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Ecogenomic profiling of spatial variations in sediment microbial communities of a freshwater lake

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ABSTRACT

Freshwater lake sediments integrate physicochemical conditions and provide sensitive indicators of spatial variation in microbial community structure. This study investigated sediment bacterial communities from four sites in Singanallur Lake, Coimbatore, using 16S rRNA V3–V4 amplicon sequencing to characterize spatial heterogeneity in sediment microbial communities under uniform seasonal conditions. Across all samples, a total of 44 phyla, 114 classes, 257 orders, 466 families, and 1107 genera were detected, reflecting high taxonomic richness and spatial variability within the lake sediments. The community was dominated by Pseudomonadota, which ranged from 80.9 % in S1–51.8 % in S4, followed by Bacillota, Bacteroidota, and Cyanobacteriota. At the genus level, Caulobacter decreased from 30.6 % in S1–12.5 % in S4, along with notable genera such as *Bosea* and *Phreatobacter*. Alpha diversity increased steadily from S1 to S4, with observed OTUs ranging from 1722 to 13,796 and Shannon index values increasing from 5.14 to 8.44. Sequencing coverage ranged from 0.34 to 0.74, indicating incomplete sampling depth and representing a methodological limitation, while Gini coefficients (0.64–0.83) reflected uneven community structures, particularly in S1. Several low-abundance and site-enriched genera, including *Akkermansia*, *Helicobacter*, and *Candidatus Saccharimonas*, showed localized enrichment, indicating site-specific environmental conditions within the lake. Venn diagram analysis showed a core of five shared genera representing 31.3 % of total abundance, while rare and unique taxa exhibited minimal overlap (4.0 % and 3.8 %), highlighting strong spatial differentiation among sampling sites. Heatmap-based multivariate analysis integrating microbial OTU abundance with measured physicochemical water quality parameters and sediment heavy metal concentrations revealed clear associations between microbial assemblages and localized environmental gradients. These patterns indicate that sediment microbial communities respond sensitively to present-day physicochemical heterogeneity within the lake. This study provides a baseline spatial ecogenomic framework for Singanallur Lake and highlights the value of integrating microbial community profiling with water quality and metal measurements for future monitoring and comparative assessments.

1. Introduction

Rapid expansion of urban landscapes has increased pressure on freshwater ecosystems, leading to spatial heterogeneity in physicochemical conditions and biological organization within lakes and reservoirs.Freshwater lakes integrate physicochemical inputs from their surrounding catchments and therefore exhibit measurable variation in water quality and sediment characteristics (Wang et al., 2023). Variations in physicochemical parameters, nutrient availability, and sediment composition can influence microbial community structure and ecosystem functioning (D. Zhang et al., 2019). Microorganisms play a central function in freshwater ecosystems, contributing to nutrient cycling, organic matter breakdown, and water purification. The diversity and functioning of these systems are sensitive to changes in environmental conditions (Niu et al., 2024). Environmental gradients involving nutrients, organic matter, and heavy metals can influence

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microbial community composition, potentially favoring taxa adapted to specific physicochemical conditions (Li et al., 2022). Changes in nutrient availability and chemical composition of aquatic environments have been shown to affect microbial diversity and relative abundance (Liu et al., 2021). A high dominance of a few microbial groups is a sign of ecological stress and structural imbalance of ecosystem, whereas higher species richness and uniform distribution indicate a stable and functioning ecosystem (Huang et al., 2023). Conventional ecological assessment techniques are useful but limited, because they often fail to capture the total microbial diversity since many microorganisms remain uncultured or unknown (Rodríguez-Gijón et al., 2023). Next-generation sequencing (NGS) technologies, together with environmental DNA (eDNA) sampling, offer efficient ways to directly analyze species diversity and community structure in environmental samples (Deiner et al., 2017). Among these techniques, Illumina sequencing is most prevalent because it provides large amounts of data accurately and rapidly at a relatively low cost, enabling precise detection and profiling of microbial communities within complex environments (Degnan, Ochman, 2012). Incorporating metagenomic approaches into environmental monitoring enables identification of microbial taxa and assessment of community-level ecological patterns in relation to measured environmental variables (Datta et al., 2020). Metagenomic studies commonly use bioinformatics tools such as Mothur which are commonly applied to calculate alpha diversity indices, such as Shannon, Chao1, and Simpson, helping to evaluate dominance and diversity patterns of microbial communities (Finn, 2024). These metrics facilitate comparison of microbial community structure across sites and allow evaluation of spatial variation in relation to environmental gradients (H. Zhang et al., 2018). Modern NGS methods such as 16S rRNA amplicon sequencing and shotgun metagenomics are widely used to study microbial communities. Shotgun metagenomics provides detailed taxonomic and functional information, whereas 16S rRNA sequencing offers a less complex and cost-effective approach for characterizing community composition, particularly in high-throughput studies (Tyagi and Katara, 2024). For studies primarily aimed at describing community structure, 16S rRNA sequencing represents an effective first-level approach. This approach is particularly suitable for freshwater lake systems where microbial communities vary spatially in response to physicochemical conditions and sediment characteristics. Ecogenomic patterns derived from such analyses provide insights into microbial distribution and adaptation along environmental gradients, supporting informed freshwater ecosystem management (N. Wang et al., 2024). In this context, freshwater lakes act as sensitive systems in which sediment microbial communities reflect spatial variation in present-day environmental conditions. Analysis of microbial assemblages can reveal site-specific enrichment of taxa adapted to local physicochemical conditions and provide insight into spatial heterogeneity within lake ecosystems. Such information contributes to microbial ecology research and supports evidence-based approaches to freshwater ecosystem monitoring. In this study, we investigated sediment microbial communities of Singanallur Lake, Coimbatore, using a spatially resolved, single-season ecogenomic approach. By integrating 16S rRNA gene sequencing with concurrent water quality and sediment heavy metal measurements, this work provides a baseline assessment of spatial heterogeneity in microbial community structure within the lake.

