

Perfluorooctanoic Acid (PFOA) Inhibits Methane Emissions and Reshapes Microbial Communities in Thermokarst Sediments

Siyan Li

Guangzhou University

Jing Wei

University of Oslo

Even Werner

University of Oslo

Peter Dörsch

Norwegian University of Life Sciences

Dag O. Hessen

University of Oslo

Aidong Ruan

Hohai University

Alexander Eiler

`alexander.eiler@ibv.uio.no`

University of Oslo

Research Article

Keywords: Methane emissions, Perfluorooctanoic acid, Thermokarst pond, Functional genes, Microbial community structure

Posted Date: October 8th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10500334/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Permafrost landscapes act as reservoirs for per- and polyfluoroalkyl substances (PFASs) and represent crucial carbon pools in the global carbon cycle. With the intensification of climate warming, permafrost is undergoing rapid degradation, exposing previously trapped organic carbon to microbial decomposition and thereby leading to substantial greenhouse gas emissions. However, this thaw also liberates legacy chemical pollutants into active biological zones. The role of PFAS contaminants in modulating microbial community composition and metabolic activity, in particular greenhouse gas production, in thawing permafrost landscapes remains poorly understood. A sampling campaign was conducted in a high-latitude permafrost region, and a dose-response exposure experiment was performed with concentrations ranging from ng to μg of perfluorooctanoic acid (PFOA; $\text{C}_8\text{HF}_{15}\text{O}_2$) per gram sediment with multiple cores from a thermokarst pond representing landscape features with highest methane emissions. Our findings demonstrate a high susceptibility of microbial communities to PFOA pollution in particular methane-cycling. PFOA led to distinct changes in microbial functional potential, including a dual effect on methane-cycling communities: surfactant-induced inhibitory effects on sensitive methanogenic taxa, and the inactive persistence and relative enrichment of methanotrophs. This pollutant-driven shift was paralleled by a significant, concentration-dependent suppression of net methane emissions. Path analysis revealed that this suppression was driven primarily by direct PFOA inhibition rather than community turnover, despite substantial PFOA-induced restructuring of the broader prokaryotic community. This study provides novel insights into the ecotoxicity of emerging contaminants on key biogeochemical cycles, underscoring the need to account for contaminant-induced shifts when projecting future Arctic climate feedbacks.

1. Introduction

The environmental risks of per- and polyfluoroalkyl substances (PFASs) have attracted growing global attention. Due to their exceptionally strong carbon-fluorine (C–F) bonds, these compounds exhibit high chemical and thermal stability, resistance to biodegradation, and unique hydrophobic/oleophobic properties [1, 2]. Since the mid-20th century, PFASs have been widely applied in textiles, coatings, lubricants, firefighting foams, and food packaging, leading to their ubiquitous detection in soils, surface waters, the atmosphere, and biota worldwide [3, 4]. Their combined persistence, bioaccumulation, and toxicity have earned them the designation “forever chemicals” [5, 6].

Among PFASs, perfluorooctanoic acid (PFOA) is one of the most common and extensively studied representatives. Structurally, PFOA consists of a hydrophilic carboxyl group and a fully fluorinated carbon chain, which confer surfactant properties and high recalcitrance [7]. PFOA is listed in the Stockholm Convention on Persistent Organic Pollutants [8]. Decades of use have resulted in PFOA being widely detected in aquatic environments, sediments, soils, sewage sludge, human blood, and even breast milk [9, 10]. Permafrost landscapes are a critical sink for global PFAS pollution [11], and as arctic temperatures rise, the thawing process liberates these sequestered chemicals, raising significant concerns.

High-latitude permafrost landscapes represent one of Earth's largest terrestrial carbon reservoirs, storing nearly twice the amount of carbon currently present in the atmosphere [12]. Rapid thawing triggers ground subsidence and the formation of thermokarst ponds [13], which act as intense anaerobic biogeochemical reactors where previously frozen organic matter is exposed to microbial decomposition, leading to substantial emissions of methane (CH_4) [14]. While these aquatic systems are critical conduits in the global carbon cycle [15], they also function as sinks for long-range transported contaminants [16]. Therefore, understanding the vulnerability of the microbial communities in thermokarst ponds to forever chemicals like PFOA is critical for predicting future climate feedbacks.

Emerging studies highlight that PFOA exerts profound effects on microbial communities. PFOA has been shown to induce oxidative stress, stimulate the secretion of extracellular polymeric substances, cause DNA and membrane damage, and suppress enzyme activities, thereby impairing microbial growth and metabolic performance [17]. Crucially for biogeochemical cycles, PFOA markedly decreases CH_4 production (by 11–19%) in anaerobic systems, with methanogenic archaea being more vulnerable than bacteria [18, 19]. However, some research suggests that microbial communities may gradually develop tolerance under long-term PFOA exposure, thereby maintaining partial metabolic functions [20]. This adaptation is often accompanied by the enrichment of resistant taxa such as Proteobacteria, Acidobacteria, and Bacteroidetes [21, 22].

While PFOA is known to interfere with microbial communities across different environmental settings, this research has predominantly focused on sludge environments and overall community shifts. Consequently, investigations directly addressing CH_4 emissions and their associated microbial communities remain limited [23, 24]. Although existing studies suggest that PFOA can inhibit CH_4 emissions and reshape the associated microbial community structure, the underlying mechanistic basis remains poorly understood [18, 25]. Therefore, an in-depth exploration of the microbial mechanisms through which PFOA influences CH_4 emissions is critical for advancing our knowledge of the carbon cycle and for environmental risk assessment.

The urgency of this research gap is further underscored by the importance of CH_4 as a potent greenhouse gas, with a global warming potential 28 times higher than CO_2 on a 100-year timescale [26, 27], and the prominent role of ecosystems like lakes, bogs, and permafrost as major conduits of CH_4 [28]. Global atmospheric CH_4 concentrations have risen continuously, now accounting for about 20% of observed radiative forcing since the pre-industrial era [29, 30]. Net atmospheric CH_4 emissions are regulated by the dynamic balance between two microbial processes: methanogenesis (production) and methanotrophy (oxidation) [31, 32]. Methanotrophy, driven by methanotrophs, constitutes the major biological CH_4 sink by converting it to CO_2 [33]. Consequently, PFOA and other chemicals can potentially have profound effects on CH_4 emissions if they interfere with this balance in ecosystems such as thawing permafrost landscapes.

In this study, we investigate the effects of different concentrations of PFOA on CH₄ emissions and the associated microbial community structure and functions. Using anaerobic incubation of thermokarst sediments exposed to a PFOA gradient, we combine greenhouse gas monitoring with high-throughput sequencing of the 16S rRNA gene and key functional genes (*mcrA* and *pmoA*). This approach allows us to systematically evaluate how PFOA influences the CH₄ cycle, microbial community composition, and metabolic functions. Furthermore, variance partitioning analysis (VPA), mixed-effects models, and partial least squares path modeling (PLS-PM) are employed to disentangle the independent and interactive effects of PFOA and environmental factors on CH₄ production. This work provides new insights into the CH₄ cycling and emission mechanisms in thermokarst pond sediments under PFOA stress, offering a scientific basis for ecological risk assessment and regional carbon emission management.

2. Materials and methods

2.1 Study site and sediment sampling

Sediment cores were collected from a thermokarst pond in the Iškoras permafrost peatland, Northern Norway (69.20°N, 25.17°E). This site represents a dynamic biogeochemical hotspot undergoing rapid permafrost thaw. Because standard regional GIS datasets often lack the temporal and spatial resolution required to monitor rapidly subsiding permafrost, high-resolution drone photogrammetry was utilized to accurately capture the active micro-topography of the sampling site (Fig. S1). Following collection, cores were transported at 4°C and processed under anaerobic conditions to maintain the integrity of the methanogenic community. Details about the site, sampling and physicochemical analyses are provided in the Supplementary Material 1.1–1.3.

2.2 Experimental design and PFOA treatment

An anaerobic microcosm incubation was established to evaluate the dose-dependent effects of PFOA on CH₄ cycling. Sediments were treated to achieve final PFOA concentrations of 0, 2, 4, 50, 100, and 5000 ng/g, based on environmentally relevant and worst-case legacy contamination scenarios (for details on the reasoning and references see Table S1). A pristine thermokarst site was deliberately chosen to provide a true, unexposed baseline. This experimental design was necessary to cleanly isolate the acute dose-response effects of PFOA and observe initial community restructuring, which would otherwise be masked if using sediments already adapted to historical PFAS contamination. PFOA monitoring in Finnmark (Northern Norway) by the Norwegian Environment Agency of similar ecosystems have so far resulted in concentrations below 0.08 ng/g sediment [34]. Initial geochemical measurements established a baseline sediment pH of 4.33–4.91. Because PFOA is an acidic compound, we stoichiometrically verified that our highest concentration gradient (5000 ng/g, equivalent to $1.2 \cdot 10^{-8}$ mol/g of H⁺) was several orders of magnitude below the natural buffering capacity of the highly organic peat sediment (TOC = 393.05 mg/g with a usual buffering capacity $> 10^{-4}$ mol/g of H⁺). Consequently, the trace molar addition of PFOA is incapable of altering the sediment pH, ensuring that any observed microbial inhibition was independent of experimental acidification. Microcosms were incubated at 10°C

to simulate active-layer summer temperatures. For more detail on the experimental design see the Supplementary Material 1.4.

2.3 Greenhouse gas analysis

Headspace gas samples were collected periodically over 7.5 days. CH₄ and CO₂ concentrations were quantified using a Gas Chromatograph equipped with a Flame Ionization Detector and a Thermal Conductivity Detector. Fluxes were calculated based on the linear change in headspace concentration over time, normalized to sediment fresh weight. For more details, see the Supplementary Material 1.5.

