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Article *in* Journal of Environmental Sciences · July 2025

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PII: S1001-0742(25)00485-1
DOI: https://doi.org/10.1016/j.jes.2025.07.053
Reference: JES 4802

To appear in: *Journal of Environmental Sciences*

Received date: 15 April 2025
Revised date: 23 July 2025
Accepted date: 25 July 2025

Please cite this article as: Shuzhen Li , Xue Yan , Jean Claude Ndayishimiye , Alexey Smirnov , Andrey N. Tsyganov , Elena Nassonova , Natalia G. Mazei , Yuri A. Mazei , Jun Yang , Urban park metagenomics highlights sediments as a potential hotspot for CH_4 and N_2O emission across diverse habitats, *Journal of Environmental Sciences* (2025), doi: https://doi.org/10.1016/j.jes.2025.07.053

Highlights

- The diversity of CH_4 and N_2O cycling genes and associated microbiomes in urban park ecosystems was investigated.
- Both taxonomic and functional community compositions exhibited significant divergence across the five habitat types.
- Positive biodiversity-ecosystem-functioning relationships were observed across all habitats except sediments in the CH_4 cycling process.
- Sediments were identified as potential hotspots for CH_4 and N_2O emissions within the studied habitats.
- Dissolved oxygen, nitrogen, and carbon concentrations significantly influenced both microbial community composition and functional gene abundance.

Urban park metagenomics highlights sediments as a potential hotspot for CH_4 and N_2O emission across diverse habitats

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Abstract: Urban areas contribute the vast majority of greenhouse gas (GHG) emissions, and urban greenspaces, including urban parks, are being established to promote environmental health by mitigating GHG emissions. However, the diversity of CH_4 and N_2O cycling genes and microbiomes in urban park ecosystems remains poorly understood. Here, we sampled five types of habitats in subtropical urban parks, including soil, moss, tree hole, water, and sediment, to explore the microbial communities and microbially mediated CH_4 and N_2O cycling processes using metagenomic sequencing. We found strongly positive biodiversity-ecosystem-functioning (BEF) relationships in nitrogen cycling functions, as well as in CH_4 cycling, except in sediment, indicating the microbial community in

the sediment had reached function saturation for CH_4 cycling. CH_4 cycling was driven by a few specific microbial genera, whereas many microorganisms participated in the denitrification process. Microbes in sediment exhibited the highest CH_4 and N_2O metabolic potential among the five habitats, especially for methanogenesis and N_2O production processes. Significant positive correlations were observed between the *mcrA* and N_2O cycling genes, suggesting methanogenesis could be coupled with denitrification. Environmental factors, such as dissolved oxygen, total nitrogen, and total carbon greatly affected microbial community composition and functional gene families. These results highlight that pond sediments are an overlooked potential source of CH_4 and N_2O emissions, which may undermine the role of urban greenspace in reducing GHG emissions. Reducing nitrogen pollution and eutrophication is recommended to mitigate CH_4 and N_2O emissions from pond sediments in urban environments.

Keywords: Urbanization; Subtropical urban ponds; Methane; Methanogenesis; Nitrous oxide; Denitrification

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Introduction

Ongoing global warming has become a substantial environmental issue. The urban population is forecast to increase from 56 % in 2021 to 68 % by 2050 globally, and urban areas account for nearly 70 % of anthropogenic greenhouse gas (GHG) emissions (UN-HABITAT, 2024). Methane (CH_4) and nitrous oxide (N_2O) are potent GHG, with about 34 and 296 times greater than CO_2 in global warming potential (Morales et al., 2010; Yu et al., 2020). Rapid urbanization has profoundly changed biogeochemical carbon and nitrogen cycles and associated greenhouse gas fluxes (Delden et al., 2018). For example, urban rivers are identified as substantial sources of GHG emissions, as the fluxes of CH_4 and N_2O in urban rivers are 4.2–4.7 times higher

than that in non-urban rivers (Xu et al., 2024). Moreover, enhanced nutrient and pollutant input may increase urban GHG emissions (Li et al., 2021; Strokal et al., 2021). CH_4 and N_2O are essential components of biomolecules, and their cycling is an important biogeochemical process largely affected by microbial production and consumption. Therefore, disentangling causal links between microbial diversity, key functional genes, and GHG production potential is important in light of climate change.

Microbial-driven CH_4 cycling is a critical component of the global carbon cycle. The methanogenic population can be characterized through the methyl coenzyme-M reductase (*mcrA*) gene (Barbier et al., 2012). This enzyme is ubiquitous and unique in methanogens and can be a reliable marker gene for the detection of methanogens in a wide range of environments (Qian et al., 2022). The microbial oxidation process through methanotrophs is the only biological sink for CH_4 in the biosphere (Zeng et al., 2018). The particulate methane monooxygenase (*pmoA*) gene encodes the β-subunit of the particulate methane monooxygenase, which exists in most known methanotrophs and is an excellent marker for studying methanotrophs (Zeng et al., 2018). Additionally, denitrification is one of the most important microbial processes producing N_2O. Nitrite reductases (*nirK*/*nirS*) are gene markers for denitrifiers responsible for reducing nitrite to nitric oxide (Kuypers et al., 2018). Nitric oxide reductase subunit B (*norB*) is used to generate N_2O, and *norB* is widespread throughout the tree of life (Kuypers et al., 2018). The conversion of N_2O into harmless N_2 by N_2O reductase is the only well-recognized atmospheric N_2O sink, and the nitrous-oxide reductase (*nosZ*) gene encoding the terminal reductase of anaerobic N_2O respiration has been studied extensively (Jones et al., 2022; Yang et al., 2024). In recent years, the advancements in shotgun metagenome sequencing technology have facilitated comprehensive and detailed investigations into microbial communities as well as various functional cycle gene families, and significant positive correlations have been observed between these functional microorganisms, gene abundances, and

GHG production/consumption rates (He et al., 2015; Meyer et al., 2020; Rocca et al., 2014; Sierocinski et al., 2018). For example, both the *mcrA*/*pmoA* ratio and the *nirS* − *nosZ* gene abundance difference exhibited significant positive correlations with CH_4 and N_2O fluxes, respectively (Kong et al., 2019; Morales et al., 2010). These relationships suggest their utility as quantitative proxies for GHG emission monitoring. Moreover, a large number of taxa with high abundances within a community suggest a higher likelihood of a specific pathway or function emerging (Yan et al., 2024). These enable us to rapidly assess the potential for GHG emissions by molecular evidence in specific habitats.

The interconnections between climate change and urbanization are increasingly recognized (UN-HABITAT, 2024), and mitigating GHG emissions has long been recognized as a fundamental strategy for achieving sustainable urban development and combating climate change (Xu et al., 2024). Urban greenspaces, including urban parks, are being rapidly established worldwide. Urban greenspaces aim to contribute to environmental health by mitigating urban heat island effects, curbing pollution, and mitigating GHG emissions (Delgado-Baquerizo et al., 2021). Previous studies have demonstrated that urban park ecosystems support a wide variety of microbial taxa dwelling in various habitats associated with important biogeochemical processes (Delgado-Baquerizo et al., 2021; Li et al., 2023). Positive biodiversity-ecosystem-functioning (BEF) relationships have been widely described (Meyer et al., 2020; Sierocinski et al., 2018), while the specific microbial community and functional gene diversity involved in CH_4 and N_2O cycling, as well as the BEF relationships that exist within the various habitats of urban park ecosystems, remain unclear. Currently, only a few studies have focused on the potential for GHG production in urban greenspaces, and these studies are limited to soil (Stefaner et al., 2021; Yang et al., 2024). Urban greenspaces encompass various habitats, such as terrestrial (e.g., soil, moss, and tree hole) and aquatic (e.g., pond and sediment) habitats. Ponds are identified as important inland water GHG sources (Holgerson and

Raymond, 2016; Peacock et al., 2019). However, it is unclear whether ponds in urban greenspaces are potential GHG sources.