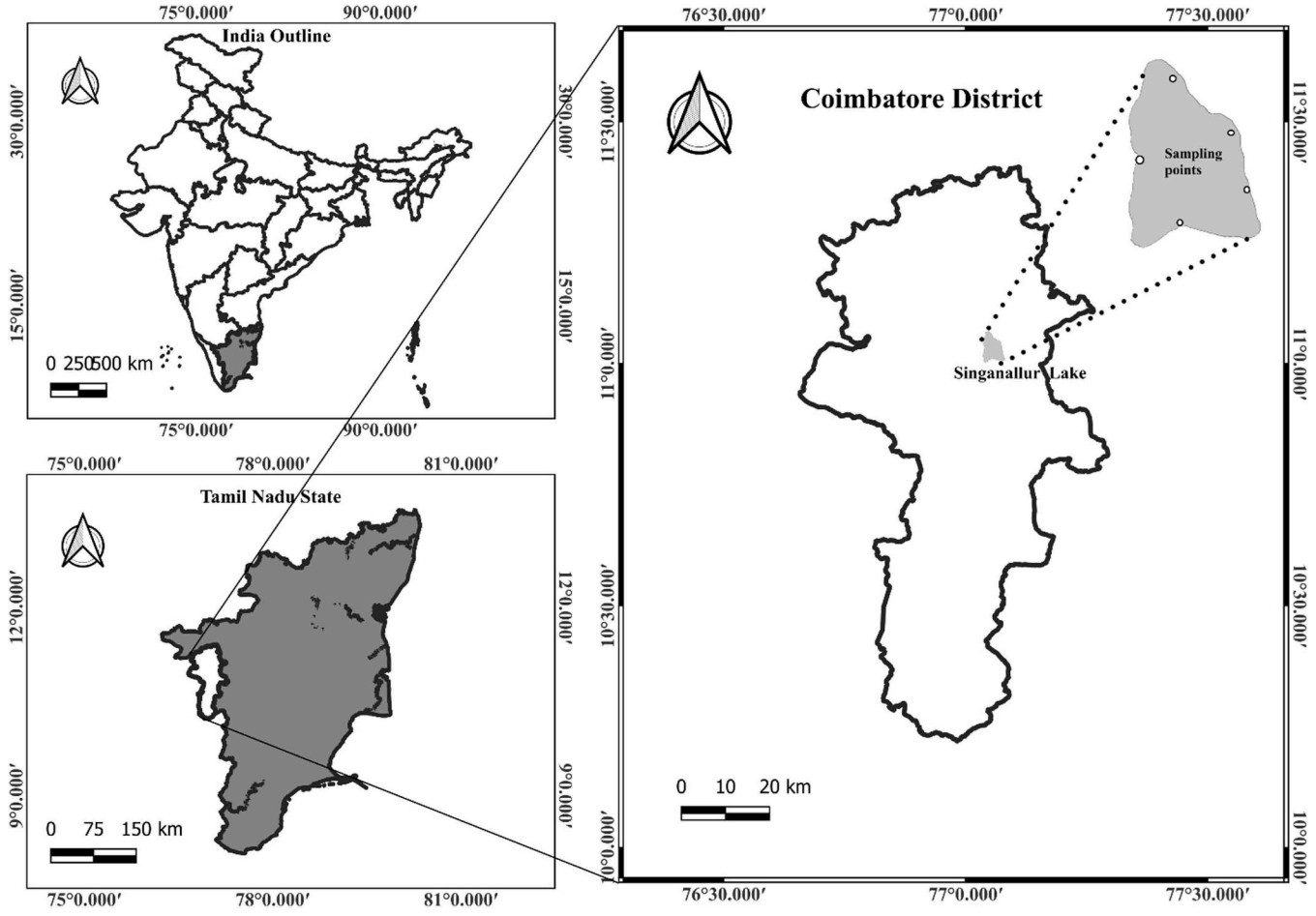

Fig. 1. Sampling site of sediment from Singanallur lake, Coimbatore, Tamil Nadu, India. Fig. 1**a.** Heatmap construction based hierarchical clustering.

2. Materials and methods

2.1. Study site and sample collection

The sediment samples were collected from Singanallur lake in Coimbatore, Tamil Nadu, India. Sampling was conducted on 3 December 2023, during the post-monsoon winter season, to reduce the influence of water disturbances. We used a sterilized grab sampler to collect the sediment samples at four spatially distinct surface spots in the lake (Fig. 1), and the sampling spots were labelled as S1, S2, S3, and S4 for downstream molecular and statistical analyses.

At each site, surface sediment samples were collected from the upper 0–5 cm layer because this layer is widely recognized to contain the most biologically active microbial communities and to be most responsive to recent environmental conditions. A single sampling event was performed at each site as part of a spatial baseline examination, designed to detect site-specific variation in microbial community structure under uniform seasonal conditions. The sediment samples were directly transferred into sterile containers and transported to the laboratory under cold conditions (on ice). All samples were processed within 24 h of collection to avoid potential alterations in microbial community composition. The subsamples were stored at –20 °C until further analysis. Water samples were collected in triplicates from the surface water layer (<1 m depth) and further used for water quality analysis.

2.2. Physicochemical characterization of water quality

Water quality parameters were measured to describe the physicochemical environment associated with the sediment microbial communities. In situ measurements of pH, water temperature (°C), Alkalinity (mg/L), Total Hardness (mg/L), Total dissolved solids (TDS), and Total suspended solids (TSS) were recorded at each sampling site using a portable multiparameter probe (Professional Plus, YSI, Yellow Springs, OH, USA), following standard operational procedures to ensure consistency across sites. Comparable physicochemical characterization approaches have been widely applied in freshwater sediment microbiome studies to interpret microbial–environment interactions (Jia et al., 2023; L. Wang et al., 2018). For laboratory-based analyses, collected water samples were processed as follows. The collected samples were filtered through 0.45 μm pore-size membrane filters (Advantec MFS membrane filter, Irvine, CA, USA) to remove suspended particulate matter prior to nutrient estimation. Standard analytical methods were used to determine the concentrations of biochemical oxygen demand (BOD) and chemical oxygen demand (COD). An automated water quality analyzer (AutoAnalyzer 3 HR, Seal Analytical Inc., Mequon, WI, USA) quantified nutrient parameters, including nitrate and phosphate. Absorbance measurements were recorded using a UV–visible spectrophotometer (GENESYS™, Thermo Fisher Scientific, Waltham, MA, USA), providing an integrated assessment of organic load and nutrient status within the aquatic system.

2.3. Heavy metal analysis

Heavy metal concentrations in sediment samples were measured to estimate potential metal-associated ecological stress. Sediment samples were air-dried, homogenized, and subjected to acid digestion with a mixture of concentrated nitric acid and perchloric acid using standard environmental protocols. The digested samples were filtered and diluted with ultrapure water before analysis.

Concentrations of selected heavy metals, including lead (Pb), copper (Cu), nickel (Ni), and zinc (Zn), were measured . Metal concentrations were quantified using atomic absorption spectrophotometry (AAnalyst 400, PerkinElmer, Waltham, MA, USA). Calibration curves were prepared using certified standard solutions, and analytical accuracy was ensured through reagent blanks and quality control samples. Metal concentrations were expressed as mg kg^{-1} dry weight of sediment (W. Wang et al., 2019).

2.4. DNA extraction from sediment samples

DNA extraction was performed following the protocol outlined by (Rangaswamy et al., 2022), with slight alterations in incubation time and reagent volumes. One gram of sediment was put into a 50 mL tube, and 10 mL of CTAB buffer was added. The sample was thoroughly mixed using a vortex mixer and then agitated at 60 °C for 10 min. Later, 15 mL of Sevag solution was added, and the mixture was vortexed at low speed for 5 min. The sample was then centrifuged at 3220 × g for 15 min to separate the layers. The clear liquid on top was carefully moved to a new 50 mL tube without disturbing the middle layer. The same amount of cold isopropanol and half the amount of 5 M NaCl were added. The mixture was shaken and then kept at −20 °C for at least 1 h and up to 12 h.

