12	6.62±0.07

Table 2 Archaeal community alpha diversities

	Observed species	ACE	Chao1	Shannon
S-0	126	126.32	126	1.71
S-BC	174±25.73	178.11±27.92	176.66±26.96	2.4±0.11
S-PA0.05	201.33±16.74	210.34±21.48	207.5±21	2.31±0.12
S-PA0.5	154±8.83	159.44±7.48	157.71±7	2.14±0.23
S-PE0.05	231.67±45.9	237.22±48.85	236.3±47.04	2.43±0.15
S-PE0.5	175.67±28.39	182.56±30.18	179.29±30.06	2.16±0.19
S-PET0.05	205±33.24	206.71±34.45	205.79±33.8	2.25±0.26
S-PET0.5	199.67±18.8	203.08±16.78	201.52±16.98	2.41±0.07
S-PP0.05	130±15.77	132.37±14.88	130.94±15.34	2.23±0.15
S-PP0.5	139.33±12.55	143.21±14.4	141.82±14.01	2.35±0.08

Relative abundances at the phylum level were analyzed to further illustrate the effects of MPs doses and styles on sedimentary bacterial and archaeal community structures. Regarding archaea, all samples were dominated by phyla Halobacterota, accounting for more than 90% of the archaeal community. Compared with the S-initial, the relative abundances of archaea species of S-BC and MPs-exposure groups were not significantly altered. On the contrary, significant changes occurred in bacterial communities after the microscopic experiment. The relative abundance of Desulfobacterota increased from 30% to 50% under PA, PE, and PET treatment, and the relative abundance of Campylobacterota was also slightly increased. The addition of PA, PE, and PET made Caldatribacteriota almost undetectable and led to a decrease in the relative abundance of Proteobacteria in the bacterial communities. However, Caldatribacteriota was enriched after PP exposure. Species belonging to Desulfobacterota and Campylobacterota are typical sulfate-reducting bacteria and sulfur-oxidizing bacteria, respectively [14, 15]. The increased abundances of Desulfobacterota and Campylobacterota suggested that MPs contribute to sulfur metabolism in cold seep sediment. Additionally, Caldatribacteriota is considered a candidate phylum with diazotrophs capacity in cold seep [16]. Proteobacteria is the mainstay of hydrocarbons degradation in the ocean [17]. The declining abundances of Caldatribacteriota and Proteobacteria revealed that

MPs could inhibit hydrocarbons metabolism and nitrogen-fixing processes in cold seep sediment. The above results implied that the presence of MPs could change the relative abundance of bacteria species related to C, N, and S metabolism but barely affected archaeal community structures.