Characterizing the taxonomy and function of the microbiome involved in CH_4 and N_2O cycling is critical for evaluating their ecological consequences and global warming implications. In this study, microbial communities and microbially mediated CH_4 and nitrogen cycling processes, especially for CH_4 (i.e., *mcrA* and *pmoA*) and N_2O (*nirK*, *nirS*, *norB*, and *nosZ*) cycling gene families determining GHG emissions in urban park ecosystems, were studied through metagenomic sequencing. The objectives of this study were to (1) explore the diversity, spatial, and co-occurrence patterns of the microbial communities and functional genes involved in CH_4 and N_2O cycling; (2) elucidate the potential of different habitats as sources or sinks of GHG; (3) identify key factors determining the microbial communities and functional genes involved in CH_4 and N_2O cycling. We hypothesize a positive correlation between microbial diversity and functional gene abundance across distinct urban habitats. Furthermore, given the prevalent eutrophication of urban water bodies coupled with anaerobic conditions in sediments, we postulate that sedimentary environments may serve as critical hotspots for greenhouse gas production compared to other habitats.

1 Materials and methods

1.1 Sample collection and processing

All samples were taken in July 2020 in Xiamen, China. The average low and high temperatures in July were 26 ℃ and 36 ℃, respectively, with a monthly precipitation of 144 mm. A total of 90 samples were collected in six urban parks, including Shuanglongtan Park (SLT, 0.55 km^2), Dapingshan Park (DPS, 1.50 km^2), Huli Park (HL, 0.11 km^2), Shangli Park (SL, 0.48 km^2), Dalunshan Park (DLS, 0.36 km^2), and Xiangshan Park (XS, 10.38 km^2). The distances between different parks range from 4.71 km to 34.87 km. For each park, five representative habitats were chosen, including approximately 100 g of mosses, 100 g of surface soils (up to a depth of 5

cm), 100 g of detritus or litter in tree holes, 2.5 L of surface water (up to a depth of 5 cm), and 100 g of surface pond sediments (up to a depth of 0.5 m). Three samples were collected from each habitat in each park. Detailed sampling processes can be found in our previous studies (Li et al., 2023; Li et al., 2024a). Briefly, moss, soil, tree hole detritus, and sediment were collected using trowels. Moss was homogenized in 40 mL PBS and sequentially processed via shaking (180 r/min, 30 °C, 2 h), ultrasonication (10 min) and static sedimentation (2 h). Then, microbial biomass was captured on 0.22 μm polycarbonate filters (47 mm diameter, Millipore, Billerica, MA, USA). Water samples (500 mL) underwent sequential filtration through 200 μm (pre-filtration) and 0.22 μm polycarbonate membranes. All materials were stored at −80 °C prior to DNA extraction.

1.2 Environmental parameters

Water temperature (WT), pH, salinity, turbidity, and dissolved oxygen (DO) were measured in the field with a Hydrolab DS5 multiparameter water quality analyzer (Hach Company, Loveland, CO, USA). Concentrations of suspended solids (SS), total carbon (TC), total nitrogen (TN), total organic carbon (TOC), ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), total phosphorus (TP) and phosphate phosphorus (PO_4-P) from water samples were determined according to standard methods (Liu et al., 2019). Chlorophyll-*a* (Chl-*a*) was measured by a PHYTO-PAM Phytoplankton Analyzer (Heinz Walz GmbH, Effeltrich, Germany). Moisture of sediment was obtained by the oven-drying method. TC and TN of sediment samples were also measured according to standard methods (Abdullah Al et al., 2022).

Since the ponds in urban parks are very shallow and the sediments can be seen directly, the transparency cannot be calculated accurately. As a result, it is impossible to use the classical trophic state index (TSI) to calculate the degree of eutrophication. We considered TN > 1.4 mg/L, TP > 0.05 mg/L, and Chl-*a* > 30 μg/L as a

hypereutrophic threshold (Liang et al., 2020). Additionally, the trophic state of Chl-*a* was assessed according to the Carlson TSI method to assess our results using the Eq. (1) (Carlson, 1977):

$$TSI\ (Chl\text{-}a) = 9.81 \times Ln\ (Chl\text{-}a) + 30.6 \quad (1)$$

Generally, $0 \leq TSI < 30$ is oligotrophic, $30 \leq TSI < 40$ is oligo-mesotrophic, $40 \leq TSI < 50$ is mesotrophic, $50 \leq TSI < 60$ is light eutrophic, $60 \leq TSI < 70$ is middle eutrophic, and $70 \leq TSI \leq 100$ is hypereutrophic (Yang et al., 2012).

1.3 Metagenomic sequencing, quality control, and assembly

Microbial DNA was extracted by the ALFA-SEQ Advanced Soil DNA Kit, and the ALFA-SEQ DNA Library Prep Kit was used to construct dual-indexed DNA sequencing libraries according to the manufacturer's recommendations. Metagenomic shotgun sequencing was performed on the MGI-SEQ-T7 platform with paired-end reads (PE150) with all 90 samples. For each sample, sequencing yielded more than 20 GB of raw data. Metagenomic sequencing data information was summarized in **Appendix A Table S1**. Trimmomatic was used for quality control (LEADING: 3, TRAILING: 3, SLIDINGWINDOW: 5:20, MINLEN: 50) (Bolger et al., 2014). Then, the high-quality clean reads were *de novo* assembled into contigs using MEGAHIT (k-min 35, k-max 95, k-step 20) (Li et al., 2015). Only contigs ≥ 500 bp were retained for subsequent analysis.

The open reading frames (ORFs) of all the assembled contigs were predicted using Prodigal (Hyatt et al., 2010), and only ORFs ≥ 90 bp were kept. Linclust (-e 0.001 -min-seq-id 0.9 -c 0.8) was applied to obtain Unigenes (non-redundant gene catalog) by clustering ORFs (Steinegger and Söding, 2018). Clean sequencing data was aligned with Unigenes through BBMap (Brain, 2014), to calculate the Transcripts Per Million (TPM) value of each Unigene in samples. These Unigenes were further aligned against the NCBI-NR database by BLASTp (-e 0.00001) to receive taxonomic annotation information. For microbial functional annotation, Unigenes were searched

against several manually curated databases, including the CH_4 cycling database (MCycDB) (Qian et al., 2022) and nitrogen cycling database (NCycDB) (Tu et al., 2019) using DIAMOND BLASTx (-e 0.00001) (Buchfink et al., 2015). To reduce false positives, the functional outputs were filtered with a hit length ≥ 25 amino acids and an identity ≥ 30 % (Liu et al., 2023). The obtained functional profiles of CH_4 and nitrogen cycling gene families were applied to the subsequent analysis. The representative key gene families were selected for further analysis, including *mcrA* and *pmoA* for methanogenesis and aerobic oxidation of CH_4, respectively, as well as *nirS*, *nirK*, *norB*, and *nosZ* for denitrification related to the formation and accumulation of N_2O.