After incubation, the sample was centrifuged at 3220 ×g for 15 min. The supernatant was removed, and the resulting pellet was retained for further analysis. Two milliliters of 70 % ethanol were added to the pellet, and the sample was centrifuged at 4000 rpm (3220 ×g) for 2 min. The ethanol was discarded, and the pellet was air-dried for 1 h. Finally, 20 μL of 10 mM Tris-HCl was added to the dried pellet. The extracted DNA samples were stored at −20 °C.

2.5. 16S rRNA gene amplification and quality assessment

DNA samples were initially evaluated for quality as outlined by (Sharma et al., 2024) using NanoDrop spectrophotometry and agarose gel electrophoresis. DNA purity was assessed from using the 260/280 absorbance ratio, which was found to be between 1.8 and 2.0. For metagenomic analysis, the 16S rRNA gene was amplified with the primers 16S F: 5′-AGAGTTTGATGMTGGCTCAG-3′ and 16S R: 5′-TTACCGCGGCMGCSGGCAC-3′, targeting a broad region of the 16S rRNA gene encompassing the V3–V4 hypervariable region. PCR mixtures were prepared by combining Taq Master Mix, 2 μL of each primer, and 20 μL of DNA template at a concentration of 40 ng.

Amplification was carried out under standardized PCR conditions: an initial denaturation at 95 °C for 7 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 46 °C for 30 s, and extension at 72 °C for 1 min, and concluded with a final extension at 72 °C for 5 min. Aliquots of 2 μL of the PCR products were assessed on agarose gel electrophoresis containing 0.5 μg/mL of ethidium bromide. The amplified 16S products were purified and again examined using agarose gel electrophoresis and NanoDrop spectrophotometry. DNA purity was reconfirmed from the 260/280 absorbance ratio, which remained within the range of 1.8–2.0. Following PCR amplification and purification, sequencing libraries were prepared using Illumina barcoded adapters. Libraries were purified using magnetic bead-based cleanup and quantified prior to sequencing.

2.6. Illumina MiSeq sequencing and data quality control

Sequencing was performed on an Illumina MiSeq platform using paired-end 2 × 300 bp chemistry, which enables high-quality sequencing of the 16S rRNA V3–V4 region with sufficient read length and overlap for accurate assembly (Illumina, n.d.). Raw sequence data were demultiplexed and subjected to quality assessment using FastQC and MultiQC. Low-quality reads, ambiguous bases, and sequencing artefacts were removed prior to downstream analysis. Chimera detection was performed, and only high-quality reads were retained for OTU clustering and taxonomic assignment.

2.7. Sequence processing and taxonomic classification

Contiguous sequences were created from raw sequence reads using Mothur's "make.contigs" function (v.1.47.0; accessed on 7th August, 2025), following the MiSeq Standard Operating Procedure (http

s://mothur.org/wiki) as described by (Kozich et al., 2013). Low-quality sequences were taken out employing the screen.seqs command. This included sequences containing ambiguous bases, homopolymer runs of ≥ 8 bp, and sequences outside the expected length range (450–500 bp) for the 16S V3–V4 rRNA region. A reference database specific to the targeted hypervariable region was constructed from the SILVA reference database (v.138.1) using the 'pcr.seqs' command, as described by (Quast et al., 2012). Sequences were aligned using the 'align.seqs' routine, and pre-clustered sequences were checked for chimeric regions with VSEARCH as described by (Rognes et al., 2016) and identified chimeras were excluded from further analysis. Taxonomic assignment was carried out using the custom reference database and the command 'classify.seqs'. Sequences that could not be properly aligned were removed during quality control. Pairwise distances between sequences were calculated using the 'dist.seqs' command, and operational taxonomic units (OTUs) were grouped using a 0.03 distance threshold with the 'cluster' command. Alpha diversity analyses were performed based on the OTU classifications.

2.8. Statistical analysis of OTUs

Microbial community diversity was assessed using alpha diversity indices derived from OTU abundance profiles generated through the Mothur pipeline (Kozich et al., 2013). All sequence data were processed using Mothur v1.47.0, following the MiSeq Standard Operating Procedure (SOP). OTUs were clustered at 97 % sequence similarity using the opti_mcc algorithm. Sample-specific.shared files were generated for downstream analysis.

Alpha diversity metrics were calculated in R v4.3.1 using the tidyverse and packages. The following indices were computed for each sample: Simpson index, Shannon diversity index, Chao1 richness estimator, observed OTUs (Sobs), and Good's coverage. Good's coverage was measured as the proportion of total sequences that are not singletons, using the formula:

$$C = 1 - \frac{F_1}{N}$$

where F1 is the number of singleton OTUs and N is the total number of sequences. Coverage values are expressed as proportions (0–1), representing sampling completeness. These indices were used to evaluate species richness, evenness, and sampling completeness.

In addition to standard alpha diversity measures, community evenness was quantified using the Gini coefficient. A Gini coefficient of 0 represents perfect evenness, where all taxa are present in equal proportions. In contrast, values closer to 1 reflect strong inequality in the community, meaning that one or a few taxa dominate while the rest occur at much lower abundances. This index was calculated using the non-parametric formula described by (Feranchuk et al., 2018):

$$G = \frac{\sum_{i=1}^{n} i \cdot x_i}{2 \cdot \sum_{i=1}^{n} x_i}$$

where x_i represents the abundance of the i^{th} OTU, sorted in ascending order, and n is the number of OTUs with non-zero abundance.

2.9. Genus-level community profiling and visualization

Genus-level microbial community analysis was performed using R (v4.3.1). Abundance data from sediment surface samples (S1-S4) were imported and merged into a single dataset. Records lacking genus annotation or containing zero read counts were excluded. Phyla were classified into three abundance-based categories: Dominant (≥1000 reads), Rare (101–999 reads), and Unique (≤100 reads). Relative abundances were calculated for each genus within these categories across all samples.

The ten most abundant genera in each sample were identified based on relative abundance. Genus-level distributions were shown using bar plots organized by abundance category and sample. These plots helped to evaluate how each genus contributed to the overall community structure within each phylum.

2.10. Comparative genus-level overlap analysis

Venn diagrams were created using Venny v2.1.0 to assess the shared microbial genera across samples within each abundance category; accessed on 27th August, 2025 (Oliveros, 2024). The top ten genera known from each of the four sediment surface samples (S1 to S4) were grouped separately for the Dominant, Unique, and Rare phylum-level categories. Four-set Venn diagrams were creaed for each category to visualize shared and unique genera among the samples.

