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Enhanced thioether formation in stormwater pipes induced by nitrogen-containing pollutants: The role of the sediment microbiome

Fangyuan Jiang [a,b,c,1], Cheng Ye [a,b,c,1], Feifei Wang [d], Tianyi Dong [a,b,c], Jiazhi Wei [a,b,c], Zoran Kapelan [e], Jan Hofman [f], Zuxin Xu [a,b,c], Wenhai Chu [a,b,c,*]

[a] State Key Laboratory of Water Pollution Control and Green Resource Recycling, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China
[b] Minisry of Education Key Laboratory of Yangtze River water Environment, Tongji University, shanghai 200092, China
[c] Shanghai Institute of Pollution Control and Ecological Security, Shanghai 200092, China
[d] School of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, China
[e] Delft University of Technology, Department of Water Management, Stevinweg 1, 2628CN Delft, the Netherlands
[f] Water Innovation and Research Centre, Department of Chemical Engineering, University of Bath, Bath BA2 7AY, UK

HIGHLIGHTS

- This study comprehensively investigates 12 thioethers in stormwater pipe systems.
- Sediments act as both a reservoir and source for thioether accumulation.
- Terrestrial-derived dissolved organic nitrogen and NH$_3$-N drive thioether formation.
- Key microbial groups include fermentative, sulfate-reducing, and denitrifying bacteria.
- Humics may facilitate thioether synthesis as methyl donors and electron shuttles.

GRAPHICAL ABSTRACT
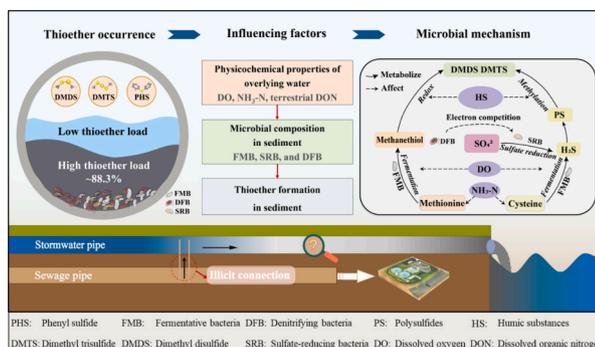
ABSTRACT

The illicit connections between sewage and stormwater pipes result in the discharge of untreated sewage into receiving rivers, posing significant odor and health hazards. While thioethers are recognized as key odorants in sewage systems, their distribution in stormwater systems remain poorly characterized. This study analyzed 12 types of thioethers in stormwater pipes sampled at 21 sites in China. Advanced analytical techniques, including Mantel analysis and Structural Equation Modeling, were employed to examine the relationships between overlying water properties, sediment microbial characteristics, and thioether concentrations. Results showed that sediment thioether loads (36.77 ± 50.14 µg S/m; range: 7.24–99.96 µg S/m) were substantially higher than those in the overlying water (12.02 ± 42.52 µg S/m; range: 0.03–92.76 µg S/m), highlighting sediment as a critical pollution reservoir. Dissolved oxygen, NH$_3$-N, and terrestrial-derived dissolved organic nitrogen were

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* Corresponding author at: National Engineering Research Center for Urban Pollution Control, Room 407, 1239 Siping Road, Yangpu District, Shanghai 200092, China.
  E-mail address: 1world1water@tongji.edu.cn (W. Chu).
[1] Fangyuan Jiang and Cheng Ye contributed equally to this study.

identified as key factors shaping sediment microbiome composition, particularly fermentative, sulfate-reducing, and denitrifying bacteria, which in turn drives thioether formation. Specifically, dominant compounds like dimethyl disulfide and dimethyl trisulfide were found to be produced through the anaerobic fermentation of methionine and redox conversion of methanethiol, as well as the anaerobic fermentation of cysteine and methylation of polysulfides. Humic substances could facilitate methanethiol redox conversion and polysulfide methylation by serving as methyl donors and enhancing electron transfer efficiency. Additionally, NH_3-N may promote microbial metabolism by providing amino groups essential for the synthesis of metabolic precursors. Therefore, effective mitigation of odorous thioethers in stormwater systems necessitates integrated strategies targeting both sulfur-containing organic precursors and nitrogen-rich pollutants.

1. Introduction

The emergence of black-odorous water bodies following rainfall events poses a significant environmental challenge. Research on urban river pollution in China has highlighted the critical role that deficiencies in urban drainage systems play in contributing to river water contamination (Xu et al., 2019). A common issue is the widespread illicit connections between sewage and stormwater pipes, which result in illicit discharge-associated (IDA) stormwater pipes receiving influent substrates from diverse sources, including urban runoff, atmospheric deposition, as well as untreated industrial and domestic wastewater (Gao et al., 2023). The end result is that during rainfall events, a diverse range of contaminants with high pollution loads are discharged into receiving water bodies, increasing the risk of odor pollution and threatening ecological safety (Yang et al., 2024)..

In recent decades, odor pollution in drainage systems has received considerable attention. Advances in odor recognition and detection techniques have enabled the identification of an increasing number of odorants in drainage systems (Jin et al., 2023). These odorants are typically classified into four categories: sulfur-containing compounds, nitrogen-containing compounds, hydrocarbons, and oxygen-containing compounds (Yang et al., 2019). Among them, thioethers are particularly notable due to their low olfactory thresholds and prevalence in sewage systems (Wang et al., 2021). Wang et al. (2014) identified dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) as the most prominent odorants in the sewage networks, while a field study by An et al. (2020) highlighted methanethiol, DMDS, and DMTS as primary odorous substances in sewer pipes. In addition to their contribution to odor issues, thioethers can also be toxic at elevated concentrations, potentially causing chronic health effects and even acute poisoning (Pandey et al., 2016). However, research on the occurrence of thioethers in stormwater drainage pipes remains limited.