1.4 Statistical analysis

Permutational multivariate analysis of variance (PERMANOVA) was used to evaluate the importance of variables affecting microbial community and functional gene families using the "adonis2" function in R. The α-diversities of microbial taxonomic community and functional gene families, including richness, Shannon index, and Pielou evenness, were calculated using the "vegan" package (Oksanen et al., 2024). A linear regression was applied to fit the community functional and taxonomic richness relationship. We proposed that functional redundancy for a specific microbial process within a given habitat was achieved when its functional richness plateaus at a stable maximum despite continued increases in microbial taxonomic richness, indicating decoupled dynamics between community taxonomic composition and functional capacity. Functional redundancy was characterized by calculating the proportion of each gene family in different habitats. A higher proportion of a gene family indicates a higher level of functional redundancy (Liu et al., 2021). Non-metric multidimensional scaling (NMDS) analyses based on the Bray-Curtis dissimilarity matrix, along with analysis of similarity tests (ANOSIM), were applied to explore the taxonomic and functional composition differences among

habitats. Distance-decay relationship (DDR) was performed based on both the similarity of microbial taxonomic composition and gene families with increasing geographical distance.

Fast expectation-maximization microbial source tracking (FEAST) analysis was conducted to track potential source habitats of microbial taxonomic and functional compositions through the "FEAST" package (Shenhav et al., 2019), and the result was visualized by using the "circlize" package (Gu et al., 2014). Relationships among functional gene families, corresponding microbial taxa, as well as habitats were shown by the "ggalluvial" package (Brunson, 2020). The importance of environmental factors to the taxonomic communities and functional gene families was assessed through the Mantel test using the "LinkET" (Huang, 2021) package and redundancy analysis (RDA) using the "rdacca.hp" package (Lai et al., 2022), respectively. Variation partition analysis (VPA) was performed to disentangle the effect of environmental variables and geographical distance on the microbial taxonomic and functional compositions.

To explore how the microorganisms and key genes involved in CH_4 and N_2O cycling processes interact in different habitats, co-occurring network analyses were performed. From each habitat, the top 12 microbial genera with the highest abundances, CH_4, and nitrogen cycling gene families were integrated into a table to construct the network. Significant Spearman correlation coefficients ($|r| \geq 0.6$; $P < 0.05$) were kept, networks were constructed, and network properties were calculated by the "ggClusterNet" package (Wen et al., 2022). Networks were visualized in Gephi 0.9.

2 Results

2.1 Diversity of microbial taxonomic communities and functional gene families

A total of 90 metagenomic datasets were obtained, covering six parks and five distinct habitats (**Appendix A Table S1**). Environmental parameters exhibited significant

differences among parks (**Appendix A Fig. S1**). According to the concentrations of TN, TP, and Chl-*a*, the water bodies in half of the parks were in a hypereutrophic state (**Appendix A Fig. S2**). PERMANOVA demonstrated that habitat type, rather than parks, was the man factor which controlled the differences in microbial community composition and functional profiles related to CH_4 and nitrogen metabolism (**Appendix A Table S2**).

The microbial richness in water bodies was relatively low as compared to the other habitats (**Fig. 1a**). The Shannon diversity and Pielou evenness index of microbial communities in soil and water were the lowest (**Appendix A Fig. S3a and b**), showing that a significant proportion of their microorganisms belonged to rare species. There was a significant ($P < 0.05$) positive correlation between functional and taxonomic richness (except in sediments), showing that the functionality of microbial communities enhanced by a greater taxon number (**Fig. 1b, Appendix A Fig. S4**). The surface water and bottom sediments exhibited the highest and lowest functional richness, Shannon diversity, and evenness, respectively (**Fig. 1c, Appendix A Fig. S3c and d**), reflecting the significant differences in biogeochemical cycling genes between water and sediment. In contrast, among the terrestrial ecosystems, soil typically displayed higher functional diversity as compared to tree holes and mosses (**Appendix A Fig. S3c and d**). Microorganisms residing in sediments exhibited significantly higher functional redundancy in CH_4 and nitrogen metabolisms compared to other habitats (**Fig. 1d**). The microbial community was dominated by Proteobacteria and *Sphingomonas* at the phylum and genus levels, respectively (**Appendix A Fig. S5**). Actinobacteria and Planctomycetes were more abundant in water, while Chloroflexi was enriched in sediment.

2.2 Spatial patterns, structure and connectivity of microbial communities

DDR analyses showed both taxonomic and functional similarity decreased with increasing spatial distance (**Fig. 2a and b**). The slopes of taxonomic composition

variation consistently exceeded those of functions across various habitats, with an exception of moss habitat, indicating that microbial functions were more spatially conserved compared to their compositions. The community turnover rates in water, tree hole, and sediment were faster than those observed in moss and soil at the level of community taxonomic composition, overall functionality, or specific CH_4 and nitrogen cycling families (**Fig. 2a and b, Appendix A Fig. S6**). NMDS analyses further revealed that terrestrial habitats (i.e., moss, soil, and tree hole), water, and sediment exhibited significant differences in both taxonomic and functional communities (**Fig. 2c and d, Appendix A Fig. S7**). FEAST analysis confirmed a certain degree of microbial taxonomic and functional connectivity among habitats (**Fig. 2e and f**). Sediments accounted for the highest proportion (19.8 %) as a source of microbial taxa for other habitats, while five habitats exhibited comparable percentages (11.3 %–12.1 %) as sources of microbial CH_4 and nitrogen cycling functions for other habitats.

2.3 Key microbial taxa and gene families involved in GHGs

Aceticlastic methanogenesis and the central methanogenic pathway were the main pathways for methanogenesis, while the serine cycle was the main aerobic oxidation of methane pathway exhibiting the highest abundances (**Fig. 3a**). Microbes in sediment exhibited the highest CH_4 metabolic potential, especially for methanogenesis processes, as evidenced by the highest *mcrA*/*pmoA* ratio (**Fig. 3c**). For nitrogen cycling pathways, organic degradation and synthesis were greatly enriched, followed by processes such as denitrification and dissimilatory/assimilatory nitrate reduction (**Fig. 3b**). Sediment was also a hotspot for nitrogen cycling, showing the highest *nirS* and *norB* gene abundances, as well as the highest *nirS* – *nosZ* value (**Fig. 3d**). The production of CH_4 and N_2O showed the highest functional potentials in sediment among the five types of habitats.

A total of 8 and 13 genera harbored *mcrA* and *pmoA*, respectively, while 76 (*nirS*), 176 (*nirK*), 137 (*norB*), and 116 (*nosZ*) genera were associated with N_2O cycling (**Appendix A Table S3**). Proteobacteria exhibited the highest abundance and were the most important microbial group responsible for CH_4 and N_2O cycling (**Fig. 4a and b**). Specifically, the *mcrA* gene was mainly from Euryarchaeota and unknown phyla, as well as *Methanobacterium*, *Methanocella*, *Methanosarcina*, and *Methanothrix* genera in sediment. The *pmoA* gene was affiliated with Proteobacteria, as well as *Methylocapsa*, *Methylocystis*, and *Methylomonas* genera belonging to sediment and water (**Fig. 4a and c**). N_2O cycling genes and microbial taxa showed complex relationships among various habitats (**Fig. 4b and d**). Actinobacteria from soil and Proteobacteria from soil, sediment, and tree hole harbored high abundances of *nirK*, while Proteobacteria from sediment enriched high abundances of *nirS*. Moreover, both *norB* and *nosZ* were also abundant for Proteobacteria from soil, sediment, and tree hole (**Fig. 4b**). At the genus level, *Bradyrhizobium* from soil and *Methylocystis* from water were the main source of *nirK*; *Janthinobcterium*, *Bradyrhizobium*, and *Mycobacterium* from tree hole contributed a lot to *norB*; *Bradyrhizobium* and *Mesorhizobium* from soil had high abundances of *nosZ* (**Fig. 4d**).