2.4 Microbial community and functional gene analysis

Total genomic DNA was extracted using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, USA). We performed DNA metabarcoding on the 16S rRNA gene (V4 region) for taxonomic profiling, alongside the *mcrA* and *pmoA* genes as functional markers for methanogenesis and methanotrophy, respectively. Sequencing was performed on the Illumina platform. Metabolic functions were predicted using the FAPROTAX database and cross-validated with functional gene abundances. Full details regarding PCR primers, thermal cycling conditions, and bioinformatic pipelines (DADA2/QIIME2) are documented in the Supplementary Material 1.6. Total microbial cell counts were monitored via flow cytometry (SYBR Green I staining) to ensure that functional shifts were not artifacts of broad biomass collapse (for more details, see Supplementary Material 1.7).

2.5 Data statistics and analysis

Alpha diversity indices of microbial communities, including ACE, Chao1, Shannon, and Simpson indices, were calculated using the vegan package in R. Beta diversity was assessed using non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity matrices computed in the same package, and community differences among treatments and sediment cores were tested by permutational multivariate analysis of variance (PERMANOVA, adonis function). Linear discriminant analysis Effect Size (LEfSe) analysis was conducted in Python 2 to identify microbial taxa with statistically significant differences between groups (LDA score > 3) [35]. Functional gene prediction was performed using Functional Annotation of Prokaryotic Taxa (FAPROTAX) (version 1.2.6) to assess microbial functional profiles before and after incubation [36]. Spearman correlation analysis was conducted to evaluate relationships between environmental factors and CH₄-associated microbial communities, and Mantel tests were performed using the ggcrr package [37] to assess associations between multiple factors and CH₄ emissions. Principal component analysis (PCA) was applied to the CH₄ emissions matrix across all sampling times, and the first principal component (PC1) was extracted as an integrated response variable for subsequent VPA and random forest modeling. VPA [38] was performed using the varpart function in the vegan package to explore the independent and interactive effects of various influencing factors on CH₄ emissions. Random forest analysis was conducted using the rfPermute package in R (with 500 trees) to further identify key biomarkers associated with CH₄ emissions. The statistical significance of the variable importance was evaluated through permutation

tests with 100 replications. PLS-PM was performed using the *pls* package to investigate the direct and indirect effects of PFOA contamination and environmental factors on CH₄ emissions [39]. Visualization of VPA and PLS-PM results was completed in Visio 2021. Statistical analyses, including analysis of variance, significance testing, and standard error analysis, as well as result visualizations, were conducted using Origin 2022. All graphs with error bars are presented as mean $\pm$ SEM. Differences among groups and the PFOA gradient were assessed by mixed-effects model using the *lme4* package in R. PFOA concentration (log-transformed) was set as the fixed effect, while the biological replicates (cores C1-C4) were assigned as a random intercept to account for spatial heterogeneity [40]. Significance levels indicated as follows: * for $p < 0.05$; ** for $p < 0.01$; and *** for $p < 0.001$.

3. Results

3.1 PFOA suppresses net methane production but not carbon dioxide production

Over the incubation period, concentrations of both CO₂ (Fig. 1A) and CH₄ (Fig. 1B) increased in all treatment groups. However, their responses to PFOA exposure differed significantly. Mixed-effects models revealed no significant response in the accumulation curves of CO₂ concentrations, using end concentration, take-off time, lag phase, or maximum production rate as curve descriptors, to increasing PFOA concentrations (Table S3; Fig. S2). No differences were observed among the four experimental cores. Subsequently, our molecular and functional analyses deliberately focused on the CH₄ cycling pathways, which demonstrated a targeted, high vulnerability to the contaminant. CH₄ accumulation curves were significantly affected, with both CH₄ end concentrations and the take-off time (the time required to initiate production) showing clear responses to rising PFOA concentrations (Fig. 1C, D; Fig. S3A, B). To accurately reflect this continuous, non-linear relationship and prevent visual distortion of the decay rate, concentration gradients were plotted on a logarithmic scale ($\log_{10}(x + 1)$ -transformed PFOA concentrations). This visually confirms the rapid, steep toxicity of PFOA on methanogenesis at the lowest, environmentally relevant concentrations (0 to 50 ng/g), while other descriptors, such as the maximum production rate, did not respond linearly (Fig. S3C, D). Analyses of Variance (ANOVAs) on the mixed-effect models confirmed these visual inspections with CH₄ end concentrations decreasing while CH₄ take-off time increasing with $\log_{10}(x + 1)$ -transformed PFOA concentrations (Table 1).

Table 1

Analysis of Variances on mixed-effects models for descriptors of CH₄ accumulation curves. Mixed-effect models were applied to assess the effects of PFOA addition and source environment (core) on the concentration of CH₄ at the end of the experiment (end concentration), the maximum production rate and lag phase, which were assessed using the framework described by Carlsen et al. [41], and take-off time. The latter was defined as the time point at which production rates exceeded a threshold determined from core-specific maximum production rates. Asterisks indicate significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Response variable	Factor	df	F-value	p-value
End concentration	core	3	638.43	<0.0001***
	Log ₁₀ (PFOA + 1)	1	177.47	<0.0001***
	core : log ₁₀ (PFOA + 1)	3	64.51	<0.0001***
Maximum production rate	core	3	18.53	<0.0001***
	PFOA	1	14.81	0.0014 **
	core : PFOA	3	9.71	0.0007 ***
Lag-phase	core	3	77.03	<0.0001***
	PFOA	1	91.25	<0.0001***
	core : PFOA	3	22.27	<0.0001***
Take-off time	core	3	1051.78	<0.0001***
	log ₁₀ (PFOA + 1)	1	92.67	<0.0001***
	core : log ₁₀ (PFOA + 1)	3	18.57	<0.0001***

Quantitatively, the control group (PFOA0) produced the most CH₄ throughout the incubation (39.71 ± 18.39 nmol/g), whereas the highest exposure group (5 µg/g) produced the least (16.66 ± 7.44 nmol/g). Relative to the control, final CH₄ concentrations decreased by 13.40% (2 ng/g), 12.96% (4 ng/g), 22.07% (50 ng/g), 31.35% (100 ng/g), and 57.70% (5 µg/g). This potent inhibitory effect was strongly described by a mixed-effects model (Adj. $R^2 = 0.99$; F-statistic = 326.6, $p < 0.0001$), which, after accounting for inter-core variability, estimated a highly significant negative effect for log₁₀(PFOA + 1) (Estimate = -7.04 ± 0.38 , $t = -18.37$, $p < 0.0001$).

3.2 PFOA induces shifts in microbial community composition

To understand the microbial basis for the altered CH₄ accumulation curves, we first analyzed the community structure. In the initial environmental (unincubated) samples, the community was dominated by Acidobacteriota (24.34%), followed by unclassified Bacteria (15.63%), Proteobacteria (9.72%), and Myxococcota (9.33%) (Fig. 2A). The incubation process (approximately 7.5 days) itself induced a marked community shift. In the incubated control (0 ng/g) group, the major phyla changed to Acidobacteriota (17.46%), Proteobacteria (15.54%), Firmicutes (15.26%) and unclassified Bacteria (14.27%).

From this control baseline, PFOA exposure amplified the dominance of Proteobacteria, which increased from 15.54% in the control to 50.15% under 5 µg/g sediment. This trend indicates a strong adaptation to high PFOA levels, likely reflecting the metabolic versatility of this phylum [42]. Conversely, the relative abundances of Acidobacteriota and Firmicutes declined with increasing PFOA concentrations, suggesting their sensitivity and ecological suppression under PFOA stress.

At the family level, this shift was even more pronounced (Fig. 2B). The family Pseudomonadaceae (a member of Proteobacteria) showed a consistent and dramatic increase with rising PFOA concentrations, expanding from 2.85% in the control to 33.80% under 5 µg/g sediment. This pattern suggests Pseudomonadaceae may play a crucial ecological role, rapidly expanding under PFOA pressure and reflecting both high tolerance and potential degradation capacity [43, 44]. Other taxa responded as well, suggesting that PFOA exposure substantially restructured the microbial community at the family level.

In contrast to these dramatic compositional shifts, PFOA did not significantly affect overall microbial abundance or α-diversity. Mixed-effects models showed no significant effect of PFOA concentration on total cell counts (Fig. S4; Table S4). Although at 5 µg/g sediment we observed the lowest average cell count ($(6.95 \pm 1.70) \times 10^4$ cells/mL) compared to the control ($(1.13 \pm 0.30) \times 10^5$ cells/mL), this difference was not statistically significant. Because the total prokaryotic numbers did not vary significantly across the PFOA gradient (ANOVA, $p = 0.12$; Table S4), the observed relative shifts in community composition can be interpreted as reflecting changes in absolute ecological contributions. Similarly, PFOA concentration exerted no significant effect on the α-diversity indices (Fig. S5; Table S5). β-diversity analysis revealed that spatial heterogeneity amongst the sediment cores (Fig. S6) exerted a stronger influence on community structure than the PFOA treatments.

3.3 Identification of microbial biomarkers responding to PFOA

To pinpoint the specific microbial taxa that characterized each treatment group, we performed a LEfSe analysis (Fig. S7). This identified key differentially abundant taxa from the phylum to family level. In the raw (unincubated) samples, Phycisphaerae and Gammaproteobacteria *Incertae Sedis* were identified as biomarker taxa. After incubation, the biomarkers shifted. The control (PFOA0) group was characterized

by Firmicutes, indicating its dominance under PFOA-free incubation conditions. Under 100 ng/g sediment, the anaerobic fermentative bacteria Peptostreptococcaceae and Peptostreptococcales-Tissierellales were enriched, likely reflecting microenvironmental shifts under moderate PFOA stress. Finally, under 5 µg/g sediment, Sphingobacteriaceae was the sole enriched family, suggesting its strong adaptability and potential for organic pollutant degradation under high PFOA exposure.

3.4 PFOA exerts a dual effect on methane-related metabolic functions

To predict the functional consequences of the observed community shifts, we used FAPROTAX functional annotation (Fig. 2C). The analysis revealed a pronounced dual effect of PFOA exposure on CH₄-related metabolism, consistent with our gas measurements. The predicted function of overall methanogenesis (via all pathways) showed a non-linear response. The relative abundance of methanogenic taxa increased slightly at low PFOA concentrations (below 50 ng/g) but then declined significantly at higher doses, reaching its lowest level under 5 µg/g. In direct contrast, the predicted function of methanotrophy increased relatively with higher PFOA levels, peaking under the highest PFOA exposure. These opposing predicted trends—the suppression of methanogens and the stimulation of methanotrophs—align with the net suppression of CH₄ emissions observed in our incubation experiment.

