Abstract

This study investigates how environmental variables, with an emphasis on chemical composition, shape microbial communities in river ecosystems across British Columbia. Rivers such as the Fraser, North Thompson, South Thompson, and Thompson are critical to regional agriculture, drinking water supply, and ecosystem function. Microbial communities in these rivers perform essential roles including nutrient cycling, organic matter decomposition, and water purification. However, these communities are sensitive to changes in environmental conditions, particularly pollution and anthropogenic disturbances. This research tracked microbial communities at 26 sites along the mentioned rivers, from near the river source to points just before they discharge into the Pacific Ocean. We hypothesized that sediment chemistry would be a stronger predictor of microbial structure than spatial proximity. Sediment, raw water, and filtered water samples were collected from each site, along with environmental data such as pH and temperature. DNA was extracted from these samples, and 16S rRNA gene sequencing was performed to characterize microbial community composition. Elemental concentrations were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Preliminary findings support our hypothesis that microbial communities at chemically similar sites were more similar in composition than those at geographically adjacent, but chemically distinct sites. Principle Component Analysis on the chemical data revealed clear chemical distinctions between water and sediment samples, and NMDS analysis showed that microbial community structure varied significantly between adjacent sites. Variance partitioning showed that chemical variables explained more variation in microbial community composition than spatial factors. These results highlight the importance of environmental chemistry, particularly sediment composition, as a key predictor of microbial community structure in British Columbia's river systems.

Introduction

Freshwater ecosystems are dynamic environments that support diverse microbial communities, which play essential roles in biogeochemical cycling, nutrient transformation, and overall ecosystem functioning (Newton et al., 2011; Battin et al, 2016). In river systems, microbial communities are particularly sensitive to changes in environmental conditions, making them valuable indicators of ecological health and water quality (Zeglin, 2015). British Columbia, with its vast and varied networks of rivers, ranging from alpine glacier-fed streams to lowland rivers influenced by agriculture and urbanization, provides a unique setting to investigate how microbial communities respond to varying environmental gradients.

Understanding the composition of microbial communities in BC's rivers is especially important given the region's reliance on freshwater resourses. These rivers support agriculture, tourism, forestry, and are a main source of drinking water. Microbial communities support many ecosystem functions that sustain these services (Wilhelm et al., 2015). However, shifts in land use, climate variability, and chemical inputs from natural and anthropogenic sources are altering river chemistry in complex ways, with unknown implications for microbial dynamics and river ecosystem function (Findlay, 2010; Crump et al., 2012).

Among various environmental factors, chemical composition has emerged as a major driver of microbial community structure in freshwater systems (Fierer et al., 2007; Ruiz-González et al., 2015). These chemical variables can directly influence microbial metabolism or alter community composition by shaping competition and niche availability. In British Columbia's rivers, where chemical profiles can vary drastically over space due to differences in geology, land cover, and human activity, a clearer understanding of chemical influences on microbial communities is essential for both predictive ecology and sustainable water resource management.

This study aims to characterize microbial community compositions across rivers in British Columbia and investigate how environmental conditions, particularly chemical composition, influence microbial community structure. Using 16S rRNA gene sequencing and detailed ICP-MS elemental analysis, we are looking to identify key environmental drivers of microbial community diversity, contributing to a greater understanding of river ecosystem health in BC.

Materials and Methods

Sample Collection and Processing

Sediment, raw water, and filtered water were collected from 26 sites along the North Thompson, South Thompson, Thompson, and Fraser Rivers following the WHONDRS sampling protocol (Stegen and Goldman, 2018). The sample locations are shown in *Figure 1*. Sediment was collected about one metre from the shoreline in triplicate subsamples about ten metres apart from each other. Raw water samples were collected in 50 mL Falcon tubes. Filtered water samples were prepared by pushing raw water through a Sterivex filter. The filters were kept for microbial community analysis. The sites were photographed, and various environmental conditions such as pH and water temperature were documented.

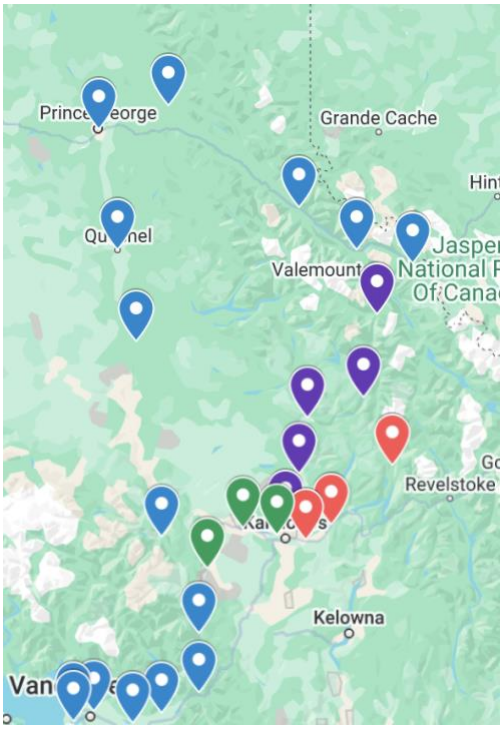

Figure 1. Map of sample sites in British Columbia with blue points representing sites on the Fraser River, purple representing the North Thompson River, red representing the South Thompson River, and green representing the Thompson River.