IDA stormwater pipes collect large amounts of bioavailable organic matter, inorganic substances, and solid particles from wastewater during dry weather (Irvine et al., 2011). The composition of wastewater fluctuates during conveyance due to in-pipe processes such as physical deposition, biochemical transformation, and migration (Li et al., 2021). In this context, sulfur-containing compounds in stormwater pipes undergo various physio-biochemical reactions (Gao et al., 2023), creating a strong endogenous potential for thioether formation.

Previous studies have demonstrated that malodorous thioethers are primarily produced through the microbial degradation of organic matter, a process considerably influenced by the concentrations of methionine and cysteine in wastewater (Wang et al., 2024; Wu et al., 2024). In addition, dissolved oxygen (DO) levels and pH values evidently affect thioether formation in surface waters (Lu et al., 2013). Accordingly, the chemical properties of dissolved organic matter (DOM) and environmental conditions play critical roles in regulating thioether formation in aquatic environments. In stormwater pipes, the sustained inflow of high-load pollutants during dry weather can alter physicochemical properties and, more importantly, reshape the diversity of sediment microbial communities (Liu et al., 2025). This drives the adaptive evolution of microbial functional traits, enabling processes such as fermentation, sulfate reduction, and denitrification (Jin et al., 2018), which ultimately

regulate thioether formation (Chen et al., 2021). Therefore, understanding the key mechanisms underlying thioether formation in stormwater pipes is essential for their effective regulation.

However, substantial knowledge gap remain regarding the specific biochemical impacts of various inflow pollutants on thioether formation and the underlying microbial mechanisms within stormwater pipes. To fill this gap, this study conducted a comprehensive analysis of thioether concentrations in both overlying water and sediment at 21 sites from seven stormwater pipes. The Mantel test and Pearson correlation analysis were employed to assess the effect of overlying water properties and sediment microbial community characteristics on thioether concentrations, while Partial Least Squares-Structural Equation Modeling (PLS-SEM) was specifically utilized to elucidate the primary pathways driving thioether formation in sediments. In addition, potential microbial pathways for the production of dimethyl disulfide and dimethyl trisulfide were proposed to clarify the mechanisms underlying thioether formation. The findings of this study provide valuable insights for the effective control of odor pollution in stormwater drainage systems.

2. Materials and methods

2.1. Sampling and chemicals

A total of 21 sampling sites were selected from seven stormwater pipes across three cities in eastern China, known for widespread illicit pipeline connections (Chen et al., 2021; Ao et al., 2024). Site selection focused on residential areas and was based on historical monitoring data indicating continuous or intermittent sewage discharge during dry weather and storm runoff during wet weather. Detailed information about the sampling locations is provided in the supplementary material. Sampling was conducted during dry weather in January 2024, following a minimum antecedent dry period of two weeks. Minor precipitation events that did not generate runoff were considered negligible for the purposes of dry weather sampling. Overlying water samples were collected first, followed by sediment samples, with careful attention to preserving the integrity of the sediment's overlying layer. Information on the suppliers of analytical standards for thioethers and other chemicals is provided in the supplementary material.

2.2. Analytical methods

2.2.1. Thioether quantification

Overlying water and sediment samples were extracted using headspace solid-phase microextraction under the following conditions: 65 °C for 30 min and a stirring speed of 150 rpm, utilizing polydimethylsiloxane/divinylbenzene fibers. Subsequently, 12 types of thioether compounds (listed in the supplementary material; however, only five were detected in the analysis) were simultaneously quantified using a Shimadzu GC–MS system (QP2020, Kyoto, Japan) equipped with an RTx-5MS capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness; Restek Corporation, USA). Samples were injected in splitless mode with the injector temperature set at 250°C. The oven temperature program was as follows: initial hold at 32°C for 5 min, increased to 110°C at 8°C/min (hold for 2 min), then ramped to 270 °C at 20°C/min (final hold for

8 min). Detailed procedures and detection limits are provided in the supplementary material. Thioether concentrations in overlying water was expressed as the mass of sulfur per liter of water (ng S/L), while concentrations in sediment were normalized to the mass of sulfur per gram of wet sediment (ng S/g).

Thioether loads in both overlying water and sediment were calculated using geometric formulas based on the pipeline's cross-sectional area, the depths of sediment and overlying water, and the respective thioether concentrations. Results were expressed as micrograms of sulfur per meter (μg S/m) of pipeline, allowing quantitative comparison of thioether contributions from each phase to stormwater pollution. Detailed calculation formulas are provided in the supplementary material.

2.2.2. Dissolved organic matter characterization

General water quality parameters, including water temperature (T), conductivity, pH, and DO were measured using a portable multiparameter meter (HQ30D, HACH, USA). The concentrations of total nitrogen (TN), ammonia nitrogen (NH_3-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), chemical oxygen demand (COD), sulfate (SO_4^{2-}), and sulfide (S^{2-}) in water samples were measured using a DR6000 UV–Vis spectrophotometer (HACH, USA) with HACH commercial reagents. The following methods were applied: the Nessler reagent method for NH_3-N, persulfate oxidation for TN, cadmium reduction for NO_3^--N, diazotization for NO_2^--N, rapid digestion for COD, the barium sulfate turbidity method for SO_4^{2-}, and the methylene blue method for S^{2-}.

Sediment organic matter was extracted using a heating and ultrasound method, as described in the supplementary material. Dissolved organic carbon (DOC) concentrations in both overlying water and sediment extracts were quantified with a total organic carbon analyzer (TOC, Shimadzu Corporation, Kyoto, Japan). Dissolved organic nitrogen (DON) concentrations were calculated as the difference between total nitrogen and inorganic nitrogen concentrations. Dissolved organic sulfur (DOS) was measured using the adsorption pyrolysis-ion chromatography method (Wang et al., 1998; Luo et al., 2006).

