CS_2 increasing CH_4-derived carbon emissions and active microbial diversity in lake sediments

Jing Wang[a], Yi-Xuan Chu[a], Hendrik Schäfer[b], Guangming Tian[a], Ruo He[c,d*]

[a]*Department of Environmental Engineering, Zhejiang University, Hangzhou 310058, China*

[b]*School of Life Sciences, University of Warwick, Coventry, UK*

[c]*Zhejiang Provincial Key Laboratory of Solid Waste Treatment and Recycling, School of Environmental Science and Engineering, Zhejiang Gongshang University, Hangzhou, 310012, China*

[d]*College of Environmental and Resource Science, Zhejiang University, Hangzhou 310058, China*

[*] Corresponding author. E-mail address: heruo@zju.edu.cn (R. He).

Abstract

Lakes are important methane (CH_4) sources to the atmosphere, especially eutrophic lakes with cyanobacterial blooms accompanied by volatile sulfur compound (VSC) emissions. CH_4 oxidation is a key strategy to mitigate CH_4 emission from lakes. In this study, we characterized the fate of CH_4-derived carbon and active microbial communities in lake sediments with CS_2 used as a typical VSC, based on the investigation of CH_4 and VSC fluxes from Meiliang Bay in Lake Taihu. Stable isotope probing microcosm incubation showed that the efficiency of CH_4-derived carbon incorporated into organic matter was 21.1% in the sediment with CS_2 existence, which was lower than that without CS_2 (27.3%). SO_4^{2-}-S was the main product of CS_2 oxidation under aerobic condition, accounting for 59.3-62.7% of the input CS_2-S. CS_2 and CH_4 coexistence led to a decrease of methanotroph and methylotroph abundances and stimulated the production of extracellular polymeric substances. CS_2 and its metabolites including total sulfur, SO_4^{2-} and acid volatile sulfur acted as the main drivers influencing the active microbial community structure in the sediments. Compared with α-*proteobacteria* methanotrophs, γ-*proteobacteria* methanotrophs *Methylomicrobium*, *Methylomonas*, *Crenothrix* and *Methylosarcina* were more dominant in the sediments. CH_4-derived carbon mainly flowed into methylotrophs in the first stage. With CH_4 consumption, more CH_4-derived carbon flowed into non-methylotrophs. CS_2 could prompt more CH_4-derived carbon flowing into non-methanotrophs and non-methylotrophs, such as sulfur-metabolizing bacteria. These findings can help elucidate the influence of VSCs on microorganisms and provide insights to carbon fluxes from eutrophic lake systems.

Keywords: sulfur-containing compounds; methanotrophs; methane oxidation; CS_2 oxidation rate; sulfur-metabolizing microorganisms

1. Introduction

Methane (CH_4) is an important greenhouse gas with a global warming potential ~34 times greater than that of carbon dioxide (CO_2) on a molar basis (Ciais et al., 2013). CH_4 emission includes natural and anthropogenic sources, of which natural sources account for approximately 40% of the global CH_4 emission (Saunois et al., 2020). Lakes are important CH_4 sources to the atmosphere due to gradually increasing emissions with global climate change (Guo et al., 2020). Recently, severe eutrophication has led to an increase of cyanobacterial blooms in lakes (Yang et al., 2021), resulting in a pronounced increase in CH_4 emission from lakes mainly due to the increase of degradation of dead algae (Bartosiewicz et al., 2021; Zhang et al., 2021).

CH_4 produced from organic matter in sediments can be partially oxidized in lake systems when it is emitted to the atmosphere. Methanotrophs are a unique group of Gram-negative bacteria capacity of utilizing -CH_4 as sole carbon and energy source (Semrau et al., 2010). Aerobic methanotrophs mainly belong to *Proteobacteria* and can be classically divided into two types: γ-*proteobacteria* and α-*proteobacteria* methanotrophs, based on phylogeny and morphological characteristics (Semrau et al., 2010). The γ-*proteobacteria* methanotrophs belong to the family *Methylococcaceae*, while α-*proteobacteria* methanotrophs are affiliated with the families *Methylocystaceae* and *Beijerinckiaceae* (Bowman, 2006; Semrau et al., 2010). In addition, *Verrucomicrobia* methanotrophs have been found in extreme environments, extending the diversity of aerobic methanotrophs (Dunfield et al., 2007; Sharp et al., 2014). CH_4 is first oxidized to methanol by methane monooxygenase (MMO), including particulate methane monooxygenase (pMMO) encoded by the *pmoA* gene and soluble methane monooxygenase (sMMO) encoded by the *mmoX* gene (Semrau et al., 2010). Most methanotrophs possess the *pmoA* gene, except *Methylocella* (Dedysh

et al., 2000) and *Methyloferula* (Vorobev et al., 2011), while the *mmoX* gene can be found in some methanotrophs, such as *Methylosinus trichosporium* OB3b and *Methylocella silvestris* BL2 (Semrau et al., 2018). Thus, the *pmoA* gene has been widely used as a biomarker to identify aerobic methanotrophs.

Volatile sulfur compounds (VSCs) widely exist in terrestrial aquatic systems and are mainly produced from the degradation of organic matter in sediments, which is usually accompanied with CH_4 emission (Liu et al., 2019). The VSCs are mainly in the form of hydrogen sulfide (H_2S), methanethiol (CH_3SH), carbon disulfide (CS_2), dimethyl sulfide ($(CH_3)_2S$), and dimethyl disulfide ($(CH_3)_2S_2$) (Bentley and Chasteen, 2004; Wu et al., 2010). H_2S and CH_3SH can be detected throughout the annual cycle in the water of Lake Taihu, China, ranging from 0.6-882.4 and 0.9-156.3 nmol L^{-1}, respectively (Liu et al., 2019). $(CH_3)_2S$ level is extremely high (up to 6000-7000 nmol L^{-1}) in May and July relative to other seasons when algal blooms form (Reese and Anderson, 2009). CS_2 is found to be the most abundant sulfur compound with a concentration of up to 60000 nmol L^{-1} in redox transition layer and water interface closed to sediment in Lake Cadagno, Switzerland (Fritz and Bachofen, 2000). In the vertical profile, VSC concentrations such as CH_3SH are shown to have depth gradients, with the maximum CH_3SH concentration just above the sediment (42 nmol L^{-1}) and very low levels (1.1-7.8 nmol L^{-1}) at the water surface (Lomans et al., 1997). In terrestrial aquatic systems, the VSC concentrations change with quality of terrestrial water, oxygen concentration, temperature, light intensity and eutrophication level (Watson and Jüttner, 2017).