Microbial Community Analyses

Total community DNA was extracted from 0.5 g of each sediment sample using the DNeasy PowerSoil Pro Kit, according to the manufacturer's instructions, except for the following steps: Step 1: 0.5 g was added rather than 0.25 g; Step 16: add 75 µL of Solution C6. DNA was extracted from the Sterivex filters using the DNeasy PowerWater Sterivex Kit. The kit

instructions were followed exactly, besides the following steps: Step 13: centrifuge at 3000 x g rather than 4000 x g.

Polymerase Chain Reaction (PCR) was completed to amplify the 16S rRNA gene for community sequencing. Each reaction contained 10.0 µL of GoTaq, 1.0 µL of 341F primer, 1.0 µL of 806R primer, 4.0 µL of PCR water, and 4.0 µL of DNA. Thermocycling conditions consisted of initial denaturation of the template DNA at 95.0°C for 4 minutes, 25 cycles of 95.0°C for 30 seconds, 53.4°C for 45 seconds, and 72.0°C for 2 minutes, and final extension at 72.0°C for 5 minutes. PCR products were cleaned using the AMPure XP Beads (Beckman Coulter) according to manufacturer's recommended protocols. The samples were stored at -25°C until further processing.

Second round PCR was then completed on the cleaned samples using a unique forward primer for each sample and an 806R primer with a P1 adaptor. Each reaction contained 10.0 µL of GoTaq, 1.0 µL of barcoded 341F primer, 1.0 µL of 806R P1 primer, 5.0 µL of PCR water, and 3.0 µL of DNA. Thermocycling conditions consisted on initial denaturation of the template DNA at 95.0°C for 4 minutes, 20 cycles of 95.0°C for 30 seconds, 65.0°C for 45 seconds, and 72.0°C for 2 minutes, and final extension at 72.0°C for 2 minutes. PCR products were cleaned using the AMPure XP Beads (Beckman Coulter) according to the manufacturer's recommended protocol. The samples were stored at -25°C until further processing.

Final library concentrations were determined using a Qubit HS DNA assay kit and measured on a Qubit fluorometer (Invitrogen). For the sediment samples, the final concentrations were recorded, and the samples were grouped into three pools (<1.00 µg/mL, 1.00-10.0 µg/mL, >10.0 µg/mL), and filter samples into two (<2.00 µg/mL, >2.00 µg/mL). Gel extraction was used to further remove any excess primers from the pools. qPCR was then performed on these pools to determine more accurate DNA concentrations before sequencing. The qPCR was prepared using the Ion Library TaqMan Quantitation Kit. Using the values determined by this analysis, the volume of each pool required for the sequencing chip was calculated to ensure an equal concentration of DNA from each sample was present. The samples were then sequenced on an Ion S5 platform.

Chemical Composition Analyses

The chemical composition of the sediment and water samples were quantified using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The sediment samples were dried, and 0.2 g of the sediment was weighed out and crushed using a mortar and pestle. The dried, crushed sediment was added to a 50 mL digestion tube, and 4.5 mL of concentrated nitric acid and 0.5 mL of concentrated hydrochloric acid were added. The

tubes were sealed and placed into a Multiwave GO microwave digestion system and run at 120°C for 15 min then 190°C for 15 min to break down the solid organic material and release the elements into solution. Once complete, the samples were filtered using gravity filtration through a filter paper into a 50 mL Falcon tube. The samples were diluted to 50 mL with 2% nitric acid. This process was complete in a triplicate for each sediment sample.

The filtered water samples' elemental compositions were also measured using ICP-MS. The water samples were measured in 15 mL volumes into 50 mL Falcon tubes and were diluted to volume with 2% nitric acid. This process was also complete in a triplicate for each water sample.

To prepare the standards, a stock solution was diluted with water to a 200 ppb intermediate stock solution. The intermediate stock solution was added to 50 mL Falcon tubes according to the volumes in *Table 1*. The standards were diluted with 2% nitric acid to a final 50 mL volume.

Table 1. Volume of 200 ppb intermediate stock solution and 2% nitric acid used to make each standard for ICP-MS analysis.

Standard	Volume of 200 ppb intermediate stock solution (mL)	Volume of 2% nitric acid (mL)
S0	0.000	50.000
S1	0.025	49.975
S2	0.250	49.750
S3	2.500	47.500
S4	12.500	37.500
S5	25.000	25.000

A 1 ppm internal standard was prepared by diluting 0.5 mL a 100 ppm stock solution with 49.5 mL of 2% nitric acid. The instrument was calibrated with an R^2 >0.99 for each run. The samples were then run on the ICP-MS instrument using the parameters shown in *Table 2*.