Excitation-emission matrix (EEM) spectra were obtained using a fluorescence spectrophotometer (HITACHI F-2700, Japan). Quantitative characterization of fluorescent DOM was based on regional integration of fluorescence, which divides the EEM into five regions: tyrosine-like proteins (region I), tryptophan-like proteins (region II), fulvic acid-like organics (region III), soluble microbial product-like substances (region IV), and humic acid-like organics (region V). Molecular weight (MW) distributions of DOM were analyzed by size-exclusion chromatography coupled with organic carbon detectors (*SEC*-OCD, Karlsruhe, Germany). Based on the elution time (i.e., apparent molecular weight), DOM in samples can be assigned to five major subfractions, including biopolymers, humic substances (HS), building blocks, low-molecular-weight acids, and low-molecular-weight neutrals. UV–visible absorption properties of the samples were determined with a spectrophotometer (HACH DR6000, USA). To further characterize the DOM, six absorption and fluorescence indices were calculated based on EEM and UV–visible spectra: specific UV absorbance at 254 nm (SUVA), fluorescence index (FI), biological index (BIX), humification index (HIX), spectral slope ratio (S_R), and a_{355} (Yin et al., 2021). Additional details on the analytical methods are provided in the supplementary material.

2.2.3. Microbial analysis

DNA was extracted in triplicate from sediment samples using the FastDNA Spin Kit (MP Biomedicals, USA) according to the manufacturer's instructions. Sequencing targeting the V3/V4 region of the 16S rRNA was performed on an Illumina-MiSeq platform (Shanghai Majorbio Bio-pharm Technology Co., Ltd). The raw sequencing data were generated in FASTQ format. Paired-end reads were then preprocessed via Cutadapt software (version 2.6), and after quality filtering, noise reduction, splicing and chimera removal were performed with QIIME2

(version 1.9.1), representative sequences and ASV abundances were obtained (Caporaso et al., 2010). Further details are provided in the supplementary material. We uploaded the sequencing reads to the NCBI Sequence Read Archive (Accession Number: PRJNA1280524).

Indicator species analysis and functional characterization of the sediment microbiome were subsequently conducted. Based on the total concentration of thioethers, samples in the top third (A2, A3, B1, B3, D3, E1, and G2) were categorized as the High Group, while the remaining samples were classified as the Low Group. The "indicspecies" R package was used to identify indicator species associated with each groups. Microbial functional profiles were predicted using the PICRUST2 package, with functional inference based on tier 2 metabolic categories in the KEGG pathway database. Additional methodological details are provided in the supplementary material.

2.3. Statistical analysis

Relationships among the physicochemical properties of overlying water, sediment microbial characteristics, and thioether formation were analyzed as follows. The Mantel test, implemented using the "vegan" package in R, was used to evaluate associations between thioether composition and water or sediment parameters, as well as between overlying water parameters and microbial characteristics. Pearson correlation analysis was used to examine the relationships between sedimentary thioether concentrations and the abundances of microbial genera and functional traits.

PLS-SEM was employed to quantify causal pathways influencing thioether formation (Zhao et al., 2023), using the "plspm" package in R. The SEM framework included inner models and outer models: the inner model described the relationships among latent variables, while the outer model illustrated associations between latent variables and their reflective indicators. This study developed an internal model to describe the relationships between three latent variables—overlying water properties, microbial taxonomic composition, and microbial functional traits—and thioether formation in sediments. Water quality parameters for overlying water were selected based on significant correlations with sedimentary thioether content ($p < 0.05$). Microbial genera and functions demonstrating statistically significant associations ($p < 0.01$) with sediment thioether levels were included as indicator variables. Further methodological details are provided in the supplementary material. All statistical analyses were conducted using R version 4.1.3 and Origin 2021b. All abbreviations used in this paper are summarized in Table S1.

3. Results and discussion

3.1. Thioether occurrence in overlying water and sediment

Five types of thioethers, diethyl disulfide, DMDS, DMTS, diethyl trisulfide, and phenyl sulfide (PHS), were detected in stormwater pipes, with their concentrations shown in Fig. 1. The other seven thioethers were not detected, likely due to the rapid transformation of structurally complex thioethers into simpler compounds (e.g., DMDS) or other byproducts under the oxygen-limited and intermittent-flow conditions within the pipes (Li et al., 2024). Notably, diethyl trisulfide and PHS were identified in the drainage systems for the first time. The total concentrations of these thioethers varied widely, ranging from 0.5 ng S/L to 2.1×10^4 ng S/L in overlying water and from below the limit of detection (LOD) to 4.9 ng S/g in sediment (see supplementary material). Variations in thioether concentrations among sampling sites likely reflects differences in environmental and water quality factors influencing thioether formation (Wang et al., 2021).