Other metabolic functions were also affected. Nitrogen fixation was particularly sensitive, showing a significant relative decrease across all PFOA treatments and its lowest relative abundance under 5 µg/g. Phototrophy and oxygenic photoautotrophy were also suppressed, with concentration-dependent relative abundances.

3.5 PFOA structuring the CH₄ cycling communities

We next examined the CH₄-cycling communities underlying the functional shifts (Fig. 3). The overall relative abundance of methanogens declined with PFOA exposure, from 4.27% in the control to 2.83% under the highest PFOA exposure (Fig. 3A), indicating a clear concentration-dependent decline. This decline was primarily driven by the dominant methanogenic group, Rice Cluster II, which comprised 77.88%-95.82% of all methanogens. Its relative abundance, after a slight increase at very low doses (2–4 ng/g), steadily dropped reaching its minimum (2.37%) under 5 µg/g. This suggests that Rice Cluster II is highly sensitive to PFOA and is a key taxon regulating CH₄ production in this system.

The overall relative abundance of methanotrophs was low (0.23% in raw samples) and declined markedly in the control incubation (0.03%), likely due to anoxic conditions unfavorable for aerobic methanotrophs. Yet, PFOA exposure progressively increased the relative abundance of methanotrophs from this low baseline (Fig. 3B). This increase was not uniform and involved a distinct community shift. The abundance of Methylomonadaceae was negligible in the raw and control groups but rose substantially with increasing PFOA, peaking at 0.18% under 5 µg/g of sediment. In contrast, Methylacidiphilaceae

dominated the methanotrophs in the raw group (0.23%) but followed a pattern of initial increase at low PFOA concentrations before declining to nearly undetectable levels under 5 µg/g.

Similar to findings on the overall community composition (Section 3.2), PFOA concentration had no significant effect on the α -diversity of methanogenic and methanotrophic communities (Fig. S8). β -diversity analysis (Fig. S9) revealed that spatial heterogeneity of the sediment cores (core identity) exerted a stronger influence on the structure of CH₄-related microbial communities than the PFOA treatments.

3.6 Integrated analysis of putative factors driving CH₄ emissions

To disentangle the complex drivers of CH₄ end concentrations (termed “emissions” from now onwards) in our experiments, we first used VPA to attribute the explained variance among PFOA, environmental factors (reflecting the conditions in the sediments amongst the four cores or biological replicates), and the CH₄ metabolism-related communities (Fig. 4A). After removing collinear variables through VIF analysis, TN, TOC, and MC were retained as environmental factors. Collectively, these three categories explained 84.35% of the total variance in CH₄ emissions. Environmental factors (TN, TOC, MC; representing core-specific environmental properties) had the largest unique explanatory power, accounting for 39.36% of the variance, while PFOA explained 5.15% of the variance in CH₄ emissions. The interaction between environmental factors and CH₄ metabolism was the second-largest component, explaining 38.37%. This indicates that environmental conditions (representing core-specific properties) largely regulate CH₄ emissions by modulating the metabolic community, whereas PFOA had a minor impact on the observed variations. The joint effect of all three categories explained an additional 2.54%. The unexplained fraction was only 15.65%, demonstrating that our selected variables effectively captured the principal drivers of CH₄ emissions in our experiment.

A random forest regression model, built to rank the importance of all variables, explained 73.4% of the variance in CH₄ emissions, indicating robust predictive power (Fig. 4B). This analysis identified the methanogenic community as the single most important predictor, followed by environmental factors. Methanogenic metabolism (function) was also identified as a key predictive factor, supporting its central role in regulating CH₄ emissions.

3.7 PLS-PM reveals comprehensive effects of multiple factors on CH₄ emissions

To clarify the causal relationships between PFOA concentration, environmental factors, CH₄-related microbial community structure, metabolic functions, and their integrated effects on CH₄ emissions, we applied a PLS-PM analysis (Fig. 5). The model showed a good fit (GOF = 0.65), indicating strong explanatory and predictive power. PFOA exerted a significant direct inhibitory effect on CH₄ emissions

(path = -0.30 , $p < 0.01$). However, the model indicated that the indirect pathway mediated by the community structure was not statistically significant (path coefficient < 0.24 , $p > 0.1$). This implies that although PFOA drives profound shifts in community composition (Figs. 2–3), these structural changes play a negligible role in regulating the observed decline in CH₄ emissions. Instead, the reduction in CH₄ is primarily driven by the direct impact of PFOA, independent of the observed community turnover.

In contrast, environmental factors had a strong and direct effect on CH₄ emissions (path = 0.68 , $p < 0.001$). This effect was also mediated indirectly, as the environment drove CH₄ emissions by determining the relative abundances and diversities of methanogens and methanotrophs. Finally, the model confirmed that methanogenic metabolism was the main functional mediator (path = 0.47 , $p < 0.001$), with its effect size far exceeding that of methanotrophic metabolism (path = 0.18) in regulating final CH₄ emissions.

4. Discussion

4.1 PFOA exhibits potent, targeted inhibition of methane emissions

The experimental results of this study demonstrate that PFOA exhibits a pronounced concentration-dependent inhibitory effect on CH₄ emissions in anaerobic thermokarst sediments. While the most dramatic suppression occurred at the highest treatment level ($5\text{ }\mu\text{g/g}$), where emissions were reduced by more than 50% compared to the control, there were also responses at the lower end of our gradient. At concentrations of 2 ng/g and 4 ng/g , which are levels directly representative of typical background contamination in remote Arctic regions, CH₄ emissions in the experiments were already reduced by 13.40% and 12.96%, respectively. This suggests that as climate warming intensifies and the thawing permafrost liberates previously trapped “forever chemicals” into active biogeochemical zones, even trace levels of pollution can significantly perturb carbon fluxes.

An important insight into the inhibitory mechanism is the differential response of carbon-based gases. While CH₄ emissions were severely suppressed, manifesting as a prolonged take-off time and a significantly lower terminal concentration, the production of CO₂ was not significantly affected. This finding strongly suggests that PFOA does not cause a general suppression of microbial activity or overall organic carbon mineralization. Instead, its toxicity appears to be specific to the methanogenic process.

Our results are consistent with the majority of findings in related systems. For instance, Jiao et al. [25] reported a significant negative correlation between PFOA concentration and cumulative CH₄ emissions. Similarly, Wang et al. [18] observed a marked decline in CH₄ emissions with increasing PFOA concentrations, and Silva et al. [45] found that PFOA specifically inhibited acetoclastic methanogenic activity. Although the experimental systems (e.g., sludge, sediments) and PFOA concentrations varied

across these studies, all consistently demonstrated that PFOA suppresses CH₄ emissions, with these inhibitory effects increasing with dose.

It is important, however, to also address contrasting findings. Li et al. [23], for example, reported an irregular increase (5–20%) in CH₄ yield with PFOA in an anaerobic sludge digestion system. They proposed that PFOA might have enhanced the biochemical CH₄ potential of their specific substrate. This discrepancy does not invalidate our findings but rather highlights the critical importance of the environmental matrix. The active sludge system used by Li et al. [23] differs substantially in substrate composition, microbial community structure, and dominant metabolic pathways from the thermokarst sediments used in our study.

Overall, our results confirm that the influence of PFOA on CH₄ release from thermokarst sediments is concentration-dependent. This effect is likely attributable to the direct toxicity of PFOA toward key methanogenic guilds, leading to a marked decline in methanogenic capacity. This finding underscores the significant potential ecological risk PFOA poses to carbon cycling and greenhouse gas budgets in thawing permafrost landscapes.

4.2 A “dual” effect on the methane-cycling community

Our results suggest a distinct dual effect on the CH₄-cycling community, which provides a clear mechanistic basis for the observed net decrease in CH₄ emissions. First, PFOA suppressed key methanogens. The abundance of methanogenic archaea declined in a concentration-dependent manner, a trend driven primarily by the suppression of Rice Cluster II. This dominant, obligately anaerobic lineage is crucial in wetlands and permafrost [46] and appears highly sensitive to PFOA. This aligns with studies showing species-specific responses of methanogens to PFOA [9, 47].

Second, and in direct contrast, PFOA stimulated methanotrophs. This effect was characterized by a distinct community shift. The relative abundance of Methylomonadaceae, known as dominant aerobic methanotrophs in polar sediments [47–49], increased significantly under high PFOA exposure. Conversely, Methylacidiphilaceae, a group of acidophilic methanotrophs, showed a variable rise-and-fall pattern, suggesting different sensitivity to PFOA stress [51]. These Type I methanotrophs possess unique structural properties such as extensive intracytoplasmic membranes and high concentrations of hopanoids, which act as functional analogs to sterols to increase membrane rigidity and decrease permeability [51–53], while their broader metabolic plasticity allows them to adapt to fluctuating environmental stressors more effectively than the highly specialized, substrate-restricted methanogens [55].

The observed suppression of Firmicutes and methanogenic archaea likely stems from their structural vulnerability to surfactant-induced stress. Unlike the resilient Proteobacteria, which utilize a double-membrane system and active efflux pumps to mitigate PFOA toxicity, Gram-positive fermenters (e.g., Firmicutes) lack an outer membrane barrier, leaving their cytoplasmic membrane directly exposed to lipid disruption [56]. Similarly, while methanogens possess unique ether-linked membranes, they often

lack the specialized multi-drug efflux systems required to prevent PFOA accumulation within the lipid bilayer (like the RND-family pumps in *Pseudomonas*) [57], likely leading to the observed functional collapse of the methanogenic pathway.