2.11. Multivariate analysis of water quality and OTUs

To investigate the relationships between the water quality parameters, heavy metal concentrations, and the microbial OTU abundance, multivariate statistical analyses and heatmap visualizations were performed using RStudio (R statistical software, version 4.3.1). Prior to visualization, the datasets were screened for missing values and outliers. To account for differences in measurement scales among the variables, data were log-transformed where appropriate and standardized using Z-score normalization. The Z-score normalization is an common approach in environmental microbiome studies to emphasize the relative variation across the samples (Jia et al., 2023).

Heatmaps were created via the pheatmap package in R, and hierarchical clustering was applied to both rows (environmental parameters and microbial OTUs) and columns (sampling sites) using Euclidean distance and the complete linkage method. This analytical framework has been widely used to identify co-variation patterns between physicochemical gradients, heavy metals, and microbial community structure in freshwater and sediment ecosystems (L. Wang et al., 2018; W. Wang et al., 2019).

The resulting heatmaps enabled visualization of statistical associations and co-occurrence patterns between physicochemical variables, heavy metals, and microbial taxa. Observed clustering patterns were interpreted as ecological associations rather than direct causal relationships, consistent with best practices in multivariate microbial community analysis.

3. Results and discussion

3.1. Overview of microbial diversity in lake sediments

Sediment microbial communities provide insight into spatial heterogeneity within freshwater lake ecosystems, reflecting variation in local environmental conditions. The analysis of sediment samples from the lake showed a highly heterogeneous bacterial assemblage, reflecting complex and spatially variable environmental conditions. By combining DNA sequences from all samples and filtering out non-bacterial reads, zero-abundance OTUs, and taxa that could not be assigned, we found 44 phyla, 114 classes, 257 orders, 466 families, and 1107 genera, indicating the coexistence of both broadly distributed and site-restricted microbial lineages. This broad taxonomic distribution highlights pronounced spatial heterogeneity within the lake sediments, consistent with the presence of multiple ecological niches maintained by localized environmental gradients. Such high taxonomic resolution at multiple hierarchical levels is characteristic of freshwater sediments exhibiting heterogeneous physicochemical conditions, where dominant taxa coexist with diverse rare and specialist groups. This taxonomic complexity provides the ecological context for subsequent analyses of richness, dominance, evenness, and site-specific community

differentiation presented in the following sections.

3.2. Statistical analysis of microbial community and diversity patterns

The community diversity results in Table 1 revealed notable differences among the samples. Sequencing coverage values were generally low, ranging from 0.34 in S3–0.74 in S1, indicating incomplete representation of the total microbial community and representing a methodological limitation. Species richness, measured by OTUs, increased steadily from 1722 in S1–13,796 in S4 progressively. The Chao1 estimator further emphasized this trend, with values rising sharply from 8221.3 in S1–185,547.4 in S4, suggesting the presence of a large pool of rare and unobserved taxa, particularly in the more diverse sites. Shannon diversity indices increased from 5.14 in S1–8.44 in S4. Although these values appear high, similar Shannon index ranges have been reported for sediment and soil microbiomes characterized by high taxonomic richness and environmental heterogeneity. In such systems, the Shannon index is strongly influenced by the presence of numerous low-abundance taxa, and elevated values do not necessarily indicate uniformly even communities. In the present study, the relatively high Shannon values are consistent with the combination of high OTU richness and moderate dominance, as further supported by high Simpson index values (0.95–0.99) and lower Gini coefficients in S3 and S4. The Shannon values, the Simpson index values and the Gini coefficients together show that the later samples support more complex and taxonomically rich communities than S1 and S2. Given the limited sequencing coverage, Shannon diversity values are therefore interpreted comparatively among sites rather than as absolute indicators of ecosystem stability, which is appropriate for spatial baseline assessments of sediments.

Chao1 is known to be highly sensitive to the number of singleton and low-abundance OTUs, and can substantially overestimate true richness, when sequencing coverage is limited or when communities harbor an extensive rare biosphere (Chao, 1984; Hughes et al., 2002; Schloss, Handelsman, 2005). The combination of low Good's coverage and a large proportion of rare taxa in S3 and S4 likely contributed to inflation of Chao1 estimates.

Similar Chao1 overestimations appear in sediment and soil microbiomes characterized by high heterogeneity, where thousands of rare taxa coexist but are incompletely sampled (Fierer et al., 2012; Delgado-Baquerizo et al., 2018). Therefore, in the present study, Chao1 values are interpreted as indicators of relative richness potential rather than absolute species numbers. Significantly, despite possible overestimation of absolute richness, the consistent increase in observed OTUs, Shannon, Simpson, and Gini indices collectively supports the conclusion that S3 and S4 harbor substantially richer and more complex microbial communities than S1 and S2.

The inequality in community composition was further assessed using the Gini coefficient as shown in Table 2. The values were ranged between 0.64 and 0.83, highlighting the differences in the evenness of taxon distributions across the samples. The highest Gini value was found in S1 (0.83), which means that few taxa were very dominant and reduced community balance. In contrast, S3 (0.64) and S4 (0.66) exhibited lower values, which indicates generally more equitable distributions of taxa despite their higher richness. S2 showed an intermediate value (0.68), reflecting low dominance patterns. These results complement the Shannon and Simpson indices, confirming that while richness increased with later samples, community evenness varied, with S1 being the most uneven and S3 and S4 reflecting greater balance in species distribution.

Table 2
Gini coefficients representing community evenness of microbial communities across sediment samples from Singanallur Lake.

Sample	Gini
S1	0.830627
S2	0.677767
S3	0.643755
S4	0.66543

3.3. Phylum-level taxonomic composition of the microbial community

Across all four lake samples (S1-S4) as seen in Fig. 2, the community was primarily shaped by a few dominant phyla, with Pseudomonadota consistently showing the highest abundance, reaching 80.87 % in S1 and remaining dominant in S2 (59.84 %), S3 (52.26 %), and S4 (51.76 %). Bacillota was more prominent in S3 and S4 (25.39 % each), while Bacteroidota (8.24–11.64 %) and Cyanobacteriota (7.02–11.32 %) maintained moderate levels across samples. Rare phyla, including Actinomycetota, Campylobacterota, Verrucomicrobiota, Patescibacteria, and Myxococcota, appeared in low proportions but were consistently represented across sites. Unique contributions varied as S1 was enriched with Patescibacteria (17.1 %) and Chloroflexota (12.2 %), S2 with Fusobacteriota (8.6 %) and Halobacteriota (9.5 %), S3 with Spirochaetota (3.1 %) and Gemmatimonadota (4.5 %), and S4 with Acidobacteriota (5.7 %) and Armatimonadota (5.7 %). Overall, the results indicate that Pseudomonadota were the dominant group, while rare and unique phyla contributed to site-specific differences in diversity.