VSCs such as H_2S, $(CH_3)_2S$ and CH_3SH have been reported to have inhibitory effects on CH_4 oxidation due to the potential toxicity of sulfur-containing compounds and their metabolic intermediates (Cooper and Brown, 2008; Lee et al., 2011; Long et

al., 2013; Zhang et al., 2016). Lee et al. (2011) found that CH_4 oxidation could be inhibited in the presence of H_2S, CS_2, $(CH_3)_2S$ or CH_3SH. The growth of methanotrophs can be inhibited at a H_2S concentration of 0.01%, while CH_4 oxidation capacity may be inhibited at a H_2S concentration of over 0.05% (Caceres et al., 2014; Zhang et al., 2016). Long et al. (2013) found competitive inhibition between H_2S and CH_4 when CH_4 was at a low concentration of 5%. Börjesson (2001) reported a negative correlation between methanotrophic activity and CS_2 concentration. Saari and Martikainen (2003) found a negligible effect on CH_4 oxidation at a $(CH_3)_2S$ concentration of 0.64 μmol in soil-water slurry, while complete inhibition was observed at a DMS concentration of 32 μmol. Yu et al. (2009) reported that H_2S in coal mine gas did not have an obvious influence on MMO activity. However, Kim et al. (2013) found that $(CH_3)_2S$ had a positive influence on methanotrophs, favoring the growth of *Methylosarcina*. Eutrophic shallow freshwater lakes are ecosystems in which CH_4 and sulfur-containing compounds coexist, especially when algal blooms form. However, little is known about the effects of sulfur-containing compounds on the activity and communities of methanotrophs in freshwater lakes.

Methanotrophs can not only oxidize CH_4, but also play a key role in metabolizing sulfur-containing compounds. Compared with pMMO, sMMO has activity with a broad range of substrates (Baker et al., 2001). Sorokin et al. (2000) identified a new type I methanotrophic strain (*Methylomicrobium)* that could oxidize CS_2 at a high pH of 9-10, suggesting that CS_2 might be an available substrate for MMO. Moreover, this strain also has the capacity for $(CH_3)_2S$ oxidation. Additionally, methanotrophs can excrete intermediates from CH_4 oxidation, such as methanol, formaldehyde and formate, which can be utilized by non-methanotrophs such as sulfur-metabolizing microorganisms as carbon sources (Xin et al., 2007; Tavormina et al., 2017). Environmental factors such

as nitrate or low O_2 level can promote flux of CH_4-derived carbon from CH_4 oxidation to other non-methanotrophs (Wei et al., 2016; He et al., 2020; van Grinsven et al., 2021). However, a clear understanding of the effects of VSCs on the metabolism of methanotrophs and the feedback on carbon cycling in lakes is still lacking.

The objective of this work was to characterize the fate of CH_4-derived carbon and active methanotrophic communities in lake sediments by applying DNA-stable isotope probing (DNA-SIP) technique. CS_2 was chosen as a typical VSC owing to the high-detected CS_2 flux from Meiliang Bay in Lake Taihu in this study. CH_4 oxidation potential was determined during the SIP incubation. Organic matter characteristics including the contents of organic matter, organic ^{13}C and extracellular polymeric substances (EPS) were analyzed during the experiment. The microbial abundance and community in the sediments were analyzed by quantitative PCR (q-PCR) and MiSeq sequencing of the 16S rRNA gene. We hypothesized that different groups of methanotroph would have different CS_2 tolerances which would affect CH_4 oxidation rates, fate of CH_4-derived carbon and microbial communities in lake sediments. A deeper investigation of the effects of CS_2 on the fate of CH_4-derived carbon and communities of methanotrophs can help elucidate the response of methanotrophs to VSCs and establish models based on environmental factors to estimate CH_4 emission from shallow eutrophic lakes.

2. Materials and methods

2.1. Study site and sampling

Lake Taihu is a typical large shallow lake with an average water depth of 1.9 m and a surface area of 2338 km^2 located in the Yangtze River Delta Plain, China (Qin et al., 2007). The study sites were located in Meiliang Bay, which is in the northwestern area

of Lake Taihu. Due to the predominance of southeasterly winds and nutrient input, Meiliang Bay suffers from severe levels of eutrophication, frequently leading to intense algal blooms and black-colored water (Duan et al., 2015; Wu et al., 2018).

CH_4 and VSC fluxes were detected in four sites (120°9′31″-120°10′42″, 31°28′10″-31°28′35″) of Meiliang Bay using the floating chamber technique (St Louis et al., 2000; Gonzalez-Valencia et al., 2014). The static chamber was cylindrical with an open surface of 0.4 m^2 and a headspace volume of 6.5×10^{-2} m^3. Each static chamber was equipped with a sampling port. Gas samples were collected from the static chambers every 20 min within a 1-h period in triplicate and stored in pre-vacuumed 20-mL vials for detecting CH_4 and VSC concentrations within 24 h. Three static chambers with 1-m interval were used to measure gas fluxes in each site.

The sediment cores were collected from four sites in Meiliang Bay with a gravity corer equipped with Perspex tubes (inner diameter, 10 cm; length, 35 cm) in November 2019 with the average temperature, pH, dissolved oxygen (DO) and chlorophyll *a* of 18℃, 7.7, 6.41 mg L^{-1}, and 2.66 μg L^{-1}, respectively. A total of eight sediment cores were obtained with two cores sampled at each site. Overlying water was retained in the core tubes to prevent the turbulence and oxidation of surface sediments. The sediment cores were immediately transported to the laboratory, carefully extruded and sliced into the top 2 cm of sediment after removing the overlying water. Then, the surface 2 cm sediment was homogenized and placed into plastic freezer bags. Approximately 1 kg sediment subsamples were stored at 4 °C for SIP incubation and analysis of physicochemical properties, including organic matter (OM), sulfur compounds (SO_4^{2-}-S, acid volatile sulfide (AVS), total sulfur (TS)) and EPS including extracellular polysaccharide (ECPS) and extracellular protein (ECP). The remaining subsamples were stored at -80 °C for further molecular analysis.