The FAPROTAX functional predictions mirrored these community shifts. The predicted potential for methanogenesis declined markedly at high concentrations, while the predicted potential for CH₄ oxidation (methanotrophy) increased. This is consistent with Li et al. [23], who observed a substantial decline in *mcrA* gene abundance under PFOA exposure. Interestingly, Cao et al. [9] reported that high PFOA concentrations upregulated key methanogenic functional genes such as *mcrA* and *mtrA*, with *mcrA* expression, yet not corresponding with increasing CH₄ production. They suggested that this may reflect a microbial stress response or selective enrichment of specific methanogenic populations [58]. These inconsistencies may arise from differences in microbial community composition, substrate types, and exposure duration among studies [24]. Overall, our results strongly support that the steep decline in CH₄ emissions resulted from PFOA simultaneously suppressing methanogens and promoting methanotrophs.

4.3 PFOA acts on the broader microbial community

Beyond the CH₄ cyclers, PFOA exposure acted as a selective pressure that reshaped the prokaryotic community in permafrost-peat sediments. We observed a high heterogeneity in the beta-diversity and taxonomic composition across replicates, where sediment incubations within the same treatment group often harbored distinct microbial assemblages. While such variability and the focus on a single site might be perceived as experimental limitations, they strengthen our conclusions regarding PFOA affecting community composition.

Proteobacteria were markedly enriched in a concentration-dependent manner consistent with a previous study [59]. This was driven by the expansion of the Pseudomonadaceae family from 2.85% in the control to 33.80% in the highest treatment group suggesting that their robust genomic repertoire for xenobiotic metabolism and stress response provides a distinct competitive advantage in PFOA-impacted sediments. This corroborates the group's renowned high tolerance, metabolic versatility, and potential to degrade organic pollutants [60]. In contrast to the enrichment, Acidobacteriota and Firmicutes decreased significantly with increasing PFOA concentrations, indicating high sensitivity and ecological niche suppression under chemical stress [61].

The observed suppression of Rice Cluster II, a lineage of archaea characterized primarily by hydrogenotrophic methanogenesis, is likely exacerbated by the concurrent decline in fermentative Firmicutes producing the H₂ and CO₂ substrates of the hydrogenotrophic pathways. This suggests that the decline of methanogens is driven by the simultaneous induction of substrate starvation by disrupting the upstream fermentation supply chain and direct PFOA toxicity.

4.4 The “diversity paradox” and overarching environmental control

Our findings resolve the apparent discrepancy between stable microbial diversity and drastically reduced CH₄ emissions. While high-throughput sequencing provides inherently compositional data which necessitates caution when linking relative abundances to absolute gas fluxes, our flow cytometry data provides a critical quantitative anchor. Because total prokaryotic cell counts remained statistically unchanged across the PFOA gradient (ANOVA, $p = 0.12$; Table S4), the profound relative decline in methanogenic taxa within the community represents a loss of absolute methanogenic biomass. Thus, even without absolute qPCR quantification, the stability of total prokaryotic cell counts serves as a quantitative anchor to interpret the relative restructuring of *mcrA* and *pmoA* guilds to the observed functional suppression of CH₄ in PFOA-impacted thermokarst sediments.

Furthermore, the “diversity paradox” of profound compositional shifts occurring without any significant change in α -diversity or total cell counts is consistent with some sediment studies [25] but contrasts work in sludge, where diversity often decreases under exposure [19]. This discrepancy might be due to lower loss rates in the complex thermokarst communities or their capability to buffer against broad cytotoxic effects. As such, PFOA appears to act as a selective filter that reshapes the community rather than a broad-spectrum biocide that reduces its richness in thermokarst sediments, similar to previous studies [2, 62].

Moreover, β -diversity analysis revealed that spatial heterogeneity in the sediment (i.e., core identity) explained the largest proportion of variance ($\sim 39\%$), which is expected in dynamic thermokarst systems. This finding is critical, as it underscores that the sediment's inherent physicochemical characteristics [63] are a primary driver of microbial composition, overshadowing any contaminant effect. However, despite PFOA explaining a smaller absolute proportion of the variance ($\sim 5\%$), its targeted inhibitory effect remained highly statistically significant ($p < 0.0001$), proving its potency as a universal physiological stressor that overrides background environmental noise.

Finally, the impact of PFOA was not limited to the carbon cycle. FAPROTAX analysis showed that functions related to nitrogen cycling, and photosynthesis were also disturbed, indicating PFOA's potential to disrupt multiple biogeochemical cycles and microbial ecological functions [23, 64].

4.5 Modeling confirms environmental modulation of PFOA's effects

Our multivariate models (VPA and PLS-PM) allowed us to disentangle the complex interplay between PFOA, environmental factors, and the microbial community. The VPA confirmed that environmental factors (e.g., DOC, pH) played a dominant role in explaining CH₄ variations, thereby reinforcing the β -diversity results. For example, DOC (a substrate for methanogenesis) showed a highly significant positive correlation with CH₄ emissions [65]. Similarly, pH was strongly correlated with the methanogenic community, and low pH can favor acid-tolerant taxa [66], potentially altering PFOA's bioavailability or toxic effects [20, 67].

The PLS-PM model clarified the causal pathways, confirming that PFOA had a significant direct negative effect on CH₄ emissions ($p < 0.01$). Furthermore, it is expected that inherent environmental heterogeneity across sediment cores explains the largest proportion of total variance. However, from an ecological modeling perspective, the detection of a highly significant PFOA inhibitory effect ($p < 0.0001$) against this massive natural background noise highlights the extreme robustness of the response. Because terminal CH₄ concentrations decreased across all biological replicates, this demonstrates that PFOA toxicity induces a universal physiological suppression of methanogenesis that supersedes local environmental variability and remained concentration-dependent.

The decoupling of taxonomic variance from functional decline provides robust evidence that PFOA acts through a conserved physiological mechanism (direct inhibition) rather than relying on the presence of a specific, sensitive community architecture. If the PFOA effect were contingent on specific community shifts, the high variability between replicates would have obscured the functional signal. Instead, the fact that CH₄ production declined uniformly across divergent communities suggests that PFOA toxicity is a fundamental physiological response, independent of the variability in microbial community composition.

Thus, the strong direct path identified by the model suggests that PFOA suppresses methanogenesis primarily through direct physiological inhibition (potentially enzyme toxicity), which overrides the influence of community composition. Furthermore, this indicates that although PFOA has the potential to directly suppress methanogenesis, its actual, realized impact in a complex sediment system is modulated by the background environmental conditions.

5. Conclusions

This study provides mechanistic evidence that PFOA exerts a pronounced, concentration-dependent inhibition on CH₄ production in thermokarst sediments. Our data demonstrate that the pervasive presence of the “forever chemical” PFOA can suppress CH₄ production by ~ 13% at background levels (2–4 ng/g) and by over 50% at contamination hotspots. This suppression was primarily associated with the decline of methanogenic archaea, especially Rice Cluster II, and the concurrent stimulation of methanotrophic taxa such as Methylomonadaceae, indicating a shift in carbon flow under PFOA stress. PFOA exposure also induced a substantial restructuring of the prokaryotic community, characterized by specific taxonomic enrichments, thereby altering the functional balance of carbon and nitrogen cycling pathways.

Integrative modeling revealed that although PFOA directly depresses CH₄ emissions, environmental factors exert dominant modulatory effects on microbial composition and metabolic responses, jointly governing CH₄ fluxes. From a policy and management perspective, these findings reveal that anthropogenic contaminants are a critical, non-climatic variable that can skew Arctic carbon feedback projections. Because permafrost acts as a critical global sink for PFAS, our work underscores the urgency of integrating contaminant-microbe-environment interactions into regional environmental monitoring and global climate models to ensure the accuracy of future carbon emission forecasts.

Declarations

Funding declaration

This research was financially supported by the China Scholarship Council (CSC, Grant No. 202106710041) and the Norwegian Research Council project Biogov (Project No. 323945).

Author Contribution

SL: Conceptualization, Methodology, Investigation, Field work, Formal analysis, Data Curation, Writing - original draft, Visualization. JW: Investigation, Methodology, Writing - review & editing. EW: Investigation, Field work, Writing - review & editing. PD: Resources, Methodology, Writing - review & editing. DOH: Funding acquisition, Writing - review & editing. AR: Conceptualization, Supervision, Writing - review & editing. AE: Conceptualization, Supervision, Project administration, Funding acquisition, Writing - review & editing.

Acknowledgement

This research was financially supported by the China Scholarship Council (CSC, Grant No. 202106710041) and the Norwegian Research Council project Biogov (Project No. 323945). We want to thank Sebastian Westermann for assistance in the field. Assistant in the lab was provided by Yu Yu, Xiang Lu, Minh Vu, and Sigrid Kjær. Siyan Li thanks Feng Guo for his support. The sequencing service was provided by the Norwegian Sequencing Centre (www.sequencing.uio.no), a national technology platform hosted by the University of Oslo and Oslo University Hospital and supported by the “Functional Genomics” and “Infrastructure” programs of the Research Council of Norway and the Southeastern Regional Health Authorities. Sequence data processing was performed on the BMKCloud platform (www.biocloud.net). The graphical abstract was created with BioRender.com.

Data Availability

The raw demultiplexed sequence data (amplicon and metagenomic reads) generated in this study have been deposited in ENA under BioProject accession code PRJEB110443. The ASV tables, metadata and method descriptions have been deposited in the OSF data repository (www.osf.io) under pj4xg.Code availabilityNo new code was developed in this study. The analyses were conducted using publicly available software and packages listed in the Methods section.

Code availability

No new code was developed in this study. The analyses were conducted using publicly available software and packages listed in the Methods section.