3.4. Genera within the dominant phyla: the case of pseudomonadota

Within dominant phyla as shown in Fig. 3**a**, *Caulobacter* was the most abundant genus across all sites, accounting for 30.55 % in S1 and decreasing to 12.53 % in S4. Other consistently present genera included *Bosea*, *Phreatobacter*, and unclassified genera of Sphingomonadaceae and Caulobacteraceae. S3 and S4 also contained smaller fractions of *Sinobaca*, unclassified genera of Lactobacillales, and *Veillonella*. The high abundance of Pseudomonadota reflects their ecological versatility in freshwater sediments, while *Caulobacter* can thrive under changing nutrient conditions and exhibits morphological adaptations under certain limitations (Heinrich et al., 2019;Hentchel et al., 2019). *Bosea* and related taxa have been reported in freshwater systems influenced by variable nutrient availability (Khanal et al., 2025). The overall dominance of Pseudomonadota is supported by their ability to use a wide range of metabolic pathways (Qiu et al., 2025). *Sphingomonadaceae* and *Caulobacteraceae* are frequently found in freshwater systems because of their physiological flexibility, ability to form biofilms, and diverse metabolic capabilities. Their adaptability allows them to colonize different habitats, survive on surfaces, and help break down contaminants, emphasizing their important role in aquatic ecosystems (de Vries et al., 2019; Nguyen et al., 2021). Their widespread presence suggests effective resource utilization and physiological adaptability. Their

Table 1
Sequencing coverage, observed species (Sobs), and diversity indices (Chao1, Shannon, and Simpson) across samples S1-S4.

Sample	OTU count	nseqs	Good's coverage	sobs	simpson	chao	shannon
S1	1722	5359	0.74	1722	0.96	8221.34	5.14
S2	2368	3708	0.41	2368	0.99	32125.03	6.75
S3	3070	4339	0.34	3070	0.99	47555.44	7.32
S4	13796	20776	0.38	13796	0.99	185547.4	8.44

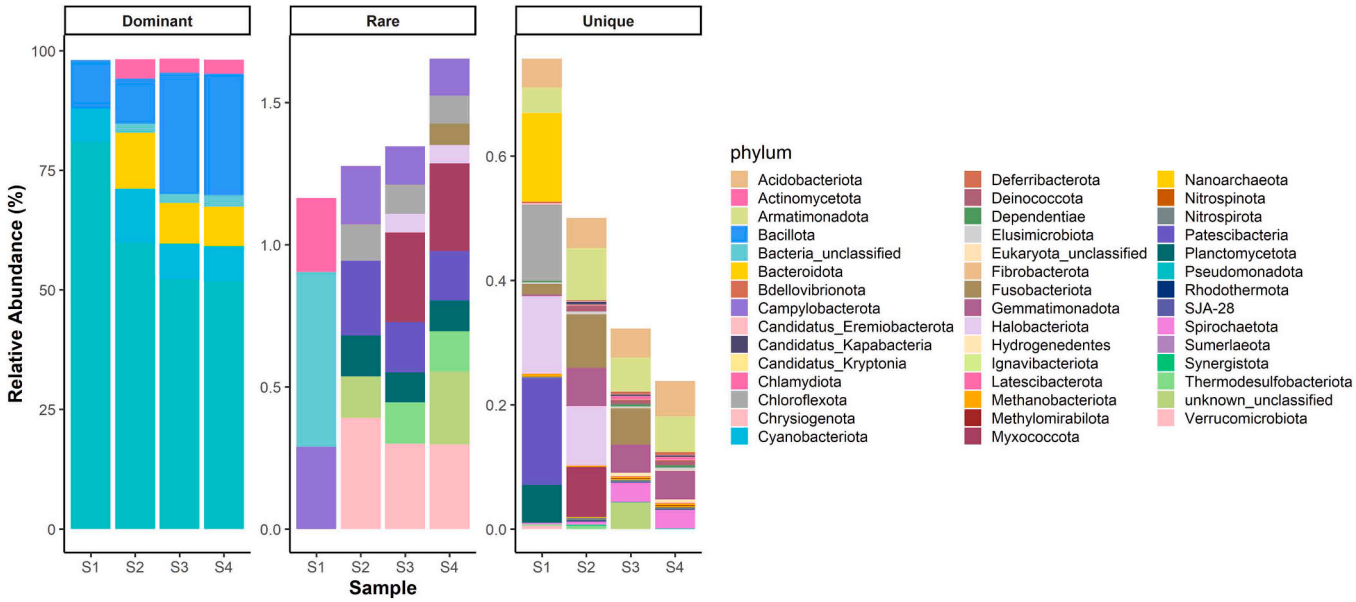

Fig. 2. Distribution patterns of microbial communities at the phylum level, highlighting compositional differences among the sampled categories.

adaptable metabolism probably drives their ecological success and impacts the microbial community structure across different sites.

3.5. Rare genera as indicators of site-specific environmental conditions

Among the rare phyla shown in Fig. 3**b**, *Akkermansia* and *Helicobacter* were the most consistent genera appearing in almost all samples with abundances reaching up to 0.36 %. These genera, commonly associated with host-associated environments, may reflect localized environmental inputs within the lake sediments (Aziz et al., 2015; T. Zhang et al., 2019). *Candidatus Saccharimonas* was found in small amounts between 0.08 % and 0.19 % but plays an important ecological role through its reduced genome and dependence on symbiosis showing that it interacts with other microbes rather than being free-living (Lemos et al., 2019; Peñalver et al., 2024). Other sample-specific rare taxa, such as *Collinsella* (S1), *Desulfovibrio* (S3), and *Fusobacterium* (S4), highlight localized ecological signals. These rare taxa provide insight into site-specific ecological conditions and microbial dependencies that shape freshwater rare biospheres.

3.6. Unique genera and site-specific dominance

Unique taxa as shown in Fig. 3**c**, revealed strong sample-specific patterns. In S1, *Candidatus Saccharimonas* dominated (14.45 %), exceeding the relative abundance of top unique genera in other sites, followed by unclassified Bacteroidia (5.63 %) and *SM1A02* (4.65 %). In contrast, S2-S4 were consistently shaped by unclassified Fimbriimonadaceae (5.11–8.30 %), alongside contributions from unclassified Gemmatimonadaceae (2.46–3.70 %) and *Treponema* (2.28 % in S4). The high dominance of *Candidatus Saccharimonas* in S1 reflects its streamlined genome and symbiotic lifestyle (Lemos et al., 2019), whereas Fimbriimonadaceae are known for their role in organic matter degradation and persistence in aquatic systems (Quan, Im, 2020). Gemmatimonadaceae likely contribute to nutrient cycling and phytoplankton interactions as described by (Mujakić et al., 2021), while the detection of *Treponema* may be linked to engineered systems and poor sanitation signaling contamination risks and potentially indicating animal-associated inputs (Mamuad et al., 2020). These findings indicate that unique microbial groups serve as indicators of localized ecological variation and site-specific environmental processes.