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167 *2.2. SIP microcosms and CH_4 oxidation potential*

168 Approximately 5 g (wet weight) of homogenized sediment was placed into 200 mL
169 sterile serum vials sealed with a butyl rubber stopper. After extracting 4 mL gas, $^{13}CH_4$
170 (99 atom % ^{13}C, Sigma-Aldrich) was injected into the serum vials to a final
171 concentration of 2% in the headspace (v/v). Two groups were set up: 1) only $^{13}CH_4$ was
172 injected into the serum vials, labeled "M"; 2) in addition to $^{13}CH_4$, CS_2 was injected to
173 the initial concentration of 350 mg m^{-3} in the headspace, labeled "MS". Controls were
174 amended with $^{12}CH_4$ (99.9% purity). Each $^{13}CH_4$ or CH_4 group was performed in 15
175 replicates. Autoclaved sediment with the addition of NaN_3 (0.13 g $kg_{\ dry\ wet}^{-1}$) was used
176 to detect abiotic CH_4 and CS_2 loss in triplicate. The microcosms were flushed with air
177 when more than 90% of CH_4 was consumed. Then CH_4 and CS_2 were added to obtain
178 the initial concentrations as described above. There were a total of four gas exchanges
179 during the whole incubation period. CS_2 was directly injected into the microcosms to
180 achieve an initial concentration of 350 mg m^{-3} when the CS_2 concentration was below
181 10 mg m^{-3} and when the concentrations of CH_4 and O_2 were higher than 5% in the
182 headspace of the serum vials. CS_2 was injected twice, 8 and 29 times when the SIP
183 samples were harvested at the CH_4 consumption of 20, 50 and 100 μmol CH_4 $g_{wet\ weight}^{-1}$
184 , respectively. Five serum vials were randomly taken for the destructive sampling of
185 sediment for physicochemical properties, ^{13}C amount and molecular analysis when the
186 CH_4 consumption reached 20, 50 and 100 μmol CH_4 $g_{wet\ weight}^{-1}$. Gas samples were
187 extracted from the headspace of the serum vials to measure CH_4 and CS_2 concentrations
188 each day. All serum vials were incubated in the dark at 30℃ to simulate the high
189 temperature of 32-34 ℃ detected in overlying water in summer. The experiment lasted
190 35 days.

2.3. Analytical methods

CH_4 concentration was detected by using a gas chromatograph with a thermal conductivity detector, which was equipped with a GDX-104 (60/80 mesh) paced column of 2 m length (He et al., 2018b). The temperatures of oven, injector and detector were 60, 80 and 100°C, respectively. N_2 was used as carrier gas at a flow rate of 20 mL min^{-1}. VSC concentrations were detected by using the HC-3 trace sulfur analyzer equipped with a flame photometric detector and a polytetrafluoroethylene column as described previously (Chen et al., 2017). The temperatures of the oven, injector and detector were 80, 90 °C and room temperature, respectively. The CH_4 oxidation potential was estimated from a zero-order decrease in the headspace of serum vials (Wang et al., 2011). The gas fluxes including CH_4 and VSCs were calculated as described by Gonzalez-Valencia et al. (2014).

The OM content of sediment was determined as described previously (Bao, 2000). The EPS content of sediment was extracted and detected as described by Wei et al. (2015). The contents of sulfur-containing compounds, including TS, AVS, and SO_4^{2-}, were determined as described previously (He et al., 2018a). The total ^{13}C, ^{13}C of organic C and ^{13}C of CO_2-C in the sediment samples were detected using a Elementar vario MICRO cube elemental analyser coupled to GV Isoprime 100 isotope ratio mass spectrometer (GV Instruments, U.K.) as described by He et al. (2020).

2.4. DNA extraction and gradient centrifugation

Approximately 0.5 g sediment subsamples were extracted using E.Z.N.A. ^{TM} soil DNA extraction kit (Omega Bio-Tek, Inc., Norcross, USA) as described in the manufacturer's instructions. DNA concentration was detected with a NanoDrop 2000

Spectrophotometer (Thermo Fisher Scientific, United States). DNA was subjected to equilibrium density gradient centrifugation using cesium chloride (CsCl). In brief, approximately 3 μg DNA was combined with CsCl solution and loaded into a centrifuge tube (Himac, Koki Holdings Co., Ltd, USA), spun in a p90NT vertical rotor (Beckman Coulter, CA) and ultracentrifuged at 50800 rpm at 20 °C for 60 h. Twenty density gradient fractions were obtained, and the buoyant density (BD) of fractions was measured by weighing aliquots. The fractionated DNA was precipitated using polyethylene glycol 6000 (PEG6000) overnight at 4 °C. Then, the pellets were purified with 70% ethanol twice and resuspended in 20 μL nuclease-free water after centrifugation at 13000 g for 30 min. Heavy DNA was identified via a quantitative PCR (qPCR) analysis of 16S rRNA gene as described by Leigh et al. (2007) with the primer set of 338F 5'-ACTCCTACGGGAGGCAGCAG-3'/515R 5'-ATTACCGCGGCTGCTGG-3' (Fierer et al., 2005). After identifying the ^{13}C-labeled DNA fractions, the fractions were combined to form the "heavy" fraction (^{13}C-labeled DNA) for further molecular analyses.