In overlying water, DMDS, PHS, and DMTS accounted for an average of 61 %, 23 %, and 14 % of the total thioether concentrations, respectively. These compounds similarly dominated in sediments, collectively comprising approximately 98 % of the total thioether concentrations. In contrast, diethyl disulfide and diethyl trisulfide constituted only a minor
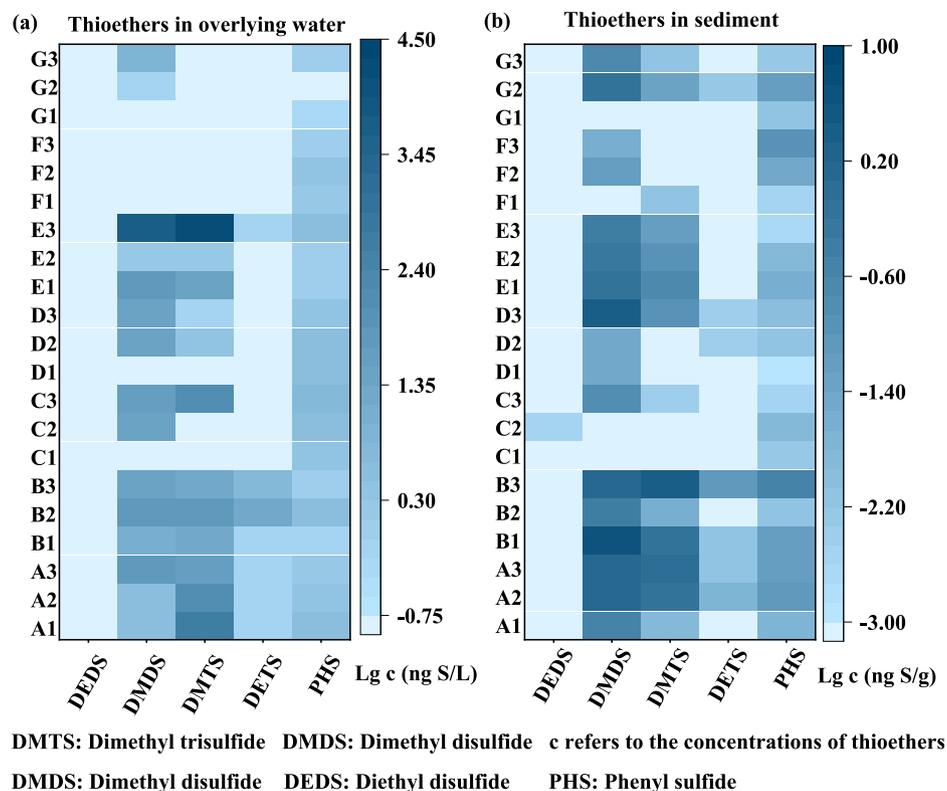
DMTS: Dimethyl trisulfide   DMDS: Dimethyl disulfide   c refers to the concentrations of thioethers

DMDS: Dimethyl disulfide   DEDS: Diethyl disulfide   PHS: Phenyl sulfide

Fig. 1. Concentrations of the five detected thioethers in (a) overlying water and (b) sediment across all sampling sites. Thioether concentrations below the detection limit are represented as Lg c < -0.75 (overlying water) and Lg c < -3 (sediment).

proportion of the detected thioether concentrations in all samples. Notably, PHS was detected in all overlying water and sediment samples, exhibiting a higher detection frequency than other thioethers (see supplementary material). Compared to other thioethers, PHS has a stable molecular structure and low volatility, as indicated by its Henry's law constant in aquatic environments (see supplementary material), making it a more persistent odor risk.

To compare thioether distribution between the water and sediment phases, thioether loads were calculated for both matrices. The thioether load in overlying water averaged 12.02 ± 42.52 µg S/m (range: 0.03–92.76 µg S/m), while the sediment showed a higher average of 36.77 ± 50.14 µg S/m (range: 7.24–99.96 µg S/m). On average, thioethers in the sediment constituted 88.3 % ± 21.1 % of the total thioether load across both the liquid and solid phases within pipes. These results indicate that sediment serves as a primary source of thioether pollution during stormwater runoff events. Two key factors contribute to this phenomenon: first, the high organic substrate content and elevated biological activity in pipe sediment promote microbial degradation of organic matter, leading to increased thioether formation (Gasperi et al., 2010; Watson and Juttner, 2017); second, the porous and rough surfaces of sediment particles facilitate adsorption of pollutants (Yin et al., 2016). Given the critical role of sediments in thioether pollution within stormwater pipes, further research is warranted to elucidate the factors and mechanisms driving thioether formation in sediments.

3.2. Effect of abiotic factors on thioether formation

The physicochemical characteristics of the overlying water were thoroughly analyzed (see supplementary material), revealing significant spatial variability among sampling sites. For example, DO concentrations ranged from 0.41 to 10.54 mg/L, NH₃-N from 0.12 to 111.00 mg/L, and DON ranged from 0.00 to 70.90 mg/L. This pronounced variability highlights the inherent complexity of water quality within urban stormwater drainage systems.

To identify the primary water quality factors influencing thioether formation in pipes, Mantel tests were performed to assess correlations between various water quality variables and thioether composition. As illustrated in Fig. 2, significant correlations were observed between thioether composition in sediments and the concentrations of DO, NH₃-N, and DON ($p < 0.05$). Moreover, total thioether concentrations were positively correlated with NH₃-N and DON concentrations ($p < 0.05$), but negatively correlated DO ($p < 0.01$) (see supplementary material). Previous studies have demonstrated that thioether formation is primarily driven by the anaerobic degradation of organic matter (Zhou et al., 2019; Wang et al., 2021), which explains the observed negative relationship between DO and thioether concentrations. In addition, a significant negative correlation was observed between DON and the FI, BIX, and the relative proportions of EEM volume for tyrosine-like and tryptophan-like proteins ($p < 0.05$). FI indicates the origin of DOM, with lower values representing terrestrial sources and higher values indicating microbial contributions (Begum et al., 2023). BIX represents autochthonous DOM production, where elevated values signify freshly produced DOM from planktonic or microbial activity (Begum et al., 2023). Tyrosine-like and tryptophan-like proteins are typically of microbial origin (Fellman et al., 2010). These results indicate that terrestrially derived DON plays a major role in influencing thioether formation in sediments (Fig. 2). Overall, low DO concentrations and high levels of NH₃-N and terrestrial-sourced DON in overlying water appear to be key factors promoting thioether formation in sediments.