Together, these patterns indicate that S1 exhibits the highest degree of community dominance and unevenness among sites, in the system. Its strong dominance by *Candidatus Saccharimonas* at S1, combined with its low alpha diversity and a high Gini coefficient, indicating a highly uneven community structure. The marked enrichment of *Pseudomonadota*, a phylum commonly associated with an increase in stress-tolerant and opportunistic taxa in impacted environments. Overall, these indicators show that S1 is likely experiencing greater environmental pressure than the other sites.

3.7. Overlap analysis using venn diagram

Venn diagram analysis provided insights into the shared and distinct genera across the lake samples (S1-S4). In the dominant phyla as shown in Fig. 4**a**, five genera were common to all sites, forming a stable core community which had a relative abundance of 31.3 %. Smaller overlaps emphasized local differences, for example S3 and S4 shared three genera (18.8 %), S1 and S2 shared two (12.5 %), while three-sample combinations such as S1-S2-S4 and S2-S3-S4 revealed only a single shared genus (6.3 % each). These patterns suggest that, alongside a strong lake-wide core, localized environmental conditions influence site-specific assemblages.

In the Venn diagram of rare phyla as presented in Fig. 4**b**, overlaps were more limited. Only single genus (4 %) was found in all samples, indicating a minimal rare core. Pairwise and group overlaps revealed uneven patterns, with S3 and S4 having the highest number of shared genera (16 %), followed by S2-S3-S4 with 3 genera (12 %). Other intersections, including S1-S2 (4 %) and S1-S2-S4 (4 %), contributed much less. This uneven distribution reflects the patchy and site-dependent nature of rare taxa.

For the unique phyla as shown in Fig. 4**c**, overlaps were even more restricted. A single genus (3.8 %) was common across all sites, while S2-S3-S4 shared three genera (11.5 %). S1-S2 had two shared genera (7.7 %), and S3-S4 shared one genus (3.8 %), whereas no overlap was detected among S1-S2-S4. These results highlight that unique phyla were weakly represented, often restricted to only specific locations.

3.8. Influence of water quality and heavy metals on sediment microbial communities

The observed correlations suggest that environmental filtering contributes to the structuring sediment microbial communities in

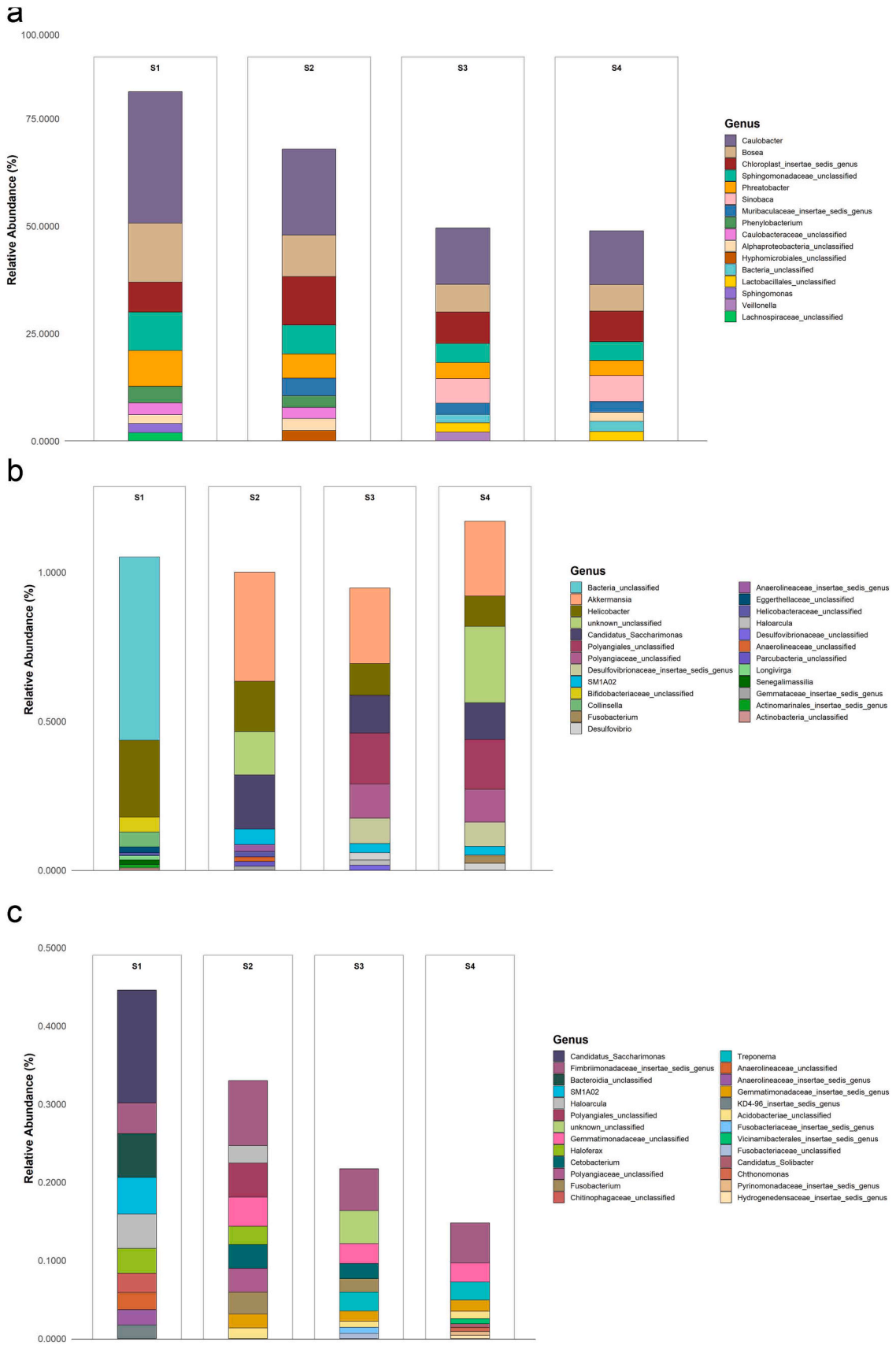

Fig. 3. a. Dominant bacterial genera across sites (S1-S4), highlighting the decline of *Caulobacter* and consistent presence of *Bosea*, *Phreatobacter*, and related taxa. Fig. 3**b.** Rare bacterial genera (≤0.5 %) showing signatures of fecal inputs (*Akkermansia*, *Helicobacter*) and site-specific taxa such as *Collinsella*, *Desulfovibrio*, and *Fusobacterium*. Fig. 3**c.** Unique genera differentiating sites, with S1 dominated by *Candidatus Saccharimonas* and S2–S4 shaped by Fimbriimonadaceae, Gemmatimonadaceae, and *Treponema*.