References

1. Buck RC, Franklin J, Berger U, Conder JM, Cousins IT, De Voogt P, Jensen AA, Kannan K, Mabury SA, Van Leeuwen SP. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. *Integr Environ Assess*. 2011;7:513–41.
2. Xu R, Tao W, Lin H, Huang D, Su P, Gao P, Sun X, Yang Z, Sun W. Effects of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonic Acid (PFOS) on Soil Microbial Community. *Microb Ecol*. 2022;83:929–41.
3. Washington JW, Rankin K, Libelo EL, Lynch DG, Cyterski M. Determining global background soil PFAS loads and the fluorotelomer-based polymer degradation rates that can account for these loads. *Sci Total Environ*. 2019;651:2444–9.
4. Lenka SP, Kah M, Padhye LP. A review of the occurrence, transformation, and removal of poly-and perfluoroalkyl substances (PFAS) in wastewater treatment plants. *Water Res*. 2021;199:117187.
5. Ding R, Wu Y, Yang F, Xiao X, Li Y, Tian X, Zhao F. Degradation of low-concentration perfluorooctanoic acid via a microbial-based synergistic method: assessment of the feasibility and functional microorganisms. *J Hazard Mater*. 2021;416:125857.
6. Sobolewski TN, Trousdale RC, Gauvin CL, Lawrence CM, Walker RA. Nanomolar PFOA Concentrations Affect Lipid Membrane Structure: Consequences for Bioconcentration Mechanisms. *Environ Sci Technol*. 2025;59:709–18.
7. Huang D, Xu R, Sun X, Li Y, Xiao E, Xu Z, Wang Q, Gao P, Yang Z, Lin H, Sun W. Effects of perfluorooctanoic acid (PFOA) on activated sludge microbial community under aerobic and anaerobic conditions. *Environ Sci Pollut Res*. 2022;29:63379–92.
8. Smorada CM, Sima MW, Jaffé PR. Bacterial degradation of perfluoroalkyl acids. *Curr Opin Biotechnol*. 2024;88:103170.
9. Cao L, Liao Y, Su C, Tang L, Qi Z, Wei L, Wu J, Gao S. Effects of PFOA on the physicochemical properties of anaerobic granular sludge: Performance evaluation, microbial community and metagenomic analysis. *J Environ Manag*. 2022;313:114936.
10. Cheng X, Wei Y, Zhang Z, Wang F, He J, Wang R, Xu Y, Keerman M, Zhang S, Zhang Y, Bi J, Yao J, He M. Plasma PFOA and PFOS Levels, DNA Methylation, and Blood Lipid Levels: A Pilot Study. *Environ Sci Technol*. 2022;56:17039–51.
11. Mahmoudnia A, Mehrdadi N, Baghdadi M, Moussavi G. Change in global PFAS cycling as a response of permafrost degradation to climate change. *J Hazard Mater Adv*. 2022;5:100039.
12. Schuur EA, McGuire AD, Schädel C, Grosse G, Harden JW, Hayes DJ, Hugelius G, Koven CD, Kuhry P, Lawrence DM. Climate change and the permafrost carbon feedback. *Nature*. 2015;520:171–9.

13. Turetsky MR, Abbott BW, Jones MC, Anthony KW, Olefeldt D, Schuur EA, Grosse G, Kuhry P, Hugelius G, Koven C. Carbon release through abrupt permafrost thaw. *Nat Geosci.* 2020;13:138–43.
14. Wik M, Varner RK, Anthony KW, MacIntyre S, Bastviken D. Climate-sensitive northern lakes and ponds are critical components of methane release. *Nat Geosci.* 2016;9:99–105.
<https://doi.org/10.1038/ngeo2578>.
15. Kuhn M, Lundin EJ, Giesler R, Johansson M, Karlsson J. Emissions from thaw ponds largely offset the carbon sink of northern permafrost wetlands. *Sci Rep.* 2018;8:9535.
16. Mahmoudnia A, Mehrdadi N, Baghdadi M, Moussavi G. Change in global PFAS cycling as a response of permafrost degradation to climate change. *J Hazard Mater Adv.* 2022;5:100039.
<https://doi.org/10.1016/j.hazadv.2021.100039>.
17. Liu G, Zhang S, Yang K, Zhu L, Lin D. Toxicity of perfluorooctane sulfonate and perfluorooctanoic acid to *Escherichia coli*: Membrane disruption, oxidative stress, and DNA damage induced cell inactivation and/or death. *Environ Pollut.* 2016;214:806–15.
18. Wang C, Wu L, Zhang Y-T, Wei W, Ni B-J. Unravelling the impacts of perfluorooctanoic acid on anaerobic sludge digestion process. *Sci Total Environ.* 2021;796:149057.
19. Choi G, Kan E. Effects of perfluorooctanoic acid and perfluorooctane sulfonic acid on microbial community structure during anaerobic digestion. *Bioresource Technol.* 2024;393:129999.
20. Yu X, Nishimura F, Hidaka T. Impact of Long-Term Perfluorooctanoic Acid (PFOA) Exposure on Activated Sludge Process. *Water Air Soil Pollut.* 2018;229:134.
21. Qiao W, Xie Z, Zhang Y, Liu X, Xie S, Huang J, Yu L. Perfluoroalkyl substances (PFASs) influence the structure and function of soil bacterial community: greenhouse experiment. *Sci Total Environ.* 2018;642:1118–26.
22. Zhang X, Tang Z, Kabir MG, Wang Q, Dong C, Song X. Effects of concurrent PFASs and CAHs on microbial communities in soil and groundwater: an insight for their ecological niches and implications for bioremediation. *Environ Pollut* (2025) 127186.
23. Li L, Li W, Xue Y, Wang Z, Wang Y, Li R, Deng M, Xu Y, Peng L, Song K. Perfluorooctanoic acid effect and microbial mechanism to methane production in anaerobic digestion. *J Environ Manag.* 2024;369:122412.
24. Chen C, Fan X, Zhang X, Cao X, Liu C, Guo L, Chen Y. Global Distribution of PFAS and Their Impacts on Microbial Greenhouse Gas Emission and Mechanisms, *Environmental Pollution* (2026) 127714.
25. Jiao Y, Zou M, Yang X, Tsang YF, Chen H. Perfluorooctanoic acid triggers oxidative stress in anaerobic digestion of sewage sludge. *J Hazard Mater.* 2022;424:127418.
26. Kirschke S, Bousquet P, Ciais P, Saunois M, Canadell JG, Dlugokencky EJ, Bergamaschi P, Bergmann D, Blake DR, Bruhwiler L. Three decades of global methane sources and sinks. *Nat Geosci.* 2013;6:813–23.
27. Li X, Li Y, Gao D, Liu M, Hou L. Methane Production Linked to Organic Matter Molecule and Methanogenic Community in Estuarine Benthic Sediments, *J. Geophys. Res. Biogeosci.* 127 (2022) e2022JG007236.

28. Miner KR, Turetsky MR, Malina E, Bartsch A, Tamminen J, McGuire AD, Fix A, Sweeney C, Elder CD, Miller CE. Permafrost carbon emissions in a changing Arctic. *Nat Rev Earth Environ*. 2022;3:55–67.
29. Mar KA, Unger C, Walderdorff L, Butler T. Beyond CO₂ equivalence: The impacts of methane on climate, ecosystems, and health. *Environ Sci Policy*. 2022;134:127–36.
30. Liu H, Pan Z, Bai Y, Xu S, Wu Z, Ma J, Wang Z, Tian Z, Chen Y. Methanogens dominate methanotrophs and act as a methane source in aquaculture pond sediments. *Ecotoxicol Environ Saf*. 2024;288:117317.
31. Evans PN, Boyd JA, Leu AO, Woodcroft BJ, Parks DH, Hugenholtz P, Tyson GW. An evolving view of methane metabolism in the Archaea. *Nat Rev Microbiol*. 2019;17:219–32.
32. Yang S, Lv Y, Liu X, Wang Y, Fan Q, Yang Z, Boon N, Wang F, Xiao X, Zhang Y. Genomic and enzymatic evidence of acetogenesis by anaerobic methanotrophic archaea. *Nat Commun*. 2020;11:3941.
33. Guerrero-Cruz S, Vaksmaa A, Horn MA, Niemann H, Pijuan M, Ho A. Methanotrophs: discoveries, environmental relevance, and a perspective on current and future applications. *Front Microbiol*. 2021;12:678057.
34. Norwegian Environment Agency. Perfluorinated alkylated substances, brominated flame retardants and chlorinated paraffins in the Norwegian Environment - Screening 2013, 2013. <https://www.miljodirektoratet.no/globalassets/publikasjoner/m40/m40.pdf> (accessed June 18, 2026).
35. Zheng Y, Zhou Z, Ye X, Huang J, Jiang L, Chen G, Chen L, Wang Z. Identifying microbial community evolution in membrane bioreactors coupled with anaerobic side-stream reactor, packing carriers and ultrasonication for sludge reduction by linear discriminant analysis. *Bioresource Technol*. 2019;291:121920.
36. Louca S, Parfrey LW, Doebeli M. Decoupling function and taxonomy in the global ocean microbiome. *Science*. 2016;353:1272–7.
37. Huang H, Zhou L, Chen J, Wei T. ggcor: Extended tools for correlation analysis and visualization. *R Package Version*. 2020;0:9 7.
38. Peres-Neto PR, Legendre P, Dray S, Borcard D. Variation partitioning of species data matrices: estimation and comparison of fractions. *Ecology*. 2006;87:2614–25.
39. Zhang Y, Wang J, Tao J, Zhou Y, Yang H, Yang X, Li Y, Zhou Q, Jeppesen E. Concentrations of dissolved organic matter and methane in lakes in Southwest China: different roles of external factors and in-lake biota. *Water Res*. 2022;225:119190.
40. Nakagawa S, Schielzeth H. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecol Evol*. 2013;4:133–42. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>.
41. Carlsen ECL, Wei J, Lejzerowicz F, Trier Kjær S, Westermann S, Hessen DO, Dörsch P, Eiler A. Redox determines greenhouse gas production kinetics and metabolic traits in water-saturated thawing permafrost peat. *ISME Commun*. 2025;5:ycaf009.