Overall, sediments were enriched with various genera carrying *mcrA* with high abundances, and aquatic habitats consisted of the highest *Methylobacter*, *Methylocaldum*, and *Methylocystis* carrying *pmoA* (**Appendix A Fig. S8**). Terrestrial habitats also exhibited higher abundances of N_2O cycling genera compared with aquatic habitats, and microorganisms with higher abundances in sediment played more important roles in both CH_4 and N_2O cycling than those in water (**Appendix A Fig. S8**).

2.4 Co-occurrence networks between microbes and functional gene families

To further explore possible interactions between microbes and functional profiles, co-occurrence networks were constructed incorporating abundant microbial taxa, CH_4

and N_2O cycling genes. Sediment and water exhibited the most network nodes (30), while tree hole (109) and water (66) had the most and least links (**Appendix A Table S4**). All habitats displayed strong microbial collaborations, as most of the links were positive (75.76 %–88.07 %). Networks with high average clustering coefficient (avgCC) (0.686–0.805), low average path distance (GD) (1.202–1.797), and high relative modularity (RM) (0.516–1.488) demonstrated a tight relationship within microorganisms and their function genes. Moreover, networks in aquatic habitats showed lower avgCC, higher GD, and higher RM values compared with terrestrial habitats. We selected the 12 most abundant genera for network construction, most of which appeared in the network and showed significant and strong positive correlations ($r \geq 0.6$; $P < 0.05$) with functional nodes, indicating that high-abundance species were important for maintaining ecological functions (**Fig. 5**). *mcrA* and *pmoA* showed no associations, and *mcrA* was specifically positively correlated with microbial genera involved in CH_4 cycling. N_2O cycling genes also showed positive correlations with genera involved in CH_4 cycling, suggesting methanogenesis could be indirectly coupled with denitrification processes in urban parks. Meanwhile, associations between *pmoA* and abundant genera, as well as between *pmoA* and genera involved in N_2O cycling, contributed most of the negative correlations in all networks.

2.5 Effects environmental variables on microbial taxonomy and function

RDA results demonstrated that water temperature, dissolved oxygen, and nitrogen were significantly correlated with the composition of microbial communities and functional gene families in the water (**Fig. 6**). In sediment, moisture, TN, and TC were all important for both community compositions and functions. The Mantel test indicated significant correlations between microbial community and dissolved oxygen, nitrogen, and TC in water and sediment environments. Dissolved oxygen significantly affected CH_4 and nitrogen cycling families, particularly these CH_4 and

N_2O cycling genes. VPA results demonstrated the combined effect of environmental variables and spatial distance played the most important roles in both microbial taxonomic and functional compositions in water (**Appendix A Fig. S9**). Environmental variables were a stronger predictor than spatial distance for microbial taxonomic and functional communities in water, while the impact of space was higher in microbial community and nitrogen cycling in sediment.

3 Discussion

3.1 Taxonomic and functional diversity of microbial communities

We systematically explored the taxonomic and functional structure of microbial communities through metagenomic sequencing, covering five different habitats in six urban parks (**Appendix A Table S1**). Our findings indicate that microbial community composition varied greatly across habitats and spatial scales with the former having greater effects (**Fig. 2, Appendix A Figs. S6 and S7, Appendix A Table S2**). These results corroborate well with those obtained based on previous amplicon sequencing data (Li et al., 2023; Li et al., 2024a). We found strongly positive BEF relationships in nitrogen and CH_4 cycling functions in all habitats, except in sediment (**Fig. 1b, Appendix A Fig. S4**), suggesting a decisive effect of microbial diversity on ecological functions (Schnyder et al., 2018). Reducing taxonomic richness in methanogenic communities can effectively decrease CH_4 production, and this impact remains robust to variations in taxonomic composition in natural communities (Sierocinski et al., 2018). CH_4 cycling is distributed among a few specific microbial genera (Evans et al., 2019) as evidenced by functional genes related to methanogenesis and methane oxidation from our results (**Appendix A Table S3**). In contrast, many microorganisms can participate in nitrogen cycling, for instance, in the denitrification process. The microbial community in the sediment reached function saturation for CH_4 cycling, as it exhibited the highest functional diversity and functional redundancy among five habitats (**Fig. 1c and d**). Approximately 10 %–20

% of microbial taxa and functions are interconnected among the habitats (**Fig. 2e and f**). This connectivity not only contributes to the maintenance of biodiversity but also facilitates the spread of critical ecological functions (Li et al., 2023).

3.2 CH_4 cycling genes and microbes in urban park habitats

Archaeal CH_4 metabolism plays a crucial role in the global carbon cycle, as CH_4 generated by this path constitutes over a half of the total CH_4 produced on Earth annually (Kirschke et al., 2013). Archaea are the main methanogenesis and methane oxidation group in urban park ecosystems, especially in pond sediment (**Fig. 4, Appendix A Fig. S8**). Generally, methanogens contain hydrogenotrophic (from H_2 and CO_2), acetoclastic (from acetate), and methylotrophic (from methylated compounds) methanogens (Evans et al., 2019), and acetoclastic methanogens can be dominant in freshwater wetlands. We observed that acetoclastic methanogenesis was the main process followed by weak hydrogenotrophic and methylotrophic methanogenesis (**Fig. 3**). Among these identified methanogens, the metabolically versatile *Methanosarcina*, which is capable of utilizing all three methanogenic pathways, has been confirmed to have a substantial association with CH_4 production (He et al., 2015; Sierocinski et al., 2018). Moreover, hydrogenotrophic *Methanoregula* and *Methanobacterium* genera were also abundant in pond sediment (**Appendix A Fig. S8**).

Methanotrophs can be classified as type I (Gammaproteobacteria) and type II (Alphaproteobacteria) based on ultrastructure, cell morphology, and carbon assimilation pathways (He et al., 2015). Type I, including *Methylobacter*, *Methylomonas*, *Methylococcus*, and *Methylocaldum*, were found to be dominant in pond sediment. Type I plays a dominant role in numerous habitats characterized by high CH_4 emissions, and *Methylobacter* serves as an indicator of environments possessing a strong CH_4 source (Krause et al., 2012). Many type I methanotrophs were observed in microbial communities and networks (**Fig. 5**), indicating their

pronounced influence on community cooperation (Zhang et al., 2019). Type II methanotrophs *Methylocystis* were prevalent in water (**Fig. 4, Appendix A Fig. S8**). Moreover, methane oxidation is greatly associated with type I methanotrophs, especially *Methylobacter* in a peatland environment (Deng et al., 2016; Zeng et al., 2018). Diversities and abundances of methanogens and methanotrophs are positively associated with CH_4 production and oxidation, and their community composition is critical to CH_4 cycling processes (Meyer et al., 2020). Hence, if the CH_4 produced in the sediments cannot be completely oxidized by methanotrophs in water, it will be released into the atmosphere.

Environmental variables such as dissolved oxygen and temperature in water, and total carbon and total nitrogen in sediment were important factors for microbial CH_4 cycling, especially for methanogenesis and methane oxidation (**Fig. 6**). Besides these factors, some other environmental variables have been found to play a significant role in CH_4 cycling, such as salinity, pH, total organic carbon, and acid volatile sulphide in the marsh or mangrove sediment (Qian et al., 2023; Yu et al., 2020), suggesting CH_4 cycling subgroups are specifically adapted to particular environments (Zhang et al., 2019). Most of the CH_4 cycling genera had low abundances (<1 %) (**Appendix A Fig. S8**), demonstrating the importance of rare species in determining CH_4 production (Schnyder et al., 2018). Overall, the highest *mcrA*/*pmoA* ratio in sediment indicates a high CH_4 fluxes in park sediment (Kong et al., 2019).