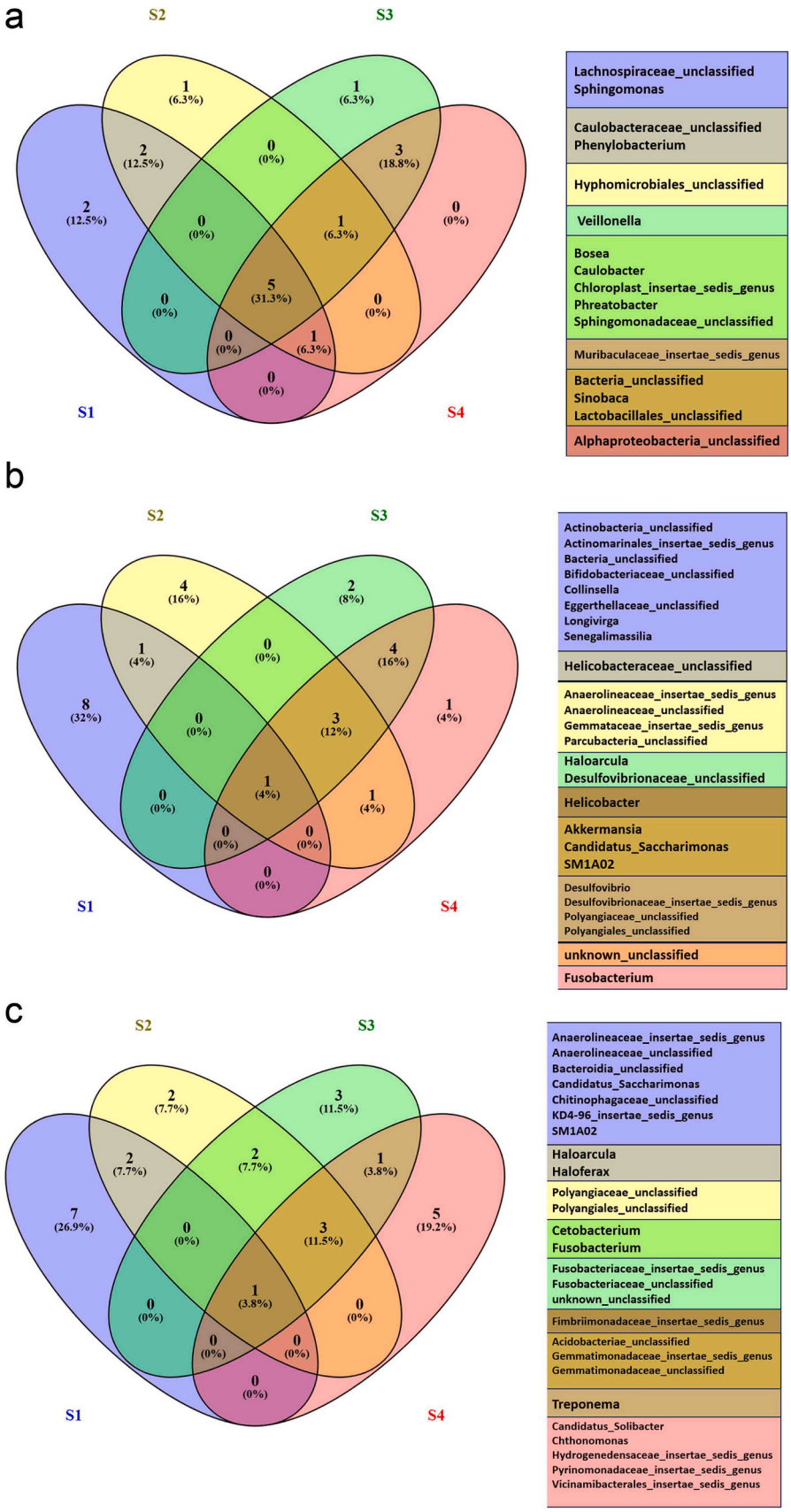

Fig. 4. a. Venn diagram showing shared dominant genera across S1-S4, revealing a stable five-genus core and smaller overlaps driven by localized conditions. Fig. 4**b.** Venn diagram of rare genera with only one genus shared across all sites, reflecting a minimal rare-core community. Fig. 4**c.** Venn diagram of unique genera showing very limited overlap, with most taxa restricted to individual sites.

Singanallur Lake. Heatmap-based clustering showed that sites with elevated organic load, nutrients, and metal concentrations supported distinct microbial assemblages, highlighting the sensitivity of sediment microbiomes to combined physicochemical stressors.

Strong associations between BOD, COD, nutrient concentrations, and specific OTU clusters are consistent with previous studies demonstrating that organic enrichment and eutrophication selectively favor copiotrophic and metabolically versatile microbial taxa in freshwater sediments. Similar enrichment of bacterial groups under high organic and nutrient conditions has been reported in urban lakes and rivers, where microbial communities shift toward taxa involved in organic matter degradation and nutrient cycling (Jia et al., 2023; L. Wang et al., 2018). These studies highlight that increased nutrient availability enhances microbial richness but often alters community structure through selective pressures.

The co-occurrence of specific OTUs with elevated concentrations of heavy metals such as Pb, Cu, Ni, and Zn further suggests that metal contamination acts as an additional ecological filter. Numerous investigations have shown that heavy metals can suppress metal-sensitive taxa while promoting metal-resistant or metal-tolerant microbial populations, resulting in distinct community assemblages in contaminated sediments (Giller et al., 2009; Gillan et al., 2015). Similar metal-associated microbial clustering patterns have been documented in lake and river sediments subjected to long-term anthropogenic inputs, where microbial communities adapt through resistance mechanisms such as efflux systems, sequestration, and enzymatic detoxification (W. Wang et al., 2019).

Importantly, the observed heatmap correlations reflect consistent ecological associations rather than direct causality, as multiple environmental variables often co-vary in urban aquatic systems. Nevertheless, the congruence between heatmap clustering patterns and alpha diversity metrics (Shannon, Simpson, and Gini indices) strengthens the interpretation that sites experiencing higher environmental stress exhibit altered community composition, increased dominance, and reduced evenness, whereas less impacted sites support more diverse and complex microbial assemblages. Comparable patterns linking physicochemical stress gradients with microbial diversity shifts have been reported in sediment microbiomes across diverse freshwater ecosystems (L. Wang et al., 2018: Delgado-Baquerizo et al., 2018).

Collectively, these findings indicate that sediment microbial communities respond sensitively to spatial variation in water quality and metal concentrations and heavy metal contamination. Integrating multivariate heatmap analyses with diversity indices and environmental measurements provides a robust framework for assessing ecological condition and identifying zones of anthropogenic impact in urban freshwater lakes.