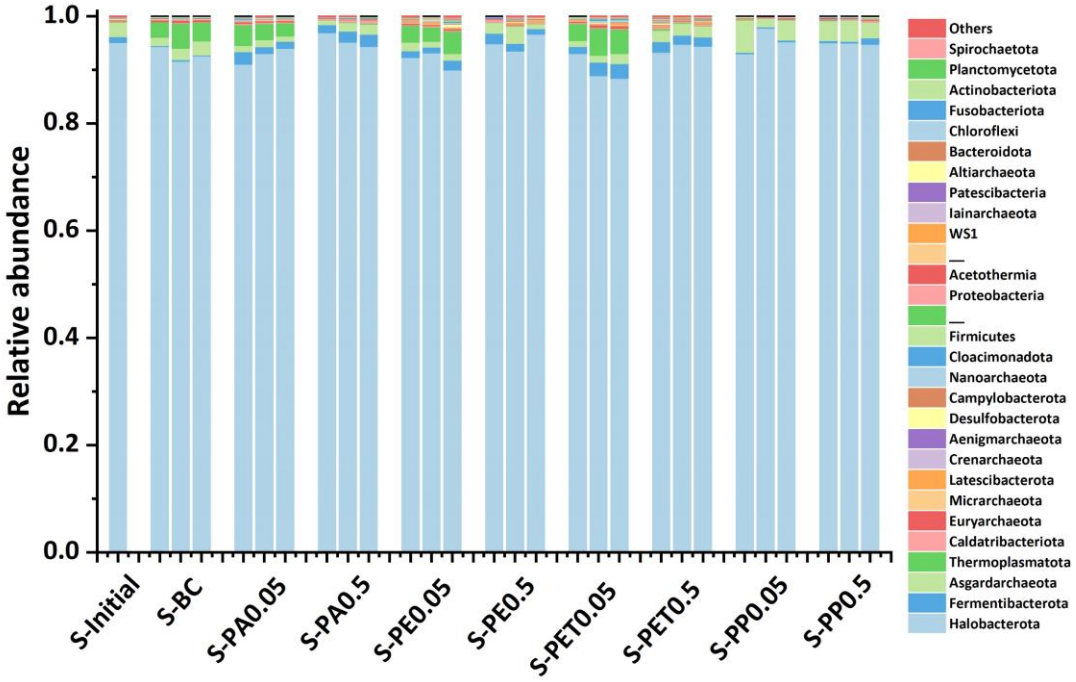

Fig. 3 Archaeal community composition and MPs treatment effects

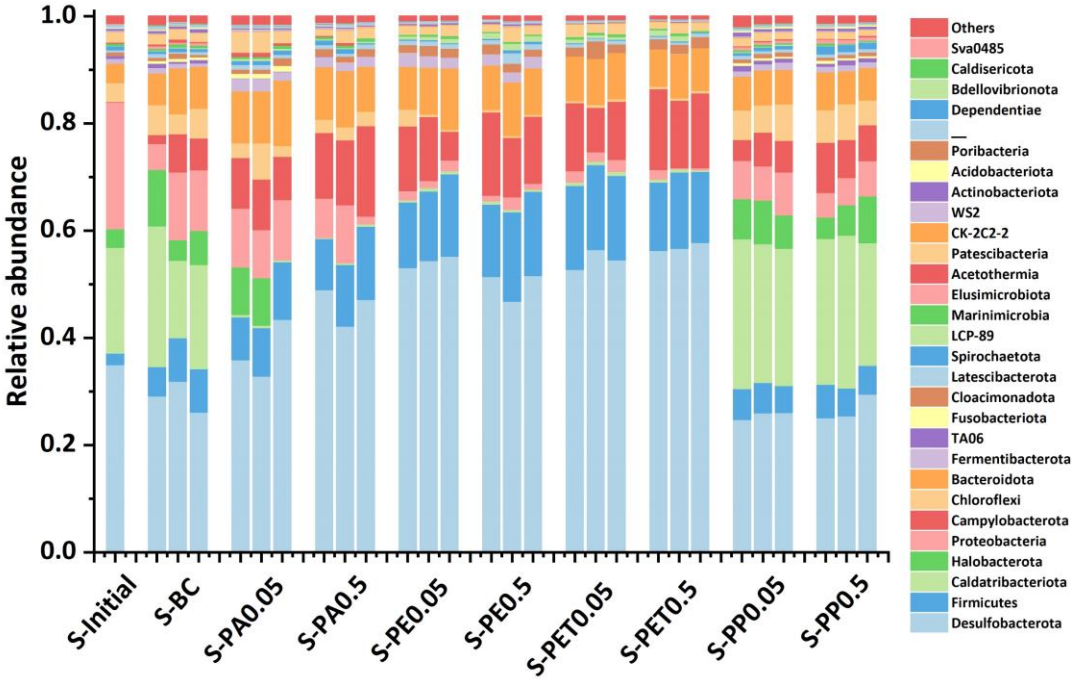

Fig. 4 Bacterial community composition and MPs treatment effects

4. CONCLUSIONS

In this study, the effects of different MPs styles (PA, PE, PET, and PP) and doses (0.05 % and 0.5 %) on anaerobic oxidation of methane and prokaryotic communities in cold seep sediments were investigated. The results indicated that the addition of all MPs reduced the rate of methane oxidation in the sediments. The lowest methane oxidation rates were 0.54 and 0.58 μmoL/L/day observed in S-PA-0.05 and S-PET-0.05, respectively, which was about 33% of that of S-BC. Based on both alpha and beta diversity measures, the different MPs treatments resulted in significant differences in the overall sediment bacterial community diversities but had little effect on the archaeal community structures. Except

for the PP treatment, MPs could reduce microbial α-diversity and affect the relative abundance of species associated with C, N, and S metabolism. The addition of PA, PE, and PET significantly increased the relative abundance of Desulfobacterota and Campylobacterota, but decreased the proportions of Caldatribacteriota and Proteobacteria in the bacterial communities.

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DECLARATION OF INTEREST STATEMENT

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. All authors read and approved the final manuscript.

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