42. Cai Y, Chen H, Yuan R, Wang F, Chen Z, Zhou B. Metagenomic analysis of soil microbial community under PFOA and PFOS stress. *Environ Res.* 2020;188:109838.
43. Wang W, Li Q, Zhang L, Cui J, Yu H, Wang X, Ouyang X, Tao F, Xu P, Tang H. Genetic mapping of highly versatile and solvent-tolerant *Pseudomonas putida* B6 -2 (ATCC BAA -2545) as a 'superstar' for mineralization of PAHs and dioxin-like compounds. *Environ Microbiol.* 2021;23:4309–25.
44. Chiriac FL, Stoica C, Iftode C, Pirvu F, Petre VA, Paun I, Pascu LF, Vasile GG. Nita-Lazar, Bacterial biodegradation of perfluorooctanoic acid (PFOA) and perfluorosulfonic acid (PFOS) using pure *Pseudomonas* strains. *Sustainability.* 2023;15:14000.
45. Silva AR, Duarte MS, Alves MM, Pereira L. Bioremediation of perfluoroalkyl substances (PFAS) by anaerobic digestion: effect of PFAS on different trophic groups and methane production accelerated by carbon materials. *Molecules.* 2022;27:1895.
46. Douglas MM, Lingappa UF, Lamb MP, Rowland JC, West AJ, Li G, Kemeny PC, Chadwick AJ, Piliouras A, Schwenk J, Fischer WW. Impact of River Channel Lateral Migration on Microbial Communities across a Discontinuous Permafrost Floodplain. *Appl Environ Microbiol.* 2021;87:e01339–21.
47. Cao L, Su C, Wu J, Wei L, Zhou Y, Tang L, Wang Q, Xian Y. Impact of perfluorooctanoic acid on treatment wastewater by a tandem AnSBR-ASBR system: performance, microbial community and metabolism pathway. *Process Saf Environ Prot.* 2022;164:373–83.
48. Roldán DM, Carrizo D, Sánchez-García L, Menes RJ. Diversity and effect of increasing temperature on the activity of methanotrophs in sediments of fildes Peninsula freshwater lakes, King George Island, Antarctica. *Front Microbiol.* 2022;13:822552.
49. Kravchenko I, Grouzdev D, Sukhacheva M, Minayeva T, Sirin A. Microbial communities of peaty permafrost tundra soils along the gradient of environmental conditions and anthropogenic disturbance in pechora river delta in the eastern european arctic. *Diversity.* 2023;15:251.
50. Oswald K, Milucka J, Brand A, Hach P, Littmann S, Wehrli B, Kuypers MMM, Schubert CJ. Aerobic gammaproteobacterial methanotrophs mitigate methane emissions from oxic and anoxic lake waters. *Limnol Oceanogr.* 2016;61:S101–18.
51. Khmelenina VN, Murrell JC, Smith TJ, Trotsenko YA. Physiology and biochemistry of the aerobic methanotrophs, in: *Aerobic Utilization of Hydrocarbons, Oils and Lipids.* Springer; 2018. pp. 1–25.
52. Sáenz JP, Grosser D, Bradley AS, Lagny TJ, Lavrynenko O, Broda M, Simons K. Hopanoids as functional analogues of cholesterol in bacterial membranes, *Proc. Natl. Acad. Sci. U.S.A.* 112 (2015) 11971–11976. <https://doi.org/10.1073/pnas.1515607112>
53. Bale NJ, Rijpstra WIC, Sahonero-Canavesi DX, Oshkin IY, Belova SE, Dedysh SN. Sinninghe Damsté, Fatty acid and hopanoid adaption to cold in the methanotroph *Methylovulum psychrotolerans*. *Front Microbiol.* 2019;10:589.
54. Van Winden JF, Talbot HM, Kip N, Reichart G-J, Pol A, McNamara NP, Jetten MS, den Camp HJO, Damsté JSS. Bacteriohopanepolyol signatures as markers for methanotrophic bacteria in peat moss. *Geochim Cosmochim Acta.* 2012;77:52–61.

55. Yang S, Anthony SE, Jenrich M. M.H. in 't Zandt, J. Strauss, P.P. Overduin, G. Grosse, M. Angelopoulos, B.K. Biskaborn, M.N. Grigoriev, D. Wagner, C. Knoblauch, A. Jaeschke, J. Rethemeyer, J. Kallmeyer, S. Liebner, Microbial methane cycling in sediments of Arctic thermokarst lagoons, *Global Change Biology* 29 (2023) 2714–2731. <https://doi.org/10.1111/gcb.16649>
56. Wu E, Wang K, Zhou J, Wang J, Liu Z, Yan H, Zhu X, Chen B. Fatty Acid Metabolic Impairment in Soil Microbes Induced by PFAS: Dependence on the Fluorocarbon Chain Length, Headgroups, and Ether Attached. *Environ Sci Technol*. 2025;59:5452–62. <https://doi.org/10.1021/acs.est.4c10654>.
57. Ramos JL, Duque E, Gallegos M-T, Godoy P, Ramos-González MI, Rojas A, Terán W, Segura A. Mechanisms of Solvent Tolerance in Gram-Negative Bacteria. *Annu Rev Microbiol*. 2002;56:743–68. <https://doi.org/10.1146/annurev.micro.56.012302.161038>.
58. Zhao Z, Li Y, Yu Q, Zhang Y. Ferroferric oxide triggered possible direct interspecies electron transfer between *Syntrophomonas* and *Methanosaeta* to enhance waste activated sludge anaerobic digestion. *Bioresource Technol*. 2018;250:79–85.
59. Chen C, Fang Y, Cui X, Zhou D. Effects of trace PFOA on microbial community and metabolisms: microbial selectivity, regulations and risks. *Water Res*. 2022;226:119273.
60. Hu F, Wang P, Li Y, Ling J, Ruan Y, Yu J, Zhang L. Bioremediation of environmental organic pollutants by *Pseudomonas aeruginosa*: Mechanisms, methods and challenges. *Environ Res*. 2023;239:117211.
61. Qian X, Huang J, Li X, Cao C, Yao J. Novel insights on ecological responses of short-and long-chain perfluorocarboxylic acids in constructed wetlands coupled with modified basalt fiber bio-nest. *Chemosphere*. 2024;365:143384.
62. Huang H, Lyu X, Xiao F, Fu J, Xu H, Wu J, Sun Y. Three-year field study on the temporal response of soil microbial communities and functions to PFOA exposure. *J Hazard Mater*. 2024;476:135008.
63. Senevirathna S, Krishna KB, Mahinroosta R, Sathasivan A. Comparative characterization of microbial communities that inhabit PFAS-rich contaminated sites: a case-control study. *J Hazard Mater*. 2022;423:126941.
64. Sun T, Wang F, Xie Y, Liu X, Yu H, Lv M, Zhang Y, Xu Y. Biochar remediation of PFOA contaminated soil decreased the microbial network complexity. *J Environ Chem Eng*. 2023;11:109239.
65. Zhang Y, Huang X, Zhang Z, Blewett J, Naafs BDA. Spatiotemporal dynamics of dissolved organic carbon in a subtropical wetland and their implications for methane emissions. *Geoderma*. 2022;419:115876.
66. Goodwin S, Zeikus JG. Ecophysiological adaptations of anaerobic bacteria to low pH: analysis of anaerobic digestion in acidic bog sediments. *Appl Environ Microbiol*. 1987;53:57–64. <https://doi.org/10.1128/aem.53.1.57-64.1987>.
67. You C, Jia C, Pan G. Effect of salinity and sediment characteristics on the sorption and desorption of perfluorooctane sulfonate at sediment-water interface. *Environ Pollut*. 2010;158:1343–7.