3.3 N_2O cycling genes and microbes in urban park habitats

Denitrification was the most abundant nitrogen cycling pathway except for organic degradation and synthesis (**Fig. 3**), indicating that urban parks are hotspots for microbial denitrification. More microbial taxa carried the *nirK* than *nirS* gene (**Appendix A Table S3**), consistent with previous findings that microorganisms containing *nirK* are predominant in these environments (Bothe et al., 2000; Jones et al., 2014). Both *nirK* and *nirS* display positive correlations with process rates, and

communities with higher diversity or abundance carrying *nosZ* are correlated with lower N_2O emissions (Rocca et al., 2014). A previous study has demonstrated that *nirS* – *nosZ* gene abundance can serve as a proxy for N_2O emissions (Morales et al., 2010). From our results, this proxy value was negative in terrestrial habitats, whereas it was positive in aquatic environments, especially in sediment (**Fig. 3**), suggesting that sediment is the most important N_2O source among the five types of habitats.

Microbial communities are the predominant source of N_2O and the proportion of N_2O producers and consumers play important roles in determining N_2O emissions (Jones et al., 2022). Proteobacteria and *Bradyrhizobium* were the dominant microbial taxa and the primary subgroups responsible for N_2O production and consumption in our study (**Fig. 4, Appendix A Fig. S8**), consistent with previous findings in soil (Jones et al., 2014; Nelson et al., 2016). N_2O cycling genes and genera were positively correlated with each other, and total carbon, total nitrogen, and moisture were important factors for microbially mediated N_2O production and consumption in sediment (**Figs. 5 and 6**). Other studies have demonstrated that pH, temperature, organic carbon, and total carbon are determinants of N_2O emissions through field experiments or global meta-analysis (Jones et al., 2022; Wang et al., 2017; Yang et al., 2024). Additionally, various anthropogenic impacts, such as alterations in land use types, fertilization of urban landscapes, and disposal of urban waste, have been regarded as significant factors influencing urban N_2O emissions (Stefaner et al., 2021). We found geographical distance was more important in shaping microbial communities and nitrogen cycling gene families in sediment (**Appendix A Fig. S9**), partly due to the limited number of environmental variables measured there, as the residuals accounted for about half of the community variation. At a continental scale, both environmental factors and geographical distance affect N_2O cycling microbial communities and N_2O emissions (Yang et al., 2024). Small lentic water systems have been revealed to play a vital role as hotspots for N_2O emissions through their sensitive response to terrestrial nitrogen inputs caused by global change or anthropogenic

activities (Li et al., 2024b), suggesting that more attention should be paid to emerging small inland water systems in cities, such as urban ponds.

3.4 Coupling of CH_4 and N_2O cycling and future implications

Significant positive correlations were observed between the *mcrA* gene family and *nirK*/*nirS*/*nosZ*, suggesting methanogenesis could be coupled with denitrification (**Fig. 5**). This coupling has been identified frequently, such as in rice paddy fields and mangrove sediment (Chen et al., 2024; Qian et al., 2023). Additionally, positive relationships between CH_4 oxidation and denitrification activities and genes across various climatic regions are observed (Chen et al., 2024). Inconsistent effects, including stimulation or inhibition, have been observed in methane oxidation activity in response to nitrogen amendments, which may be attributed to the specific influence of microbial community composition or nitrogen concentration (Ho et al., 2012). Different microbial methane oxidation taxa respond variably to nitrogen availability, suggesting their varying degree of tolerance to or dependency on nitrogen.

Most of the water bodies in urban parks were under hypereutrophic states with high concentrations of nitrogen, phosphorus, and Chl-*a*, which not only influence microbial community taxonomic composition but also significantly affect CH_4 and N_2O cycling gene abundances (**Fig. 6, Appendix A Fig. S2**). Environmental factors greatly shape CH_4 and nitrogen cycling communities, suggesting the potential to reduce GHG emissions by regulating local environmental conditions. Furthermore, global surveys have found high emissions of CH_4 and N_2O in urban rivers, which are closely associated with widespread eutrophication and altered nutrient cycling (Li et al., 2021; Xu et al., 2024). Ponds in urban greenspaces, as emerging urban aquatic environments, showed great potential for being sources of CH_4 and N_2O in pond sediments compared to other terrestrial habitats. Small ponds contribute largely to inland water GHG emissions, which may be due to shallow waters, high nutrients, and high sediment and edge-to-water volume ratios (Holgerson and Raymond, 2016;

Peacock et al., 2019). In the future management of urban ponds, increasing the mobility of water bodies, intermittently draining the pond, and changing the hydraulic design to minimize backwater area may be beneficial in reducing GHG emissions (He et al., 2015; Li et al., 2021; Peacock et al., 2019). Additionally, it is crucial to prioritize the reduction of nitrogen and phosphorus concentrations in ponds and sediments, thereby mitigating the degree of eutrophication in the entire aquatic environment, enhancing water quality, and maintaining adequate dissolved oxygen levels in the water bodies, thereby contributing to the effective diminishment of CH_4 and N_2O emissions.

4 Conclusions

The expansion of urban green spaces, including parks, represents a critical strategy for mitigating GHG emissions and enhancing urban livability. Our study demonstrated that habitat-specific characteristics predominantly regulate GHG dynamics in these ecosystems. Notably, urban park ponds were identified as underappreciated sources of CH_4 and N_2O emissions, with sediment microbiomes exhibiting strong linkages to eutrophication risks. While metagenomic sequencing revealed functional gene profiles associated with CH_4 and N_2O cycling, it inherently captures both active and dormant microbial communities, thereby reflecting potential rather than actual process rates. Furthermore, the detected genetic potential requires validation through substrate availability assessments and *in situ* gas flux measurements, as environmental conditions ultimately govern gas production/consumption dynamics. Our findings nevertheless underscore the necessity of habitat-tailored management strategies, including eutrophication control and sediment remediation. Future studies should integrate direct GHG flux monitoring with multi-omics approaches (e.g., transcriptomics) to resolve microbial activity patterns and improve emission models for urban aquatic ecosystems.

Data availability statement

Metagenomic sequencing data have been uploaded to the public NCBI Sequence Read Archive (SRA) (PRJNA1192737) and NODE database (OEP00005379), respectively.

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.

Author contributions

Shuzhen Li: Conceptualization, Data curation, Formal analysis, Methodology, Software**,** Resources, Visualization, Writing – original draft. **Xue Yan**: Data curation, Formal analysis, Investigation, Visualization, Writing – review & editing. **Jean Claude Ndayishimiye**: Investigation, Writing – review & editing. **Alexey Smirnov**: Writing – review & editing. **Andrey N. Tsyganov**: Writing – review & editing. **Elena Nassonova**: Writing – review & editing. **Natalia G. Mazei**: Writing – review & editing. **Yuri A. Mazei**: Writing – review & editing. **Jun Yang**: Conceptualization, Funding acquisition, Investigation, Supervision, Writing – review & editing.

Acknowledgments

This study was financially supported by the National Natural Science Foundation of China (No. 32361133557), and the Russian Science Foundation (No. 24-44-00096). We thank the AEHG members for field sampling and experiment assistance.

Appendix A Supplementary data

Supplementary data associated with this article can be found in the online version.