Variations in water quality and heavy metal concentrations across sampling sites were reflected in corresponding shifts in sediment microbial community structure. Differences in metals such as Pb, Cu, Ni, and Zn were associated with changes in OTU abundance and clustering patterns, indicating sensitivity of microbial communities to spatial metal gradients. Although metal concentrations at sites S1–S4 did not consistently exceed sediment quality guideline values, previous studies have shown that prolonged exposure to low or moderate metal levels can influence microbial community composition by favoring stress-tolerant taxa.

In this study, sites with relatively higher metal enrichment and organic load supported distinct microbial assemblages compared to less enriched sites, suggesting chronic environmental pressure rather than acute contamination. Together, the observed associations highlight that sediment microbial communities respond to combined effects of water quality and metal enrichment and can serve as sensitive indicators of subtle ecosystem disturbance.

3.9. Heatmap-based correlation analysis of environmental parameters and microbial OTUs

Heatmap-driven hierarchical clustering showed clear associations between water quality parameters, heavy metal contents, and microbial OTU patterns in the sediment samples. The results revealed that the environmental factors and microbial taxa clustered into distinct groups, indicating non-random co-variation and strong site-specific structuring of the sediment microbial communities.

Several physicochemical parameters such as pH, Alkalinity, correlates with specific OTU clusters. These OTUs were predominantly associated with alkalinity, TDS, TSS, BOD, COD, and nutrient concentrations, exhibited strong positive enriched in sites characterized by elevated organic load and nutrient availability, suggesting that microbial community composition is closely linked to eutrophic and organically enriched sediment conditions, as shown in Fig. 5. Average water quality parameters of sampling sites indicated in Table 3.

Heavy metals like Pb, Cu, Ni, and Zn formed a distinct but partially overlapping cluster with subsets of OTUs, indicating metal-associated microbial assemblages. OTUs co-occurring with elevated metal concentrations were predominantly restricted to specific sampling sites, suggesting localized metal stress or selection pressure within the sediments. The clustering profile suggests a major role of metal-tolerant or metal-associated microbial taxa in site-specific community divergence. Correlation coefficients derived from the standardized dataset demonstrated that several OTUs showed moderate to strong correlations ($|r| \geq 0.6$) with individual water quality parameters and heavy metals, supporting statistically meaningful associations rather than random co-occurrence. Hierarchical clustering of samples showed that sites with similar physicochemical and metal profiles also harbored similar microbial community structures, confirming that environmental filtering shapes sediment microbiomes.

Overall, the heatmap analysis demonstrates that spatial variation in water quality parameters and metal concentrations is closely associated in microbial OTU distribution and abundance, and that microbial communities respond in a structured manner to combined organic and metal stressors in urban freshwater sediments.

Spatial variation in water quality parameters and sediment heavy metal concentrations within Singanallur Lake corresponded with distinct microbial community patterns across sampling sites. Differences in nutrient-related parameters and metal enrichment across the spatially distinct sampling sites (S1–S4) were associated with changes in OTU

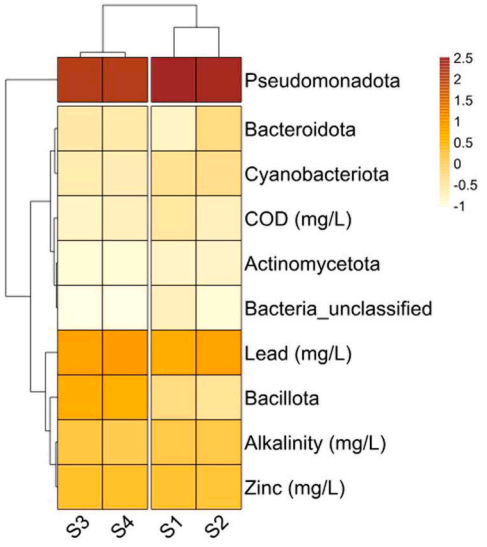

Fig. 5. Heatmap based correlation analysis of water quality parameters and microbial OTUs.

Table 3
Physicochemical parameters of water quality.

Parameters	Sample sites (Avg)
COD (mg/L)	109.25
Alkalinity (mg/L)	355.5
Temperature (°C)	28.3
pH	6.35
BOD (mg/L)	124.25
TDS (mg/L)	966.25
TSS (mg/L)	46
Total Hardness (mg/L)	358
Total Nitrogen (mg/L)	5.725
Total Phosphorous (mg/L)	15.25
Copper (mg/L)	1.8825
Zinc (mg/L)	1.498
Lead (mg/L)	2.1775
Nickel (mg/L)	2.035

abundance and clustering, indicating sensitivity of sediment microbial communities to localized physicochemical gradients within the lake. Although the study does not capture temporal dynamics, the observed associations suggest that microbial assemblages respond to environmental conditions within the lake. These patterns highlight the value of sediment microbiomes as indicators of spatial heterogeneity in water quality under uniform seasonal conditions.

4. Conclusion

This study provides a spatial ecogenomic characterization of the sediment bacterial community of Singanallur Lake, characterized by a stable spatially structured core of dominant phyla Pseudomonadota, Bacillota, Cyanobacteriota, Bacteroidota, and Actinomycetota, which together constitute over 90 % of the relative abundance. Within these, the genera *Caulobacter*, *Bosea*, and *Phreatobacter* were identified as highly adaptable and ecologically relevant groups that are widely reported in freshwater sediments and exhibit physiological flexibility under varying environmental conditions. Meanwhile, the consistent presence of rare genera such as *Akkermansia*, *Helicobacter*, and *Candidatus Saccharimonas* highlights localized ecological variability and potential niche specialization within the sediment microbial community. The occurrence of unique and unclassified taxa within Fimbriimonadaceae and Gemmatimonadaceae highlighted environmental heterogeneity and the impact of urban runoff.

Overall, spatial variation in microbial community composition, diversity indices, and OTU distribution patterns was closely associated with measured water quality parameters and sediment heavy metal concentrations. These associations indicate that sediment microbial communities respond sensitively to present-day physicochemical gradients within the lake rather than providing evidence of long-term or causal environmental impacts.

From a management perspective, integrating microbial community assessments with routine water quality and sediment monitoring can strengthen understanding of spatial ecological variability within freshwater lakes. Regular monitoring of sediment microbial communities using high-throughput sequencing approaches can provide a sensitive framework for tracking changes in lake ecosystem condition over time.

Overall, the results demonstrate that sediment microbial communities function as sensitive indicators of spatial environmental heterogeneity within freshwater lake systems, and their ecogenomic patterns provide a baseline reference for future temporal, functional, and comparative studies aimed at freshwater ecosystem assessment and management.

CRediT authorship contribution statement

Surya Balakrishnan: Writing – original draft, Methodology, Formal analysis, Data curation. **Bharathkumar Rajagopal:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Abiraj Velusamy:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Lathika Shanmugam:** Writing – review & editing, Writing – original draft, Visualization, Data curation. **Boobal Rangaswamy:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Consent to publish

All authors approved the manuscript and gave their consent for submission and publication.

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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.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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