2.5. Q-PCR analyses

Total DNA was used to determine the abundance of 16S rRNA gene (Fierer et al., 2005), *pmoA*, *mmoX* and *mxaF* using qPCR analysis. qPCR was performed in triplicate with a 15 μL reaction mixture for each sample including 1 × qPCR mix (GeneCopoeia, USA), 0.4 μmol L^{-1} primers, and 1 μL DNA template. The primers for the *pmoA*, *mmoX*, and *mxaF* genes were A189F 5'-GGNGACTGGGACTTCTGG-3'/Mb661R 5'-CCGGMGCAACGTCYTTACC-3' (Kolb et al., 2003), mmoX206F 5'-ATCGCB AARGAATAYGCSCG-3'/mmoX886R 5'-ACCCANGGCTCGACYTTGAA-3' (Hutchens et al., 2004), and mxa1003F 5'-GCGGCACCAACTGGGGCTGGT-

3'/mxa1561R 5'-GGGCAGCATGAAGGGCTCCC-3', respectively (McDonald et al., 1997). The thermal conditions set for 16S rRNA gene were as follows: initial denaturation at 95 °C for 10 min followed by 40 cycles of 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s. The annealing temperatures of *pmoA*, *mmoX* and *mxaF* were 58 °C, 56 °C, and 55 °C, respectively. The standard curves were performed in a range of 10^{-1}-10^{-7} ng μL^{-1}. The amplification efficiencies of these genes were between 90% and 93% with R^2 values of 0.90-0.99.

2.6. 16S rRNA gene MiSeq sequencing

Total DNA and ^{13}C-DNA retrieved from "heavy" fractions were used for PCR amplification. The PCR mixture in a total volume of 50 μL contained 5 μL 10 × buffer, 0.2 mM dNTPs, 0.06 U DNA Polymerase, 0.8 mM 338F/806R primers with barcodes, and 2 μL DNA template. PCR amplification was performed at 95 °C for 3 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, with a final extension at 72 °C for 10 min. PCR products were verified with 1.2% (w/v) agarose gels and sequenced using the Illumina MiSeq platform at Shanghai Majorbio Bio-Pharm Technology Co., Ltd.

The sequencing data were analyzed on the online Majorbio Cloud platform (www.majorbio.com) for quality filtering, chimera removal, and operational taxonomic unit (OTU) clustering on QIIME (v1.9.1). The paired-end reads were trimmed with Flash (v1.2.11). OTUs were clustered on the basis of a 97% similarity using Uparse (v7.0.1090). Sequencing data was classified with RDP Classifier (v2.11). The sequences obtained in this study have been deposited in the National Omics Data Encyclopedia (NODE) database under accession number OER221345-OER221422.

2.7. Data analysis

Statistical analysis was performed by SPSS Statistics 23.0 based on Student's t-test and analysis of variance (ANOVA). Alpha diversity was calculated in Mothur (v1.30.2) and visualized with the ggplot2 package in R (v4.0.3). Redundancy analysis (RDA) was performed based on the OTU level measured in R (v4.0.3) with the vegan package and visualized with the ggplot2 package.

3. Results and discussion

3.1. CH_4 and VSC fluxes

CH_4 and VSC fluxes from the Meiliang Bay were detected in four seasons (i.e., March, May, August and November) using floating static chambers. The CH_4 fluxes from the Meiliang Bay ranged from 16.8 to 78.7 mg m^{-2} d^{-1} (Fig. 1). Compared with the other seasons, the CH_4 fluxes were lower in March, due to the lower temperature of 14°C which influenced the degradation of organic matter. Similar studies have reported that exponential increases in CH_4 emissions with increasing temperature in freshwater systems (Aben et al., 2017; van Bergen et al., 2019). H_2S and CS_2 were the two detected VSCs with the fluxes of 10.7-65.4 and 14.4-72.7 mg m^{-2} d^{-1}, respectively, from the Meiliang Bay. The H_2S fluxes were similar to the CH_4 emissions with the lowest occurring in March. Statistical analysis showed that the H_2S emissions had a strong linear correlation with the CH_4 fluxes from the Meiliang Bay. The CS_2 fluxes were low with the average of 22.1 mg m^{-2} d^{-1} in March, May and August. However, the CS_2 flux was in range of 35.9 to 72.7 mg m^{-2} d^{-1} in November, which was significantly higher than the other seasons. This might be photochemical CS_2 production from the precursor molecules such as cysteine, cystine and $(CH_3)_2S$ of Cyanobacterial dying and degradation (Modiri Gharehveran and Shah, 2018) and a biological production of

phytoplankton species (Xie et al., 1999). Further studies such as CS_2 formation need to be conducted to illustrate the high CS_2 fluxes from the Meiliang Bay in November.

3.2. CH_4 oxidation and fate of CH_4-derived carbon

After exposure to CH_4, the CH_4 oxidation potential (MOP) of sediment increased in the first two days (Fig. 2A). The MOP of sediment reached the peak value (2.8 μg g^{-1} h^{-1}) in the M group on day 2, which was significantly higher than that in the MS group, likely due to the toxicity of CS_2 to microorganisms (Cooper and Brown, 2008). Börjesson et al. (2001) also found that high concentrations of CS_2 addition could lead to limited methanotrophic activity. Thereafter, the MOP of sediment was not obviously different between the MS and M groups and fluctuated from 2.5-2.8 μg g^{-1} h^{-1}, indicating that a CS_2 concentration of 350 mg m^{-3} had no significant effect on MOP in a longer term.

During CH_4 oxidation, CH_4-derived carbon was mainly converted into CO_2-C, biomass-C and organic-C such as EPS, formate, acetate and lactate (He et al., 2020). The OM content was 26.3 g kg^{-1} in the original sediment (Fig. 2B). After exposure to CH_4, the OM content of sediment increased with continuous CH_4 consumption and reached 31.2 and 32.8 g kg^{-1} in the MS and M groups, respectively, when the CH_4 consumption was 100 μmol $g_{wet\ weight}^{-1}$, owing to the secretion of organic matter such as acetate and oxalate during CH_4 oxidation (Kalyuzhnaya et al., 2013; Ma et al., 2021). Compared with the M group, the increase of the OM content was slightly less in the MS group, however, no significant difference in the OM content of sediment was found between the M and MS groups. This indicated that CS_2 addition could influence the distribution of CH_4-derived carbon and reduce organic carbon deposited into the sediment.