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List of figures

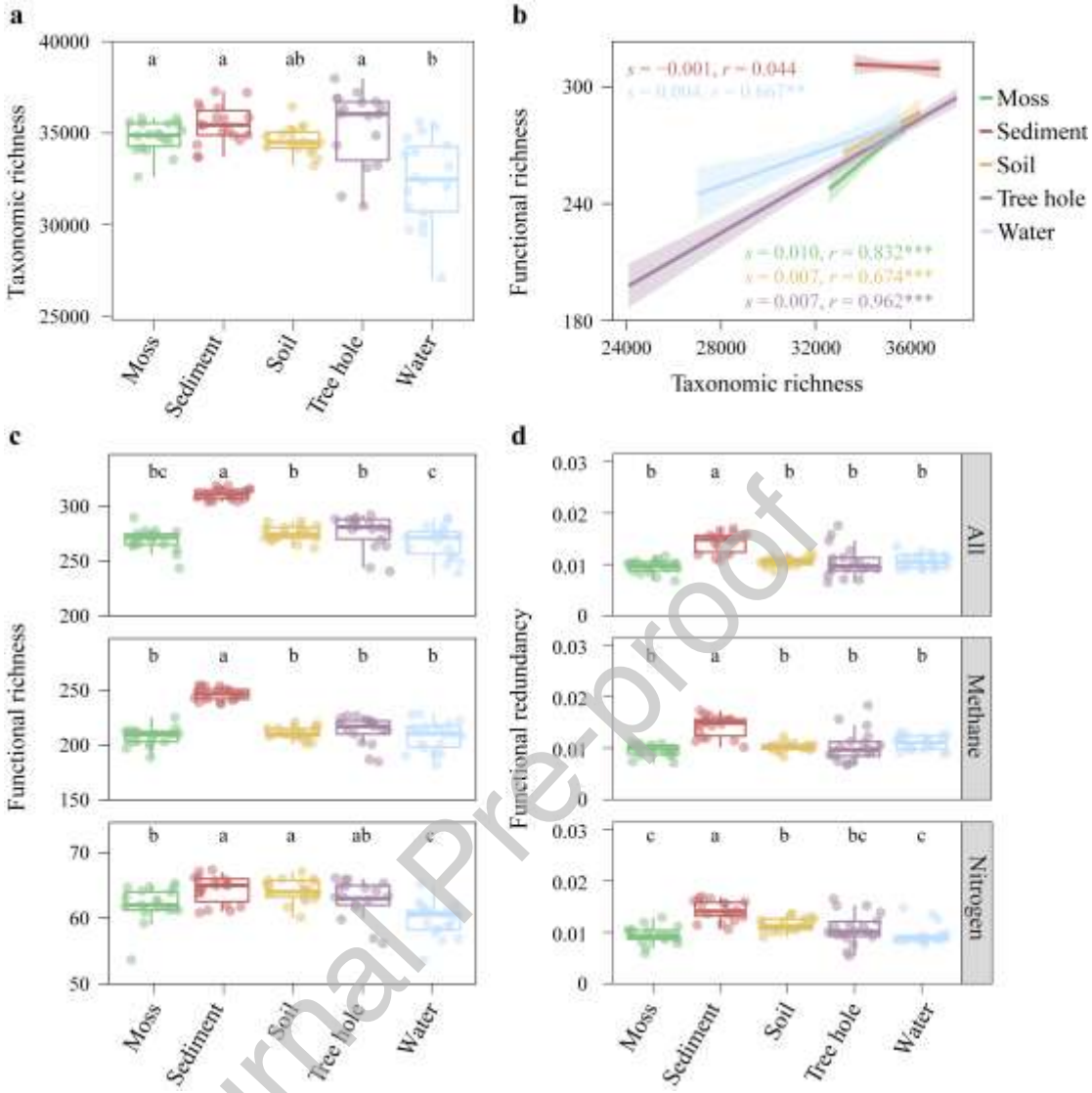

Fig. 1 Taxonomic and functional diversity of microbial community. (a) Taxonomic richness of microbial community. (b) Relationship between functional and taxonomic richness. *s* represents the slope of the linear regression, and shadow represents the 95 % confidence interval of the linear regression. *r* value is Pearson's correlation coefficient (***P* < 0.01, ****P* < 0.001). (c) Functional richness of microbial community based on functional gene families. (d) Functional redundancy of methane and nitrogen cycling genes. "All" refers to considering all methane and nitrogen functional gene families present in the microbial community. The Wilcoxon test is performed, and different letters indicate a significant difference (*P* < 0.05) between the two habitats.

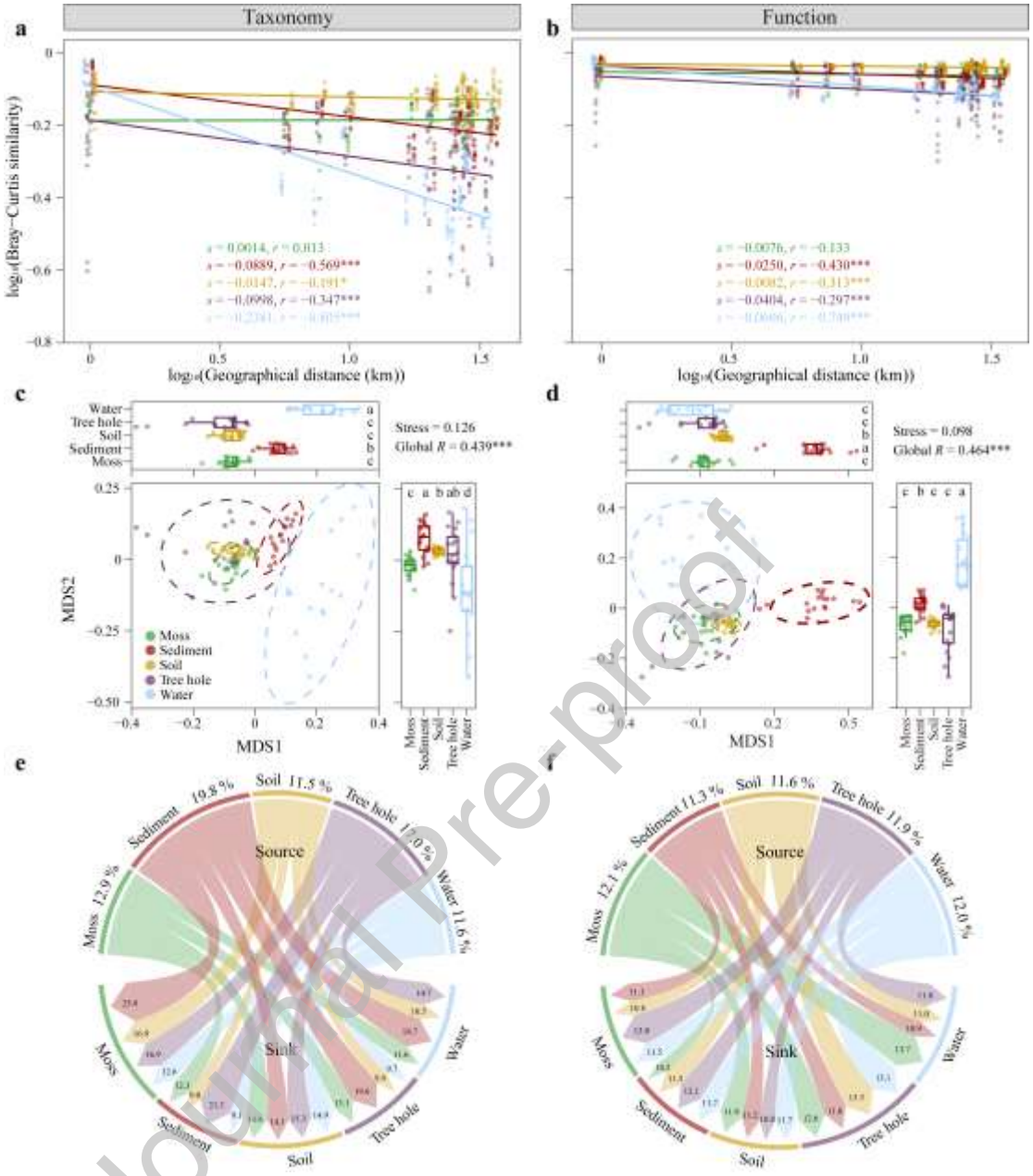

Fig. 2 Distance-decay relationship (DDR) of (a) taxonomic and (b) functional compositions with geographical distance. A linear regression is fitted between Bray-Curtis similarity and spatial distance of pairwise samples. *s* value is the slope, and *r* value is Pearson's correlation coefficient (**P* < 0.05, ****P* < 0.001). Nonmetric multidimensional scaling (NMDS) ordination of (c) taxonomic and (d) functional compositions based on the Bray-Curtis dissimilarity. The global *R* and significance (****P* < 0.001) among the samples are examined via the analysis of similarities (ANOSIM). The Wilcoxon test is performed, and different letters indicate significant

differences ($P < 0.05$) among habitats in the boxplot. Fast expectation-maximization microbial source tracking (FEAST) analysis for (e) taxonomic and (f) functional compositions. The arrow direction represents the source-sink relationships, and percentages represent the contribution that each source provides.