3.3. Effect of biological factors on thioether formation

Sediment-associated microbial communities play pivotal roles in mediating pollutant transformation within stormwater pipes (Zuo et al., 2021). Taxonomic profiling identified Proteobacteria, Actinobacteriota, and Chloroflexi as the three dominant phyla, together accounting for 54 ± 14 % of total sequences (see supplementary material). Among the 30 most abundant genera (see supplementary material), indicator species
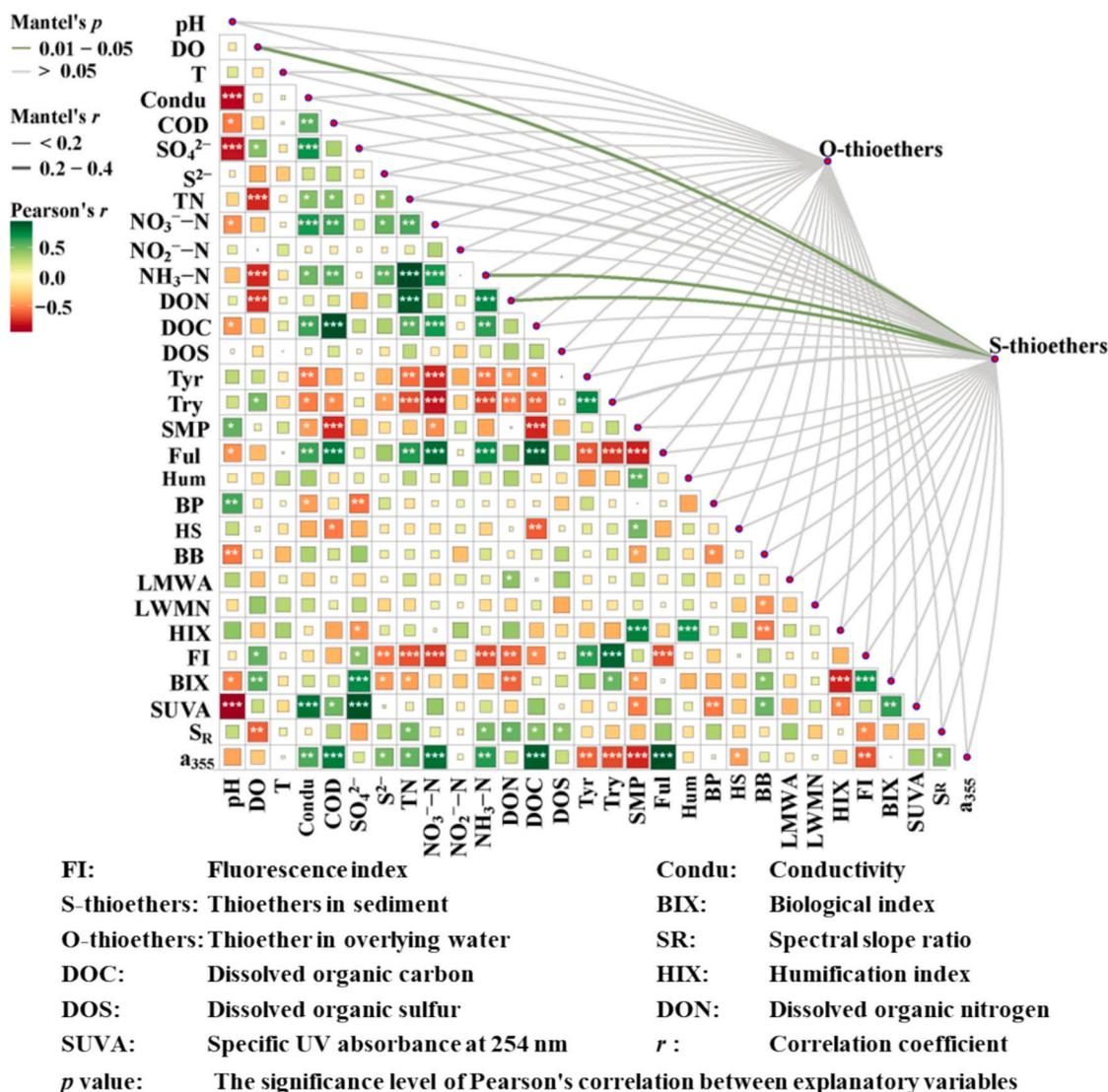
Fig. 2. Relationships between thioether concentrations and various physicochemical parameters of the overlying water, analyzed using the Mantel test. BP, HS, BB, LMWA, and LWMN present proportions of biopolymers, humic substances, building blocks, low-molecular-weight acids, and low-molecular-weight neutrals in *SEC-OCD*. Tyr, Try, SMP, Ful, and Hum represent proportions of EEM volume for five regions (tyrosine-like proteins, tryptophan-like proteins, fulvic acid-like organics, soluble microbial product-like substances, and humic acid-like organics).

analysis revealed *unclassified_f_Comamonadaceae, unclassified_f_Rhodobacteraceae, Caldisericum,* and others exhibited significant between-group differences (indicator value > 0.6, p < 0.05; see supplementary material). The association between these genera and thioether concentrations was further evaluated using Pearson's correlation (Fig. 3a). Sedimentary thioether concentrations were significantly positively correlated with the relative abundances of *norank_f_Bacteroidetes_vadinHA17, Trichococcus, norank_f_Anaerolineaceae, Christensenellaceae_R-7_group, Lactivibrio,* and *Caldisericum* (p < 0.05). In contrast, significant negative correlations were observed with *unclassified_f_Rhodobacteraceae, Hydrogenophaga, Flavobacterium,* and *unclassified_f_Comamonadaceae* (p < 0.05). These findings suggest that these indicator species are functionally involved in sulfur-metabolizing pathways responsible for thioether formation.