Carbon conversion efficiency (CCE) refers to the amount of organic carbon (OC) produced from CH_4 oxidation, which can be determined by a regression analysis of the amount of OC produced from CH_4 oxidation with CH_4-C consumption (Trimmer et al., 2015). In this study, a strong linear relationship was found between the organic ^{13}C amount and $^{13}CH_4$ consumption with the correlation coefficients (R) of 0.997 and 0.999 for the M and MS groups, respectively (Fig. 2C). Compared to the CCE of 21.1% in the MS group, the CCE was higher (27.3%) in the M group, indicating that more CH_4-derived carbon was converted into organic carbon and deposited into the sediments in the M group than in the MS group. This result was in accordance with the OM content (Fig. 2B) of sediment, which might be because CS_2 might prompt more CH_4-derived carbon used for the growth and metabolism of CS_2-degrading microorganisms. Previous studies have also shown that CH_4-derived carbon can be served as nutrients to higher trophic levels and be influenced by various environmental factors such as O_2 concentrations, nitrogen sources, and the existence of toluene (Medvedeff and Hershey, 2013; He et al., 2015; He et al., 2020).

The efficiency of CH_4-derived carbon conversion into total carbon (TC) of sediment (CE_{TC}) was 31.5% when the CH_4 consumption was 20 μmol $g_{wet\ weight}^{-1}$, increased with CH_4 consumption and reached 41.8% in the M group when the CH_4 consumption was 50 μmol $g_{wet\ weight}^{-1}$ (Fig. 2D). Relative to the M group, the CE_{TC} level was significantly lower in the range of 24.7%-27.5% in the MS group. The efficiency of CH_4-derived carbon conversion into CO_2-C ($CE_{CO2\text{-}C}$) was 52.5%-57.1% and 62.5%-64.7% in the M and MS groups, respectively (Fig. 2E). The $CE_{CO2\text{-}C}$ levels in the MS group were significantly higher than in the M group. This indicated that CS_2 addition could promote more CH_4-C converted into CO_2-C and decrease the TC content of the sediment.

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342 *3.3. Secretion of EPS*

343 EPS is a complex mixture formed by biochemical processes from high molecular
344 weight organic compounds, including cell exudates, autolysis and cell surface material
345 shedding (Costa et al., 2018). EPS is comprised of carbohydrates, proteins, nucleic
346 acids, humic acids and other substances, of which ECPS and ECP account for 75-89%
347 (McSwain et al., 2005; Ni et al., 2009). The ECPS and ECP contents were about 71.8
348 and 227.3 mg kg^{-1}, respectively, in the original sediment (Fig. 3). After exposure to CH_4
349 and CS_2, the ECPS content increased and reached 215.0 and 155.3 mg kg^{-1} in the MS
350 and M groups, respectively, when the CH_4 consumption was 100 μmol $g_{wet\ weight}^{-1}$. The
351 ECP content was 2-3 times of the ECPS content measured in the experimental
352 sediments, which might be attributed to the fact that the availability of substrates could
353 trigger different biosynthesis pathways of EPS. Bozal et al. (1994) reported that
354 *Alteromonas* strain secreted more protein when growing on a mineral medium, while
355 higher carbohydrate levels of EPS were obtained in a complex medium. Compared with
356 the M group, the ECPS and ECP contents were significantly higher in the MS group
357 when the CH_4 consumption was 100 μmol $g_{wet\ weight}^{-1}$. This may be attributed to the
358 toxicity of CS_2 and its intermediates to microorganisms, which might prompt EPS
359 secretion for survival and defensive functions (Marvasi et al., 2010). Li (2020) also
360 found that *Methylomonas koyamae* could secrect more EPS into the culture under the
361 CH_3SH stress of 200-1000 mg m^{-3} than the group without CH_3SH. Moreover, CH_3SH
362 and CS_2 could be converted between each other (Li, 2020). Previous studies have also
363 reported that microorganisms could secrete EPS to improve resistance under harsh
364 environments in the presence of heavy metals, chemicals and antibiotics (Fang et al.,
365 2002; Coburn et al., 2016).

366

367 *3.4. Variation of sulfur-containing compound contents*

368 To assess CS_2 metabolism, sulfur-containing compounds, including SO_4^{2-}-S, AVS
369 and TS, were determined in the experimental sediments. The TS content was 69.3 mg
370 kg^{-1} in the original sediment. With CS_2 addition, the TS content of sediment increased
371 and reached the maximum of 287.1 mg kg^{-1} when the CH_4 consumption was 100 μmol
372 $g_{wet\ weight}^{-1}$, while the TS content in the M group almost stayed constant (Fig. 4A). The
373 TS increment in the sediments accounted for 79.8%, 96.7% and 88.0% of the CS_2-S
374 input when the CH_4 consumption was 20, 50 and 100 μmol $g_{wet\ weight}^{-1}$, respectively.
375 This indicated that the increase of TS content in the sediments was attributed to the
376 CS_2-S input. Thus, we inferred that CS_2-S could be oxidized or metabolized by
377 microorganisms in the sediment. Hartikainen et al. (2001) also found *Thiobacillus* could
378 remove CS_2 and produce SO_4^{2-} in a peat biofilter. AVS is a product of sulfate reduction
379 such as the biodegradation of OM with SO_4^{2-} serving as an electron acceptor (Richard
380 and Morse, 2005). The AVS content increased with incubation time and reached 12.6
381 mg kg^{-1} in the MS group when the CH_4 consumption was 50 μmol $g_{wet\ weight}^{-1}$ (Fig. 4B),
382 which was significantly higher than the others ($P < 0.05$). The AVS content decreased
383 slightly when the CH_4 consumption was 100 μmol $g_{wet\ weight}^{-1}$ in the MS group, likely
384 due to increased AVS oxidation into SO_4^{2-}.