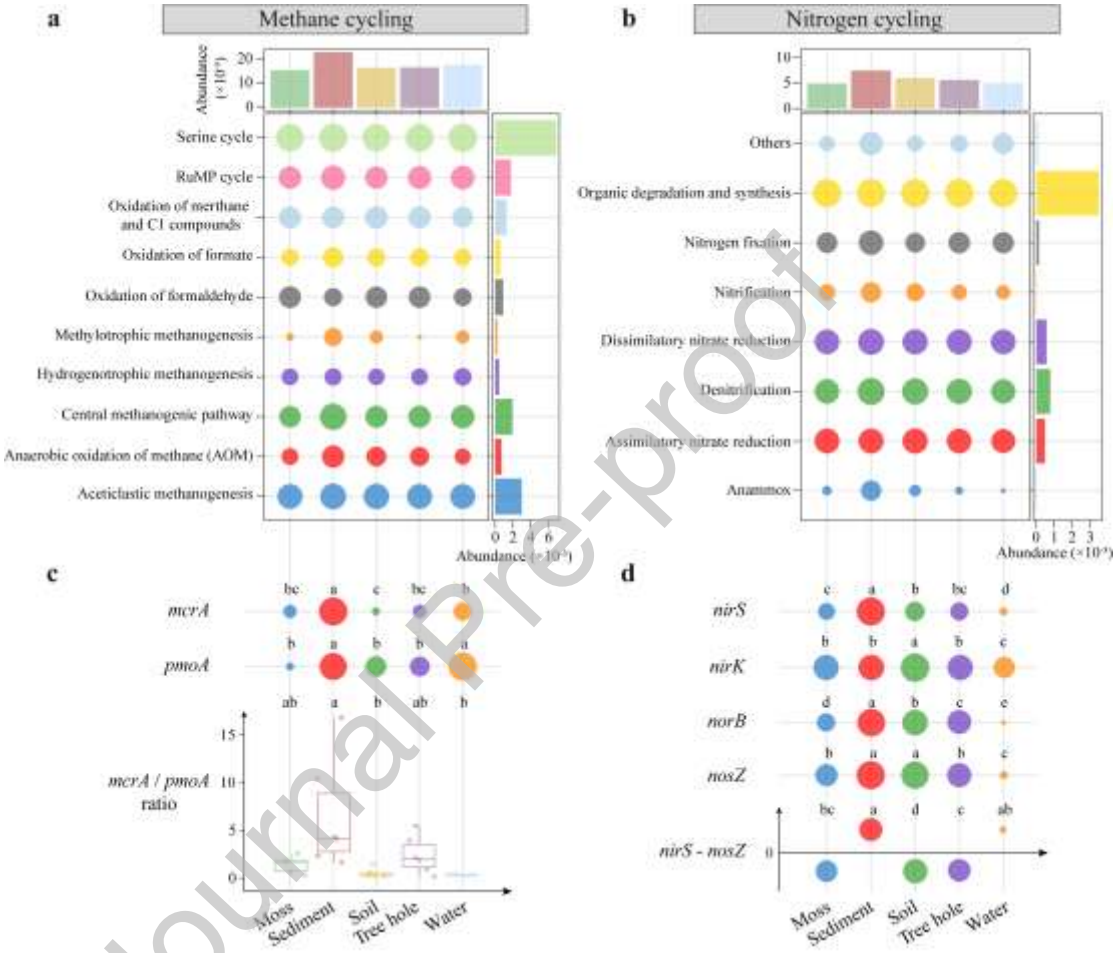

Fig. 3 Relative abundances of (a) methane and (b) nitrogen cycling pathways, and relative abundances of (c) methane and (d) nitrogen key gene families. Node size is proportional to its relative abundance. The bar chart on the right represents the sum of abundance for each functional pathway across the five habitats. The bar chart above depicts the average abundance of functional pathways within each habitat. The Wilcoxon test is performed, and different letters indicate significant differences ($P < 0.05$).