The aforementioned genera can be categorized into three main functional groups: fermentative bacteria (FMB), denitrifying bacteria (DFB), and sulfate-reducing bacteria (SRB) (see supplementary material). Fermentative bacteria, including *Norank_f_Bacteroidetes_vadinHA17, Trichococcus, norank_f_Anaerolineaceae, Christensenellaceae_R-7_group,* and *Lactivibrio,* drive anaerobic decomposition of macromolecular organics (Baldwin et al., 2015; Chen et al., 2021). *Caldisericum,*

as an SRB, facilitates the reduction of sulfate to H_2S through the oxidation of organic substrates (Chen et al., 2021). Genera such as *unclassified_f_Rhodobacteraceae, Hydrogenophaga, Flavobacterium,* and *unclassified_f_Comamonadaceae* are recognized as denitrifying bacteria and may compete with SRB for electron donors (Li et al., 2020). These findings suggest that microbial genera associated with organic fermentation, denitrification, and sulfate reduction play a central role thioether formation in pipe sediments.

Functional profiling of microbial communities revealed 46 metabolic functions related to in-pipe processes, with carbohydrate metabolism, amino acid metabolism, and energy metabolism being the most predominant (see supplementary material). Correlation analysis between microbial functional frequency and sedimentary thioether concentration revealed a significant positive correlation between carbohydrate metabolism and thioether concentrations (p < 0.01), whereas signal transduction and the metabolism of other amino acids showed significant negative correlations with thioether concentrations (p < 0.05; Fig. 3b). Carbohydrate metabolism is essential for microbial energy production and amino acid biosynthesis (Wang et al., 2024), supplying both energy and the necessary precursors for thioether formation. Signal transduction reflects the accumulation of signaling molecules and utilization
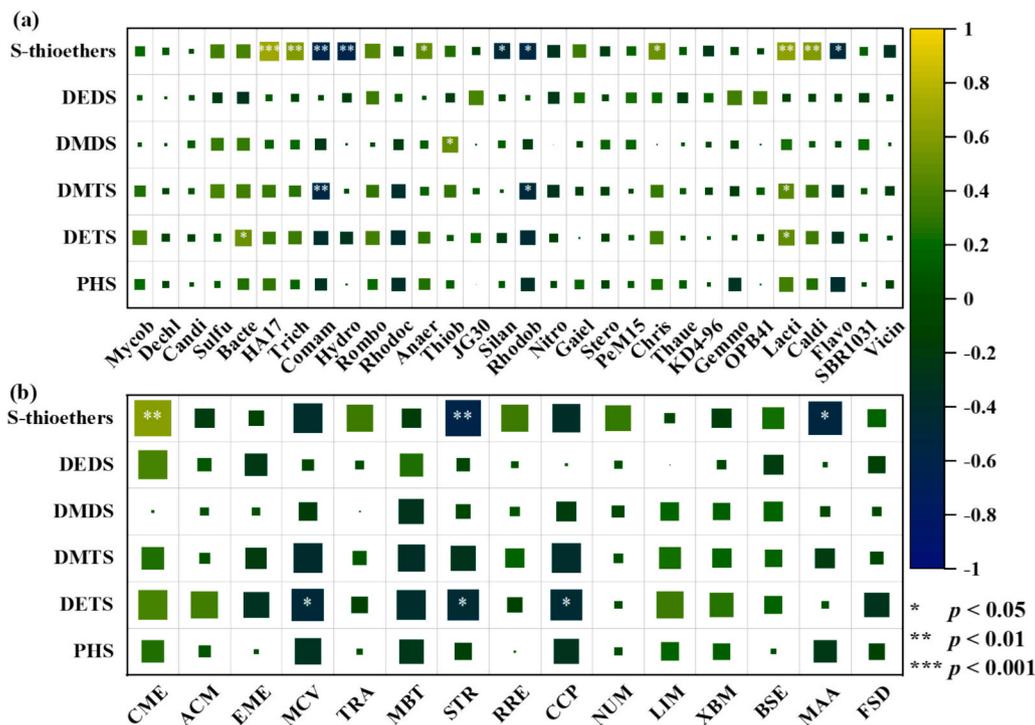
Notes: (1) S-thioethers: Thioethers in sediment; (2) DEDS: Diethyl disulfide; (3) PHS: Phenyl sulfide;

(4) DETS: Diethyl trisulfide; (5) DMTS: Dimethyl trisulfide; (6) Other abbreviations are listed in Table 1.

Fig. 3. Pearson's correlations between the thioether concentrations in sediment and (a) predominant microbial genera and (b) functional features of the sediment microbiome. The analysis included the 30 most abundant microbial genera and 15 principal microbial functions, based on average abundances in all samples. *p* denotes the significance level of Pearson's correlation.

of low-concentration substrates under nutrient-limited conditions, facilitating microbial adaptation to oligotrophic environments (Ding et al., 2022; Yin et al., 2024). Such strategies are typically associated with nutrient limitation and are unfavorable for thioether formation (Li et al., 2022; Noell et al., 2023). The observed negative correlation between thioether concentrations and the metabolism of other amino acids may be due to competition between the utilization of various amino acid derivatives and conventional amino acids, particularly methionine, cysteine, and cystine, which serve as precursors for thioether formation. Overall, these results provide new insights into the microbial mechanisms underlying thioether formation in sediments.