Figures

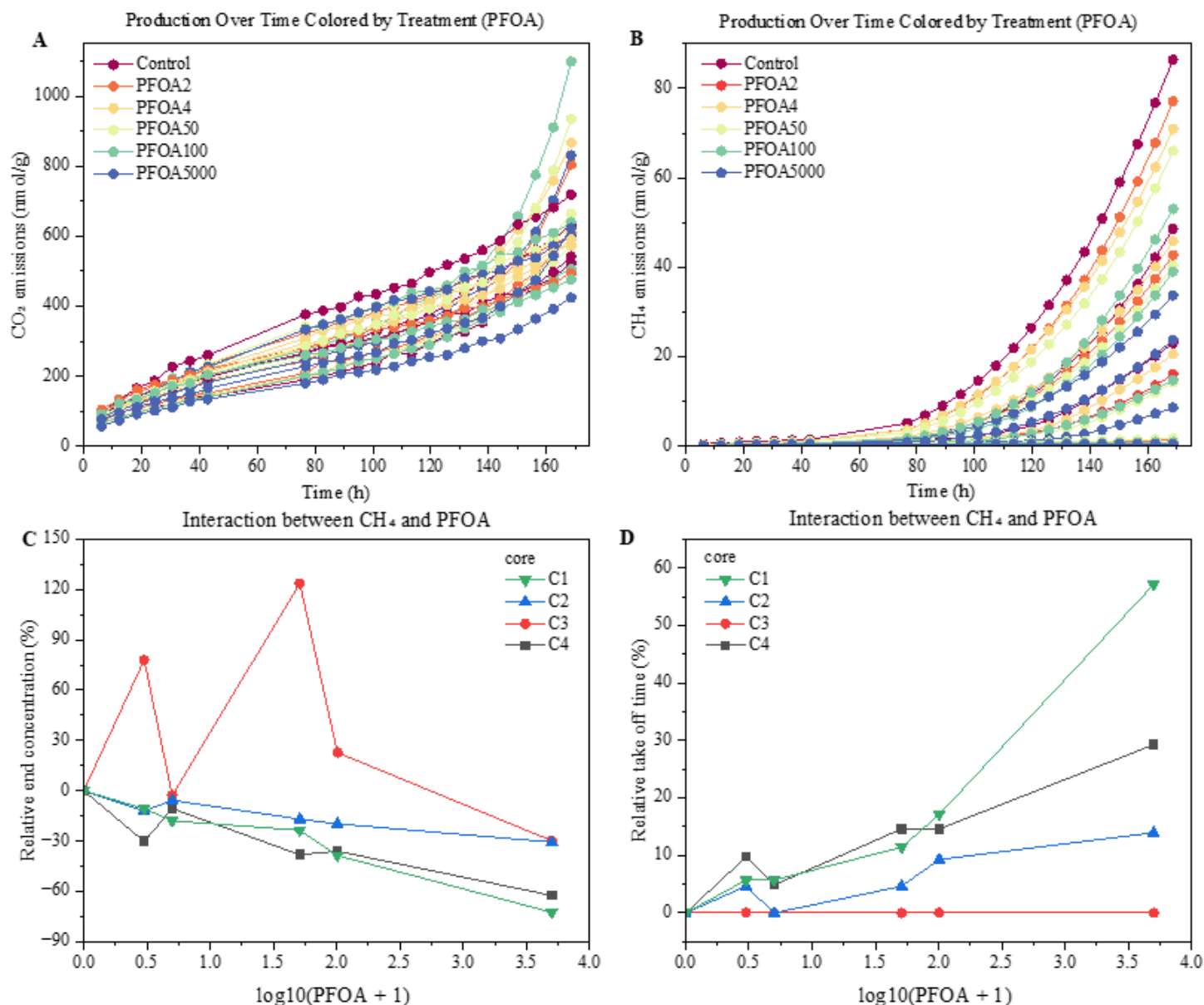


Figure 1

CO₂ and CH₄ accumulation curves and their descriptors under different PFOA concentrations based on mixed-effects model analysis. The colors indicate the various cores (C1-C4). (A) CO₂ accumulation curves, and (B) CH₄ accumulation curves under different PFOA treatments; (C) relative CH₄ end concentration (%) and (D) relative take-off time (%) across log₁₀(x+1)-transformed PFOA concentrations. Take-off time is defined as the time point at which CH₄ production rates exceeded a threshold defined by core-specific maximum production rates. To accurately reflect the continuous, non-linear relationship and prevent visual distortion, PFOA concentrations (x-axis in C and D) were transformed using log₁₀(x+1) to accommodate the control group (0 ng/g). To account for natural core-to-core variance, the response variables (y-axis in C and D) were normalized against each core's individual control providing the percentage change relative. The absolute values of CH₄ end concentrations and take-off time with

log₁₀(x+1)-transformed PFOA concentrations are provided in Supplementary Figure S3. Statistical significance based on mixed-effects model analysis is given in Table 1.

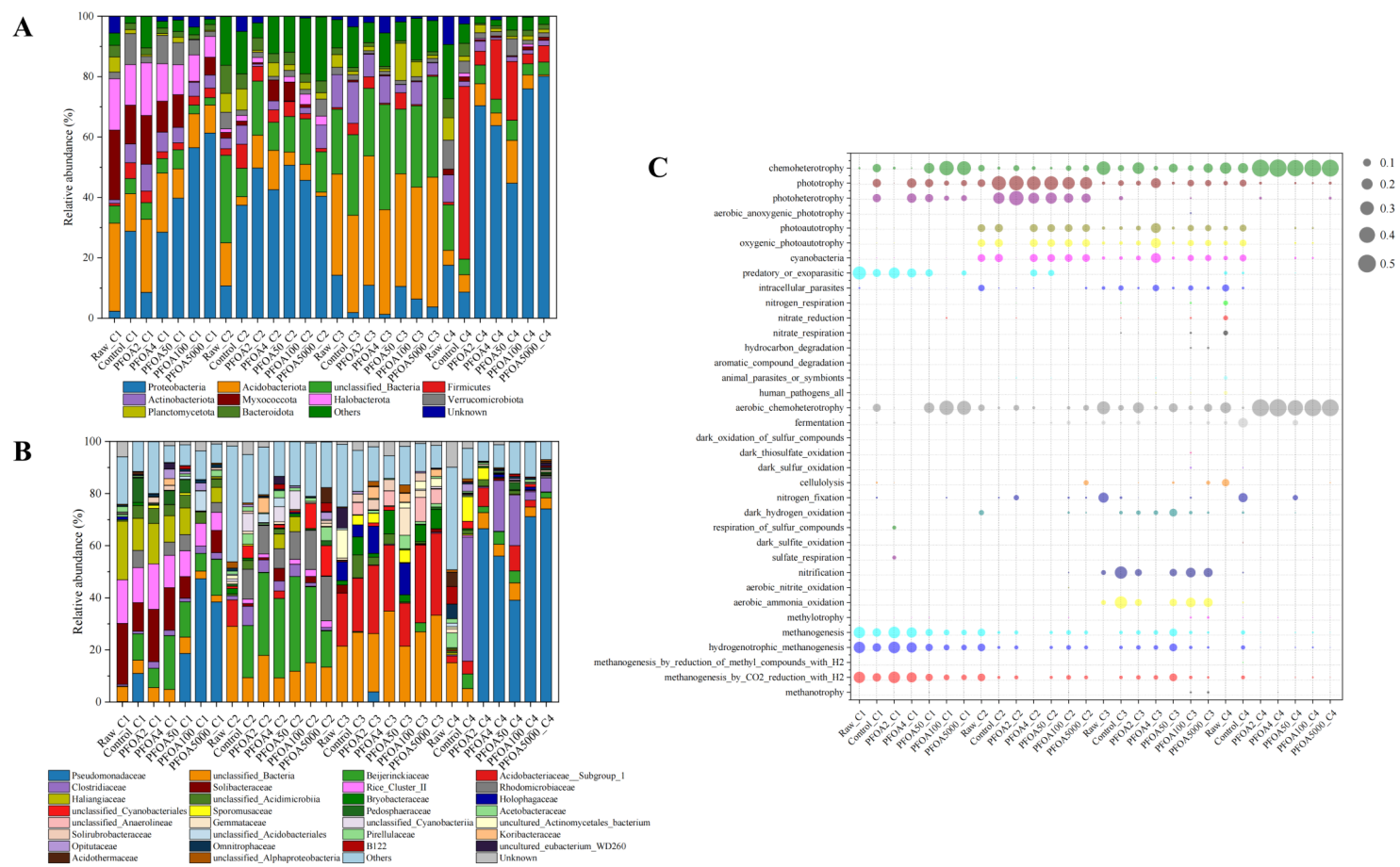


Figure 2

Relative abundance of dominant microbial taxa and metabolic functions under different PFOA concentrations averaged across four biological replicates or cores. C1-C4 indicate the four cores (biological replicates) taken from the thermokarst pond. (A) Relative abundance of the top 10 microbial phyla; (B) Relative abundance of the top 30 microbial families; and (C) Metabolic functions of microbial communities based on FAPROTAX functional annotation under different treatment conditions: raw (original samples), control (0 ng/g) and PFOA concentrations (2-5000 ng/g). The color of each bubble represents a specific metabolic function, and the bubble size indicates the relative abundance of that function within each PFOA treatment.

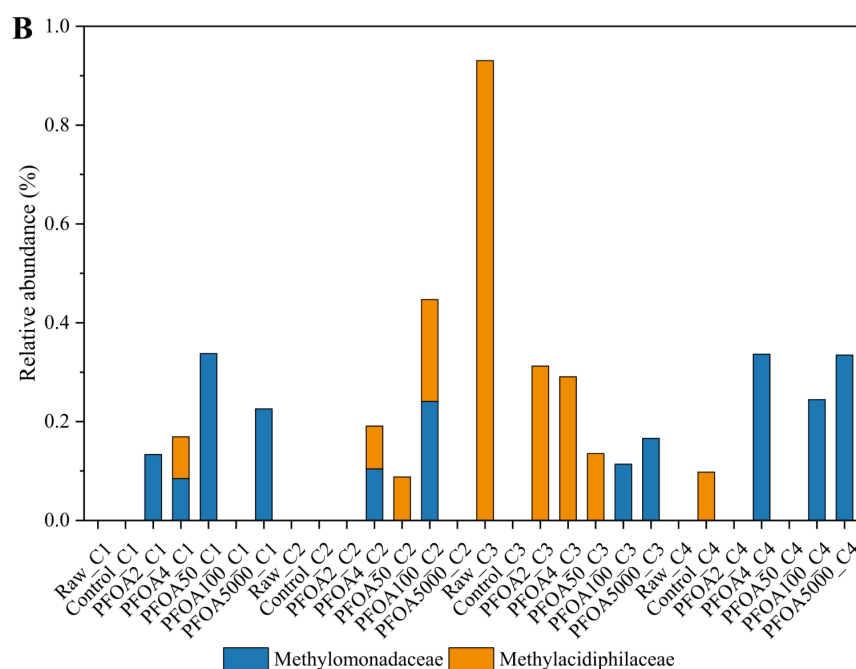
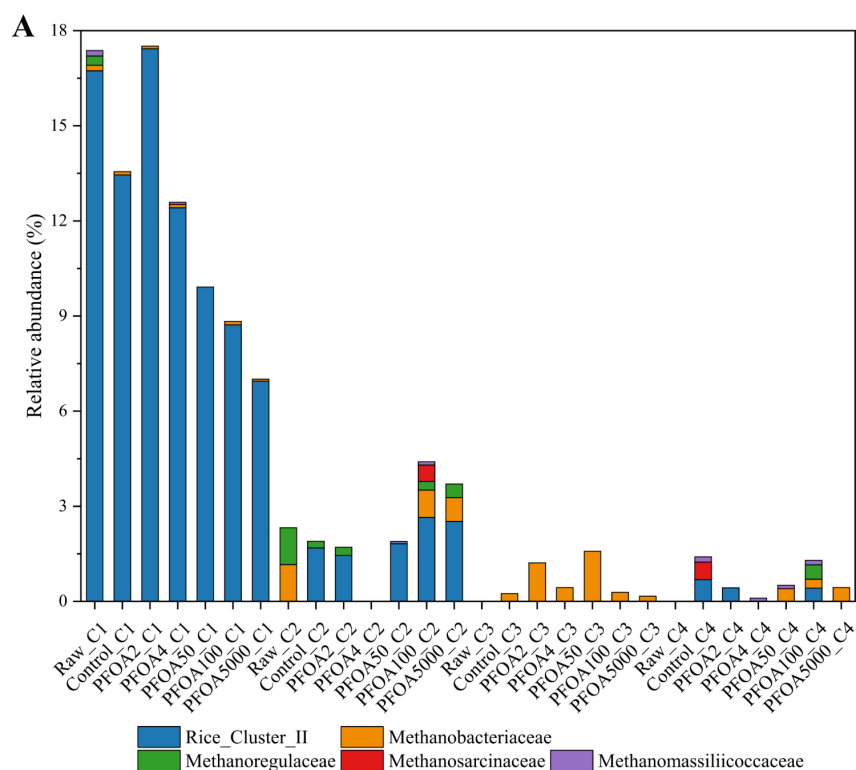


Figure 3

Community composition of CH₄ metabolism-related microorganisms under different PFOA concentrations averaged across four biological replicates or cores. C1-C4 indicate the four cores (biological replicates) taken from the thermokarst pond. (A) Relative abundance of methanogens; (B) Relative abundance of methanotrophs under different conditions: raw (original samples), Control (0

ng/g) and PFOA concentrations (2-5000 ng/g). Colors represent different microbial families within each functional group.