385 The SO_4^{2-}-S content was 57.8 mg kg^{-1} in the original sediment (Fig. 4C). With CS_2
386 addition, the SO_4^{2-}-S content of sediment increased with incubation time and reached
387 67.3, 97.5 and 223.4 mg kg^{-1} when the CH_4 consumptions were 20, 50 and 100 μmol
388 $g_{wet\ weight}^{-1}$, respectively, in the MS group, which was in accordance with trends of TS
389 content in sediment (Fig. 4A). The percent of SO_4^{2-}-S increment relative to CS_2-S were
390 59.3%, 61.1%, and 62.7% when the CH_4 consumptions were 20, 50 and 100 μmol g_{wet}

391 $_{weight}^{-1}$, respectively, in the MS group (Fig. 4D). This suggested that CS_2-S was mainly
392 oxidized to SO_4^{2-} as the final product in the experimental sediments, owing to the fact
393 that CS_2 was the only S source in MS group. Alcantara et al. (1999) also found that the
394 end-product of microbial CS_2 oxidation was SO_4^{2-} accompanied by the intermediates
395 such as H_2S, COS and elemental S. Additionally, sulfur-containing compounds such as
396 H_2S, CH_3SH and $(CH_3)_2S$ could also be mainly oxidized to SO_4^{2-} under aerobic
397 condition (Fang et al., 2015; Wang et al., 2021).
398
399 *3.5. Abundance of bacteria, methanotrophs and methylotrophs*
400 The abundance of bacterial 16S rRNA gene was $3.88\times10^{10} \pm 1.38\times10^{9}$ copies g^{-}
401 ^{1} in the original sediment (Fig. 5A). After exposure to CS_2 and CH_4, the abundance of
402 bacterial 16S rRNA gene decreased to $2.34\times10^{10} \pm 3.55\times10^{8}$ and $1.74\times10^{10} \pm 3.06\times$
403 10^{8} copies g^{-1} in the M and MS groups when the CH_4 consumption was 20 μmol g_{wet}
404 $_{weight}^{-1}$, respectively, likely due to only CH_4 or CH_4 and CS_2 being available as carbon
405 sources for the sediment microbial community. Compared with the M group, the
406 abundance of bacterial 16S rRNA gene was lower in the MS group during the
407 incubation ($P < 0.05$), suggesting that CS_2 could strongly inhibit microbial growth
408 owing to its toxicity. It has similarly been found that CS_2 can inhibit the growth of
409 bacterial species in the rhizosphere (Hartel and Haines, 1992).
410 The abundances of *pmoA* and *mmoX* encoding pMMO and sMMO were 1.77×10^{8}
411 $\pm 6.94\times10^{6}$ and $7.56\times10^{7} \pm 8.03\times10^{6}$ copies g^{-1}, respectively, in the original
412 sediment. After exposure to CH_4, the abundance of *pmoA* and *mmoX* gradually
413 increased to $2.26\times10^{9} \pm 1.05\times10^{8}$ and $0.38\times10^{9} \pm 0.21\times10^{8}$ copies g^{-1}, respectively,
414 in the M group when the CH_4 consumption increased to 100 μmol $g_{wet\ weight}^{-1}$ (Fig. 5B).
415 However, in the MS group, the abundances of *pmoA* and *mmoX* reached the maxima of

$1.65\times10^9 \pm 8.28\times10^6$ and $0.26\times10^9 \pm 0.31\times10^8$ copies g^{-1}, respectively, when the CH_4 consumption was 50 μmol $g_{wet\ weight}^{-1}$ and decreased slightly at a CH_4 consumption of 100 μmol $g_{wet\ weight}^{-1}$ (Fig. 5C). This might be attributed to the inhibitory effect of CS_2 and its metabolic intermediates and products such as H_2S and SO_4^{2-} on the methanotrophic growth (Long et al., 2013; Caceres et al., 2014). Compared with the M group, the abundance of *pmoA* and *mmoX* was significantly higher in the MS group when the CH_4 consumption was 50 μmol $g_{wet\ weight}^{-1}$ ($P < 0.01$), indicating that CS_2 could enhance methanotrophic growth to a certain extent. However, the stimulating mechanism of CS_2 for methanotrophic growth was not clear. Further studies such as the effect of CS_2 on gene expression need to be conducted to understand the effect of CS_2 on methanotrophic growth and metabolism.

Methanol is an important intermediate product during CH_4 oxidation and can be converted into formaldehyde catalyzed by methanol dehydrogenase (MDH) (Islam et al., 2021). The *mxaF* gene encoding the large subunit of MDH is considered a functional marker gene for identifying methylotrophs. The *mxaF* abundance was $1.77\times10^8 \pm 1.74\times10^7$ copies g^{-1} in the original sediment, identical to the abundance of the *pmoA* gene. After exposure to CH_4 and CS_2 (Fig. 5D), the *mxaF* abundance increased and reached $7.78\times10^8 \pm 1.36\times10^7$ copies g^{-1} in the M group when the CH_4 consumption was 100 μmol $g_{wet\ weight}^{-1}$, while it reached the maximum of $6.97\times10^8 \pm 3.68\times10^7$ copies g^{-1} in the MS group when the CH_4 consumption was 50 μmol $g_{wet\ weight}^{-1}$. This was in accordance with the variation of *pmoA* and *mmoX* abundance in the sediment samples. Similar results were obtained by He et al. (2012, 2020), who reported a linear relationship between methanotrophs and methylotrophs during CH_4 oxidation.

3.6. Variation of microbial community structure

SIP microcosms were applied to track the active microbial community assimilating CH_4 and CH_4-derived carbon. The "heavy" and "light" DNA fractions were separated by CsCl gradient ultracentrifugation and identified according to the relative abundance of the 16S rRNA gene by qPCR analysis (Fig. S1). MiSeq sequencing results showed that a diverse bacterial community was present in total DNA and ^{13}C-DNA from the experimental sediments. After exposure to CH_4 and CS_2, the Chao index of the microbial community in the experimental groups increased relative to the original sediment in the total DNA (Fig. 6A). The Chao index of ^{13}C-DNA was significantly higher in the MS group than in the M group when the CH_4 consumption was 50 and 100 μmol $g_{wet\ weight}^{-1}$ (Fig. 6B). This indicated that after exposure to CS_2, the richness of the active microbial community increased due to more CH_4-derived carbon flowing into the microbial community, such as CS_2-metabolizing microorganisms.