### 3.4. Key driving pathway for thioether formation

Based on the findings presented in Sections 3.2 and 3.3, thioether formation is significantly influenced by nitrogenous pollutants in the overlying water and by sediment microorganisms. Furthermore, Mantel tests revealed significant correlations between microbial community composition, microbial functions, and overlying water parameters, including DO, TN, NH₃-N, DON, and FI ($p < 0.05$; supplementary material). As TN primarily comprises NH₃-N and DON—which together account for approximately 71 % of TN—and given the significant negative correlation between DON and FI ($p < 0.01$; Fig. 2), the principal factors affecting sediment microbial communities (both composition and function) are identified as DO, NH₃-N, and terrestrially derived DON. These factors are consistent with those influencing thioether concentrations in sediment. Therefore, it is speculated that nitrogenous pollutants in the overlying water may influence thioether production in sediment by modulating the microbial community structure and function.

To further quantify the causal relationships among overlying water properties, microbial characteristics, and thioether formation, a deeper

analysis using PLS-SEM was employed. Drawing on the significant correlations between thioether concentrations and overlying water properties, the abundance and functional frequency of microbial taxa (as presented in Sections 3.2 and 3.3), the outer model incorporated the following indicators: overlying water properties (such as DO, NH₃-N, DON, and FI); microbial composition (including *unclassified Bacteroidetes_vadinHA17*, *Lactivibrio*, *unclassified Comamonadaceae*, and *Caldisericum*); and microbial functions (e.g., carbohydrate metabolism and signal transduction).

As shown in Fig. 4a, the high loadings in the outer model indicate that these reflective indicators effectively represent the latent variables. Specifically, DO (loading = -0.643), NH₃-N (loading = 0.839), DON (loading = 0.845), and FI (loading = -0.807) strongly reflect the properties of overlying water. Key microbial group, including DFB (*unclassified_f_Comamonadaceae*, loading = -0.747), FMB (*Lactivibrio*, loading = 0.900; *norank_f_Bacteroidetes_vadinHA17*, loading = 0.945), and SRB (*Caldisericum7*, loading = -0.747), effectively represent the microbial community. Microbial functions was well characterized by carbohydrate metabolism (loading = -0.960) and signal transduction (loading = 0.954).

The loadings of the inner model suggest that the overlying water has a strong directly and indirect effects on the formation of sedimentary thioethers ($p < 0.05$; Fig. 4a). Notably, the most important pathway involves two interrelated components: the association between overlying water properties and microbial composition (loading = 0.781; $p < 0.01$), and the relationship between microbial composition and thioether formation (loading = 0.534; $p < 0.05$). Therefore, the primary mechanism is that the characteristics of the overlying water shape the sedimentary microbiome, which in turn drives thioether formation.
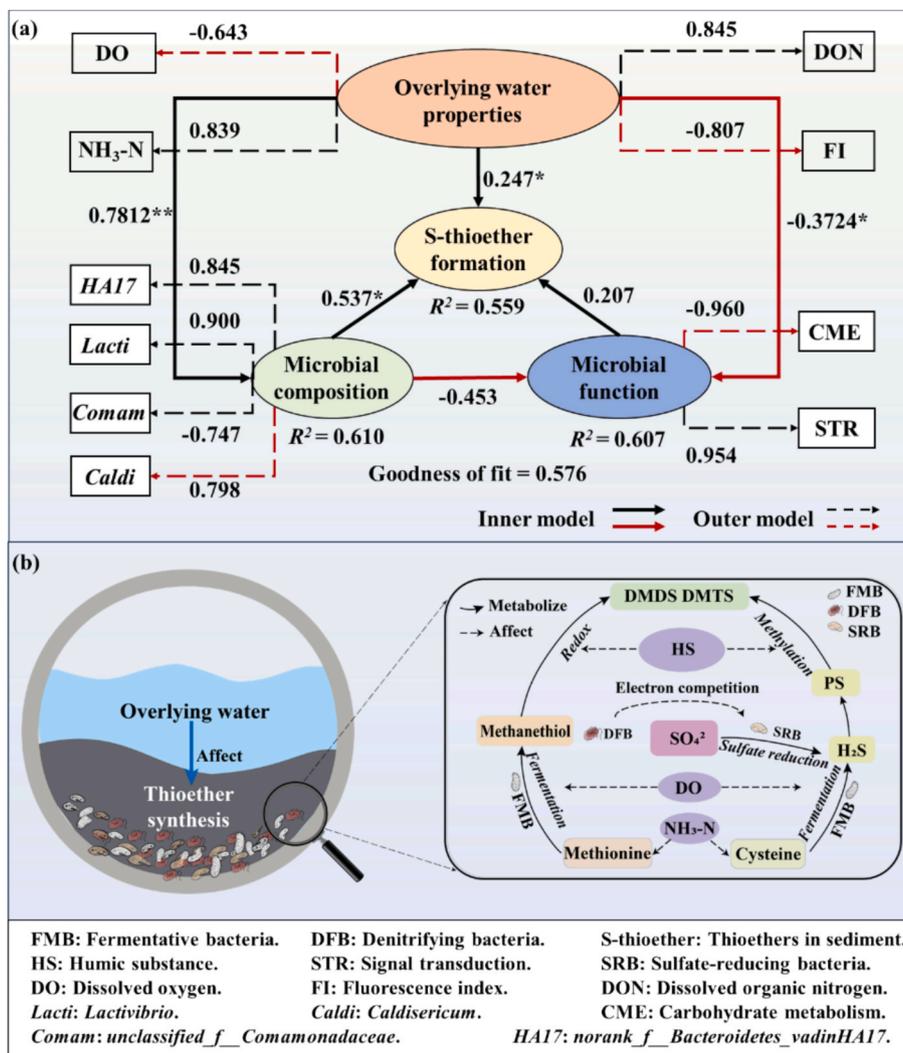
Fig. 4. (a) Partial Least Squares-Structural Equation Modeling illustrating the direct and indirect effects of overlying water properties on thioether formation in sediment. Numbers adjacent to the arrows represent the standardized factor loadings for reflective indicators associated with their respective variables; black and red arrows indicate positive and negative relationships, respectively. R^2 indicates the explained variance proportion of each variable. * and ** indicate $p < 0.05$ and < 0.01, respectively. (b) Proposed microbial pathways for the production of dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) in pipe sediments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. Proposed metabolic pathways of DMDS and DMTS formation