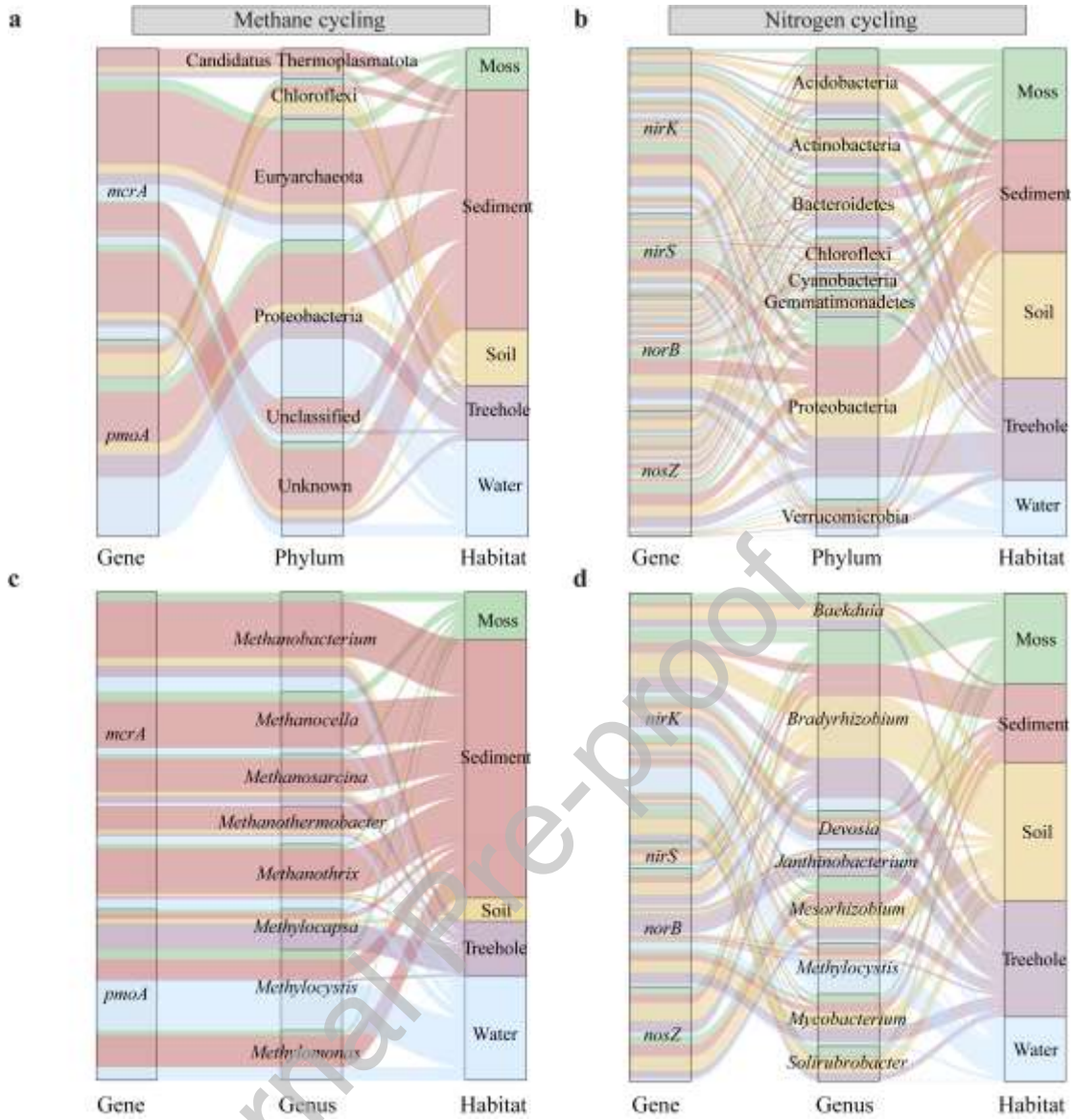

Fig. 4 Relationships of (a, c) methane, and (b, d) nitrogen cycling gene families, microbial taxa, and corresponding habitats. Line thickness is proportional to the relative abundance.

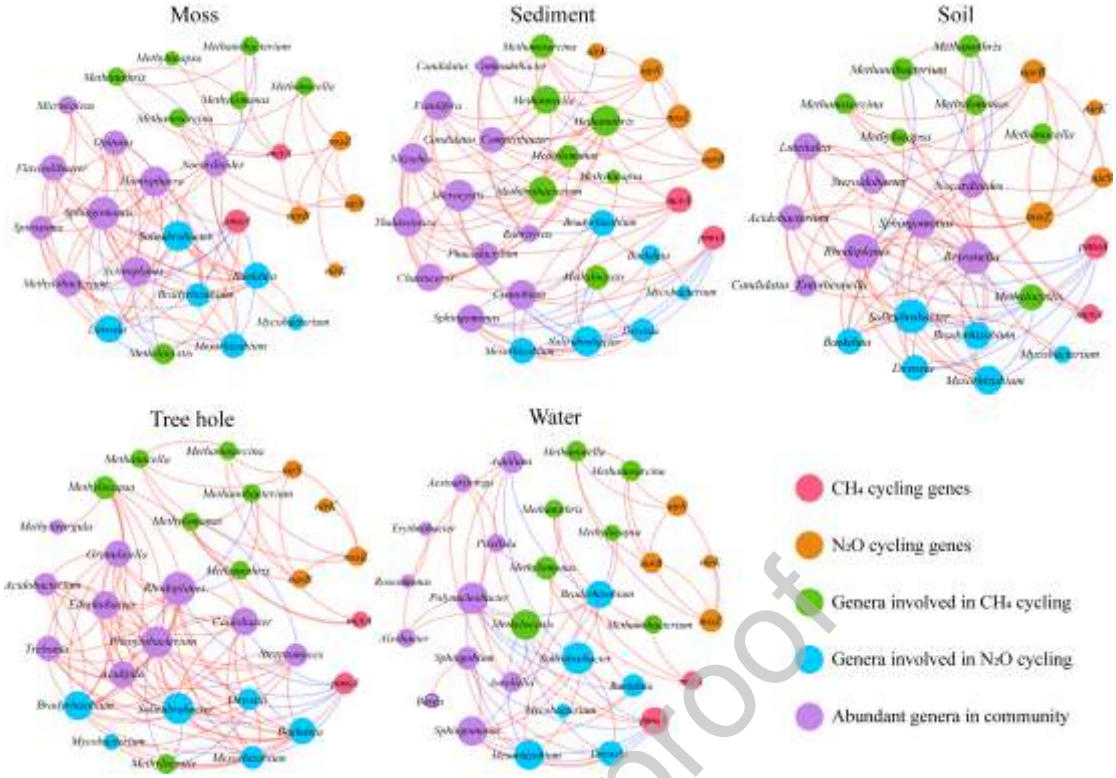

Fig. 5 Co-occurrence networks between microbes and functional gene families. Nodes are colored by different species or gene families. Node size is proportional to the number of connections (degree). Orange and blue links represent positive and negative correlations, respectively.

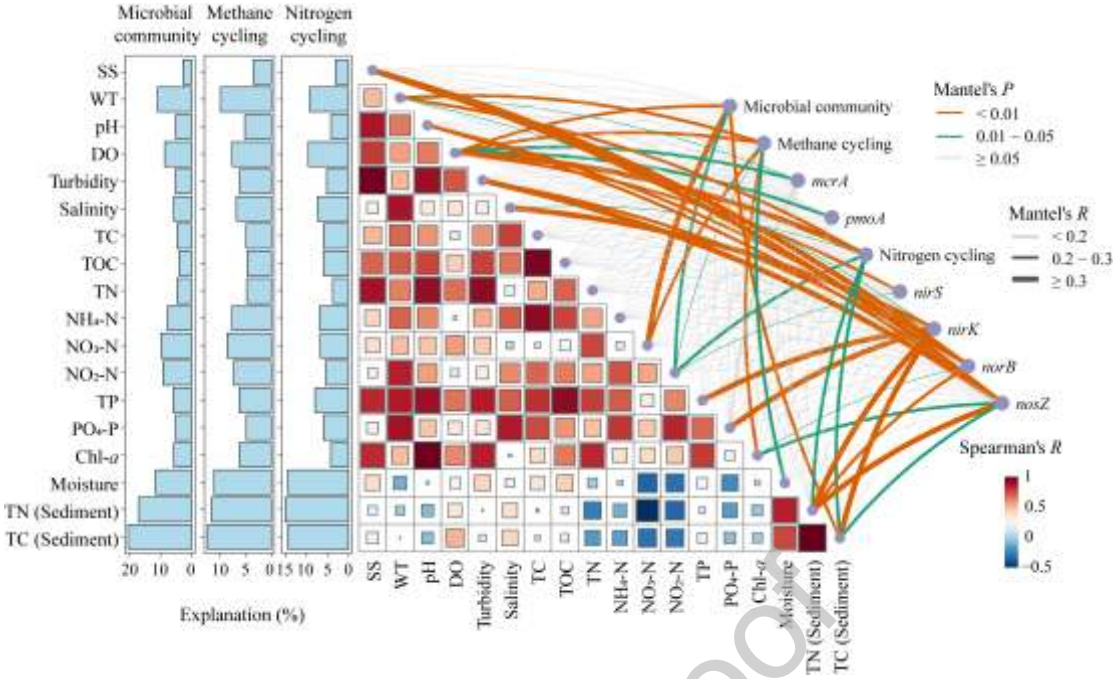

Fig. 6 Relationships between environmental variables and community taxonomic and functional compositions revealed by redundancy analysis (RDA) and Mantel test. The bar plots on the left are from RDA and represent the explanation (%) of each environmental variable. Pairwise correlations of environmental factors are shown on the right with a color gradient denoting Spearman's correlation coefficient (*R*). Edge width represents Mantel's *R* value, and the edge color corresponds to significance. SS: suspended solids; WT: water temperature; DO: dissolved oxygen; TC: total carbon; TOC: total organic carbon; TN: total nitrogen; NH_4-N: ammonium nitrogen; NO_3-N: nitrate nitrogen; NO_2-N: nitrite nitrogen; TP: total phosphorus; PO_4-P: phosphate phosphorus.

Declaration of Interest Statement

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

☐ The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for this journal and was not involved in the editorial review or the decision to publish this article.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical Abstract