Proteobacteria, *Nitrospirota*, *Bacteroidota*, *Desulfobacterota*, *Chloroflexi*, *Acidobacteriota*, *Verrucomicrobiota*, *Myxococota* and *Bdellovibrionota* were the main phyla in the sediment samples, accounting for 81.1%-89.4% of the sequencing reads in the total DNA during the whole experiment (Fig. 6C). After exposure to CH_4 and CS_2, the relative abundances of *Proteobacteria*, *Bacteroidota* and *Acidobacteriota* decreased from 54.1%, 15.6% and 7.5% in the original sediment to 31.2%-33.6%, 10.5%-10.9%, and 4.6%-5.0% in the sediment samples, respectively, when the CH_4 consumption was 100 μmol $g_{wet\ weight}^{-1}$. However, the relative abundances of *Nitrospirota*, *Desulfobacterota* and *Chloroflexi*, respectively, increased to 11.2%-12.2%, 6.6%-10.8% and 7.6%-8.8% in the sediment samples, being 2-4 times of the levels of the original samples. The relative abundances of *Verrucomicrobiota*, *Myxococcota* and *Bdellovibrionota* increased slightly after exposure to CH_4 and CS_2. The community

structure in phylum level was in accordance with Chao index that there was significant difference between the original sample and the others, while no obvious difference was observed between the MS and M groups.

Proteobacteria was the most dominant phylum in the ^{13}C-DNA, accounting for 39.3%-71.8% of the sequencing reads during the whole experiment, exceeding that of the total DNA (28.4-35.8%) (Fig. 6D). The relative abundances of *Bacteroidota* and *Chloroflexi* were respectively 1.2%-3.3% and 2.3%-5.6% in the ^{13}C-DNA of the two groups when the CH_4 consumption was 20 μmol $g_{wet\ weight}^{-1}$ and respectively increased to 5.9%-12.1% and 3.3%-11.5% when the CH_4 consumption was 100 μmol $g_{wet\ weight}^{-1}$. This indicated that more CH_4-derived carbon flowed into *Bacteroidota* and *Chloroflexi* with increasing CH_4 consumption. *Acidobacteriota* and *Myxococcota* were also abundant in the ^{13}C-DNA of the two groups, and a higher relative abundance was found in the ^{13}C-DNA of the MS group when the CH_4 consumption reached 20 μmol $g_{wet\ weight}^{-1}$ than in the others, suggesting that *Acidobacteriota* and *Myxococcota* might be related to CS_2 metabolism. Masuda et al. (2016) aslo found *Acidobacteriota* dominated in the metabolism of sulfur-compounds in paddy soil. Compared with the total DNA, the relative abundances of *Nitrospirota*, *Desulfobacterota* and *Verrucomicrobiota* were lower in the ^{13}C-DNA of the two groups during the whole incubation period, suggesting that *Nitrospirota*, *Desulfobacterota* and *Verrucomicrobiota* took part in assimilating CH_4-derived carbon, but their activities were not pronounced.

To illustrate the relationships of the sediment samples among environmental factors including TS, SO_4^{2-}, AVS, ECP, ECPS and OM, RDA was conducted based on the OTUs of the total DNA and ^{13}C-DNA (Fig. 7) at a 97% similarity level. After exposure to CH_4 and CS_2, the microbial community structure of the sediment samples changed and differed from that of the original sediment. Among the six environmental

490 factors, TS, SO_4^{2-} and AVS were the main environmental factors influencing the
491 microbial communities in the total DNA and ^{13}C-DNA of the MS group, while OM was
492 the main factor affecting the microbial communities in the M group. The total and active
493 microbial communities in the MS and M groups respectively clustered together,
494 suggesting that CS_2 had an important influence on the microbial community structure
495 of total and active microorganisms in assimilating CH_4-derived carbon. A similar result
496 was obtained by Shi et al. (2011) who found that the sulfur addition could increase of
497 the abundance of *Thiobacillus* to oxidize sulfur.
498
499 *3.7. Active methanotrophs, methylotrophs and sulfur-metabolizing bacteria*
500 α-*proteobacteria* methanotroph *Methylocystis* and γ-*proteobacteria* methanotrophs
501 *Methylomicrobium, Methylomonas, Crenothrix* and *Methylosarcina* were the dominant
502 methanotrophs in the sediment samples. The relative abundance of methanotrophs was
503 0.4% in the original sediment. After exposure to CH_4, the relative abundance of
504 methanotrophs increased with CH_4 consumption and reached 7.6% in the total DNA of
505 the M group when the CH_4 consumption was 100 μmol $g_{wet\ weight}^{-1}$ (Fig. 8A). The
506 relative abundance of methanotrophs also increased in the total DNA of the MS group,
507 and reached a maximum of 6.0% at the CH_4 consumption of 50 μmol $g_{wet\ weight}^{-1}$. In the
508 ^{13}C-DNA of the M group, the relative abundance of methanotrophs was highest (17.7%)
509 at the CH_4 consumption of 20 μmol $g_{wet\ weight}^{-1}$, and decreased with CH_4 consumption,
510 indicating the growth of non-methanotrophs derived from CH_4 oxidation, such as the
511 cross-feeding observed between methanotrophs and methanol-utilizing bacteria
512 (Krause et al., 2017). However, in the ^{13}C-DNA of the MS group, the relative abundance
513 of methanotrophs increased and reached the maximum of 27.9% at the CH_4
514 consumption of 50 μmol $g_{wet\ weight}^{-1}$, indicating CS_2 addition could increase the relative

abundance of methanotrophs in the initial stage. This was in accordance with the abundance of *pmoA* and *mmoX* determined by q-PCR analysis. The relative abundance of methanotrophs decreased to 9.7% at the CH_4 consumption of 100 μmol $g_{wet\ weight}^{-1}$, which might be attributed to the toxicity of CS_2 and the intermediates (Caceres et al., 2014). The relative abundances of *Crenothrix* and *Methylosarcina* were significantly higher in the M group than in the MS group due to the sensitivity to environmental stress (Cruaud et al., 2019). Conversely, the relative abundance of *Methylocystis* reached higher proportions in the total and ^{13}C-DNA of the MS group than those of the M group owing to the existence of sMMO, which showed broad substrate availability (Lee et al., 2006).