To elucidate the formation mechanisms of thioethers in sediment, the authors proposed the microbial metabolic pathways involved the synthesis of DMDS and DMTS. First, the relationship between sediment organic matter properties and thioether concentrations was analyzed. Detailed data on the concentration, composition, and optical properties of sediment organic matter are provided in the supplementary material. Mantel test revealed significant positive correlations between thioether concentrations and the levels of DON, DOS, and DOC in the sediment organic matter (see supplementary material). These results suggest that nitrogen- and sulfur-containing organic compounds, such as sulfur-containing amino acids, may serve as potential precursors for thioethers in stormwater pipes. This inference is consistent with previous studies, which identified methionine and cysteine as the primary precursors (Wang et al., 2024; Wu et al., 2024). Additionally, other nitrogenous organics, including free amino acids and urea, may promote thioether production supplying essential nitrogen for microbial growth (Frette et al., 2009). Betaine may also contribute by acting as a methyl donor (Frette et al., 2009). Reduced sulfur compounds (e.g., meth-anethiol) can undergo disproportionation reactions to form thioethers

under anaerobic conditions (Lomans et al., 2002). Collectively, these results underscore the critical roles of nitrogen- and sulfur-containing organic compounds in thioether generation.

The microbial pathways for DMDS and DMTS production from methionine and cysteine were further elucidated·NH₃-N facilitates the synthesis of methionine and cysteine by providing essential amino groups (Reitzer, 2003). One major pathway involves the hydrolysis of methionine, followed by the oxidation of methanethiol (Fig. 4b) (Huang et al., 2024; Wang et al., 2024). Specifically, methionine is degraded into methanethiol by methionine thiolase, generating NH₃-N and 2-oxobuta-noic acid as by-products with FMB participating in this process (Spasojevic et al., 2012). Methanethiol is subsequently oxidized in the presence of nicotinamide adenine dinucleotide to form DMDS and DMTS (van den Bosch et al., 2009). HS enhance redox efficiency by acting as electron shuttles and providing methyl donors (Jin et al., 2023; Yang et al., 2024).

Another key pathway involves the anaerobic degradation of cysteine and the methylation of polysulfides. Cysteine is anaerobically degraded to yield pyruvate, H₂S, and NH₃-N (Du and Parker, 2012). H₂S reacts with elemental sulfur via disproportionation to form polysulfides (Hu et al., 2020; Li et al., 2024), and can also be produced by SRB through

sulfate reduction (Zhang et al., 2023). These polysulfides are then methylated to form DMDS and DMTS via methyltransferases, with methyl groups provided by HS (Gu et al., 2019; Jin et al., 2023). Both FMB and SRB play critical roles in these pathways. However, under conditions of elevated nitrogen pollution, DFB may compete with SRB for electron donors, potentially inhibiting H_2S production (Ontiveros-Valencia et al., 2012; Shi et al., 2018).

In addition, energy and cofactor-producing pathways are crucial for thioether synthesis. Adenosine triphosphate and nicotinamide adenine dinucleotide phosphate are generated primarily produced by FMB through glycolysis, supplying the necessary energy and cofactors for thioether synthesis (Wang et al., 2024).

In summary, the anaerobic fermentation of cysteine and methionine, methanethiol oxidation, and polysulfide methylation are critical steps in DMDS and DMTS formation. FMB, SRB, and DFB are key microorganisms involved in these processes, while HS and NH_3-N significantly promote thioether production.

4. Conclusions

This study systematically examined the occurrence of thioethers in IDA stormwater pipes and the formation mechanisms of sedimentary thioethers during dry weather. Sediment thioether loads (36.77 ± 50.14 µg S/m; range: 7.24–99.96 µg S/m) were substantially higher than those in overlying water (12.02 ± 42.52 µg S/m; range: 0.03–92.76 µg S/m), indicating sediments as major reservoirs of these odorous compounds. Overlying water parameters, especially DO, terrestrial-derived DON, and NH_3-N, directly and indirectly influence sedimentary thioether formation. More importantly, these parameters significantly shape the composition of sediment microbial communities, particularly FMB, SRB, and DFB, which collectively mediate thioether formation. Using DMDS and DMTS as representative compounds, methionine and cysteine were identified as the primary precursors. HS could facilitate the synthesis of DMDS and DMTS by acting as methyl donors and electron shuttles, while NH_3-N can support microbial metabolism by providing amino groups essential for the biosynthesis of these precursors. Therefore, effective control of odorous thioethers in stormwater systems requires addressing both sulfur-containing precursors and nitrogen-rich pollutants.

CRediT authorship contribution statement

Fangyuan Jiang: Writing – original draft, Methodology, Investigation, Funding acquisition. **Cheng Ye:** Writing – review & editing, Methodology. **Feifei Wang:** Writing – review & editing. **Tianyi Dong:** Investigation. **Jiazhi Wei:** Investigation. **Zoran Kapelan:** Writing – review & editing. **Jan Hofman:** Writing – review & editing. **Zuxin Xu:** Supervision. **Wenhai Chu:** Supervision, Funding acquisition.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2025.132878.

Data availability

Data will be made available on request.

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