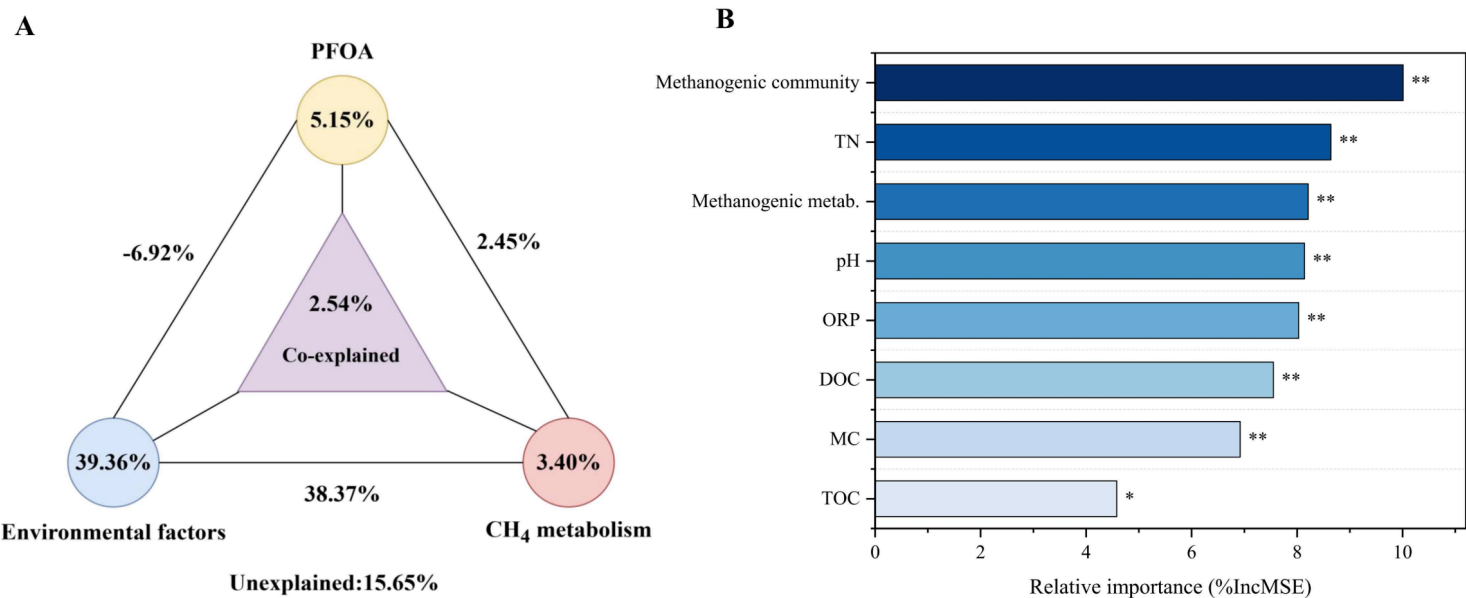


Figure 4

Key indicators of CH₄ emissions identified by VPA and random forest analyses. (A) VPA showing the relative contributions of PFOA, environmental factors, and CH₄-related metabolism to CH₄ emissions. Colored circles represent the three explanatory variables, and their overlapping areas indicate the proportions jointly explained. (B) Random forest analysis showing the relative importance of environmental and microbial indicators affecting CH₄ emissions. Asterisks indicate the significance levels (* $p < 0.05$; ** $p < 0.01$), and colors represent different variables according to their importance ranking.

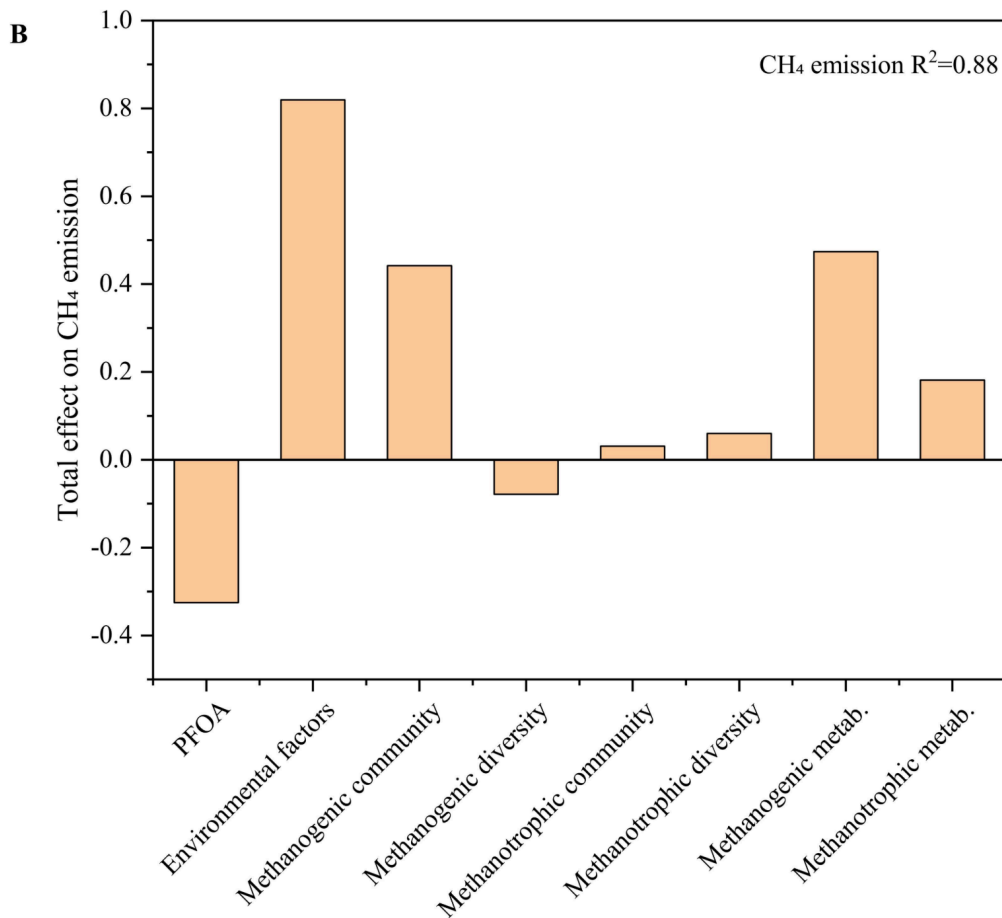
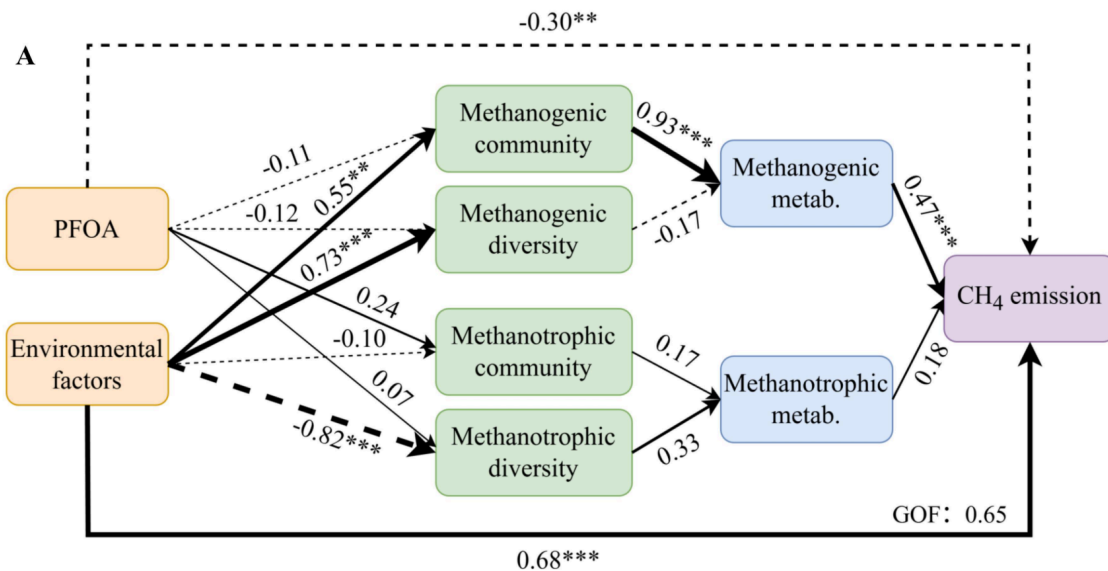


Figure 5

Direct and indirect effects driving CH₄ emissions under different PFOA contamination levels. (A) Path analysis diagram showing the direct and indirect effects of PFOA and environmental factors on CH₄ emissions through methanogenic and methanotrophic communities, diversity, and metabolic potentials. Solid lines indicate positive path coefficients, whereas dashed lines indicate negative coefficients. Arrow thickness corresponds to the strength of standardized path coefficients. Asterisks

indicate significance levels (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). (B) Bar plot of standardized total effects of major variables on CH₄ emissions, indicating the overall contribution of each variable. The direction of each bar indicates whether the relationship is positive or negative, and the bar length represents the effect size.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementalmaterials2.docx](#)