Methylotenera, *Methyloversatilis*, *Methylophilus*, *Hyphomicrobium* and *Methylobacillus* were the main methylotrophs in the total DNA of sediments (Fig. 8B). The relative abundance of methylotrophs was less than 0.2% in the original sediment. After exposure to CH_4, the relative abundance of methylotrophs increased and reached the maximum of 5.1% in the total DNA of the M group at the CH_4 consumption of 50 μmol $g_{wet\ weight}^{-1}$, while it increased with incubation time and reached 4.1% in the total DNA of the MS group at the CH_4 consumption of 100 μmol $g_{wet\ weight}^{-1}$. In ^{13}C-DNA, *Methylobacterium* and *Candidatus* Methylopumilus were also detected, accounting for 0.01%-0.46% of the total sequencing reads. The relative abundance of methylotrophs was high (28.6%) in the ^{13}C-DNA of the M group at the CH_4 consumption of 20 μmol $g_{wet\ weight}^{-1}$, indicating that methylotrophs were the main microorganisms assimilating CH_4-derived carbon. With increased incubation time, the relative abundance of methylotrophs decreased in the ^{13}C-DNA of the M group, indicating that more CH_4-derived carbon flowed into the non-methylotrophs in the SIP microcosms. In the MS group, the relative abundance of methylotrophs increased and reached the maximum of

10.3% in the ^{13}C-DNA at the CH_4 consumption of 50 μmol $g_{wet\ weight}^{-1}$, which was lower than that in the M group. This suggested that more CH_4-derived carbon flowed into the non-methylotrophs such as CS_2-metabolizing microorganisms during CH_4 oxidation with CS_2 existence.

The relative abundance of sulfur oxidizers including *Thiobacillus*, *Sulfuritalea*, *Sulfurifustis*, *Sulfuricella*, *Sulfuricurvum* and *Sulfuritortus* was low (0.24%) in the original sediment samples. After exposure to CS_2, the relative abundance of sulfur oxidizers increased to 1.8%-2.3% in the total DNA of sediment at the CH_4 consumption of 50 μmol $g_{wet\ weight}^{-1}$ and decreased with CH_4 consumption in the MS group (Fig. 8C). Compared with the M group, the relative abundance of sulfur oxidizers was higher in the MS group, indicating that more sulfur oxidizers took part in sulfur metabolism with CS_2 existence. In the ^{13}C-DNA, with the increasing CH_4 consumption, the relative abundance of sulfur oxidizers increased from 0.75% to 2.14% when the CH_4 consumption increased from 20 to 100 μmol $g_{wet\ weight}^{-1}$ in the MS group. *Thiobacillus* was the most abundant sulfur oxidizer in the ^{13}C-DNA of the MS group at the CH_4 consumption of 100 μmol $g_{wet\ weight}^{-1}$, suggesting that CH_4-derived carbon such as CO_2 might serve as important carbon sources for *Thiobacillus* growth. Kappler and Nouwens (2013) also found that facultative sulfur oxidizing chemolithoautotroph such as *Starkeya novella* (formerly *Thiobacillus novellus*) could utilize C1 compounds including methanol for growth and metabolism as well as sugars, amino sugars, amino acids, and organic acids. Further studies need to be conducted to illustrate what are the CH_4-derived carbon sources for *Thiobacillus*.

The diversity of sulfate-reducing bacteria was high but with a low proportion (Fig. 8D). *Pseudomonas*, *Geothermobacter*, *Desulfatiglans*, *Desulfobacca* and *Desulfobulbus* were the most abundant sulfate-reducing bacteria, accounting for 6.1%

565 of the relative abundance in the original sediment sample. After exposure to CH_4, the
566 relative abundance of sulfate-reducing bacteria decreased to 3.7% in the total DNA of
567 the M group, but increased to 6.5% in the total DNA of the MS group, when the CH_4
568 consumption was 20 μmol $g_{wet\ weight}^{-1}$. The relative abundances of sulfate-reducing
569 bacteria in the total DNA and ^{13}C-DNA decreased with CH_4 consumption, which might
570 be attributed to aerobic condition inhibiting the growth of sulfate-reducing bacteria (Xu
571 et al., 2017). Compared with sulfur oxidizers, the relative abundance of sulfate-reducing
572 bacteria was lower in the ^{13}C-DNA of the MS group, indicating that sulfur oxidation
573 dominated in the SIP microcosm, which was in accordance with the high sulfate content
574 of sediment under the existence of CS_2.
575
576 **4. Conclusion**
577 H_2S and CS_2 were the two detected VSCs accompanied with CH_4 emitted from the
578 Meiliang Bay. SIP microcosm incubation showed although a CS_2 concentration of 350
579 mg m^{-3} did not have significant effect on the MOP, the fate of CH_4-derived carbon and
580 active microbial community varied much. Sediment $CE_{CO2\text{-}C}$ was increased but
581 sediment CCE was decreased with the coexistence of CH_4 and CS_2. CS_2 was mainly
582 oxidized into SO_4^{2-}—S under aerobic condition. CS_2 could significantly change the
583 active microbial community structure involved into CH_4-dervied carbon in the
584 sediments, with TS, SO_4^{2-} and AVS levels serving as the main factors. CH_4-derived
585 carbon mainly flowed into methylotrophs in the first stage. With CH_4 consumption,
586 more CH_4-derived carbon flowed into non-methylotrophs. CS_2 could prompt more
587 CH_4-derived carbon flowing into non-methanotrophs and non-methylotrophs, such as
588 sulfur-metabolizing bacteria. These findings can help elucidate the influence of VSCs
589 on microorganisms in fresh lake sediment and the responses of methanotrophs to

environmental changes, and provide insights to reducing CH_4 emission from eutrophic lake systems.

Declaration of competing interest

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

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