Table 2. ICP-MS Instrument Parameters

Nebulizer	MicroMist
RF Power (W)	1550
Sampling Depth (mm)	10
Carrier Gas (L/min)	1.04
Nebulizer Pump (rps)	0.1
S/C Temperature (°C)	2
He Gas (mL/min)	5

Extract 1 (V)	0
Extract 2 (V)	-195
Omega Bias (V)	-80
Omega Lens (V)	8.8
Cell Entrance (V)	-30
Cell Exit (V)	-50
Deflect (V)	11
Plate Bias (V)	-35
OctP Bias (V)	-8
OctP RF (V)	190
Energy Discrimination	5

Analysis of Microbial Community Data

Amplicon sequences were processed to generate Operational Taxonomic Units (OTUs). Samples with fewer than 10,000 sequencing reads were excluded from analysis. Beta diversity was assessed using the Bray-Curtis dissimilarity index to evaluate differences in microbial community composition across samples. Non-metric multidimensional scaling (NMDS) was performed to visualize dissimilarities in community structure, allowing for visualization of clustering patterns among sample types and sites.

Species richness, used as a measure of alpha diversity, was quantified by counting the number of OTUs detected per sample. All microbial diversity analyses were conducted in RStudio using the vegan, phyloseq, and ggplot2 packages. Additional multivariate analyses, like redundancy analysis and variance partitioning, were used to explore how environmental and spatial variables explain variation in microbial community composition.

Analysis of Chemical Composition Data

Elemental concentration data from sediment and filtered water samples were obtained using ICP-MS. Principal Component Analysis (PCA) was performed to reduce dimensionality and identify major chemical gradients across sites. PCA results were visualized using scores plots, allowing for comparison of chemical similarities and differences across sites and between sample types. The resulting PCA axes were then used as explanatory variables in subsequent multivariate models to identify relationships between chemical composition and microbial community structure.

Results

Microbial Community Composition

Beta diversity of the microbial community structure in each sample was assessed to explore variation in microbial community composition across sites using the Bray-Curtis dissimilarity index. The results were visualized using a NMDS plot, as shown in *Figure 2*. The results showed the water samples had a distinct microbial community composition compared to the sediment samples, with the water samples all being clustered in the bottom two quadrants on the NMDS plot. Most of the sediment samples were clustered together in the upper left quadrant, with some sediment samples much further away, suggesting that the sediment samples had a greater variation in microbial community composition.

There is no clear pattern of microbial composition within the same river; however, sites such as Moose (near the source of the Fraser River) and William's Lake (further downstream on the Fraser River) showed low species richness and similar microbial community composition despite geographic separation, highlighting that spatially distant samples can share similar community characteristics.

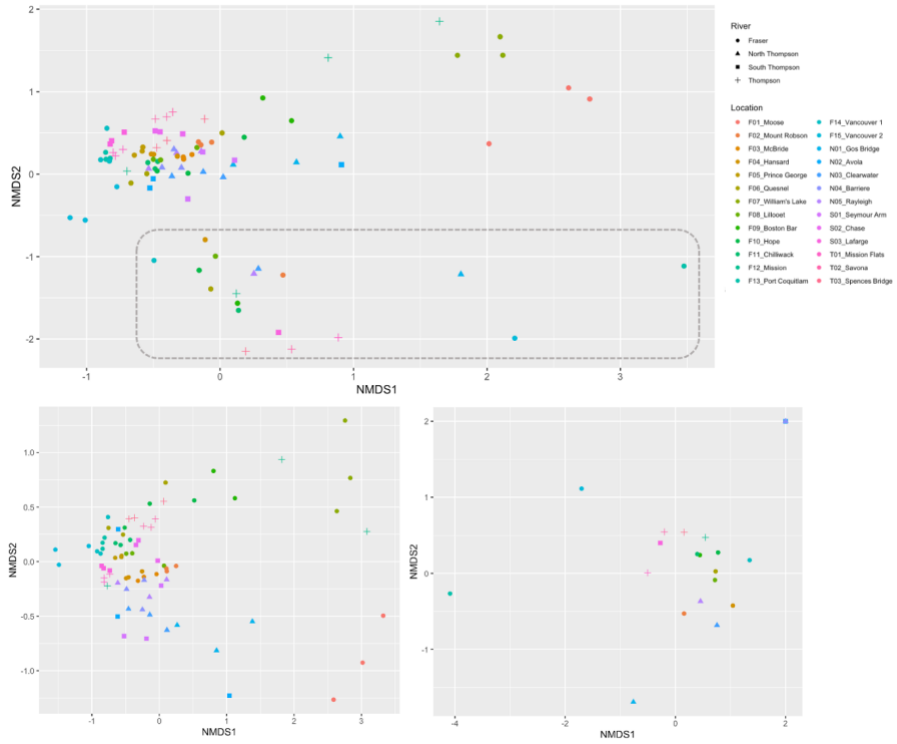

Figure 2. Multidimensional scaling plot illustrating the Bray Curtis dissimilarity in the microbial community composition of all samples with water samples outlined in grey (top), sediment samples (bottom left), and water samples (bottom right).

Species richness varied widely between sediment samples at sites located close together on the same river, as shown in *Figure 3*. Moose and William's Lake had the lowest species richness (<200 taxa), yet showed high similarity in species composition, consistent with their NMDS clustering.

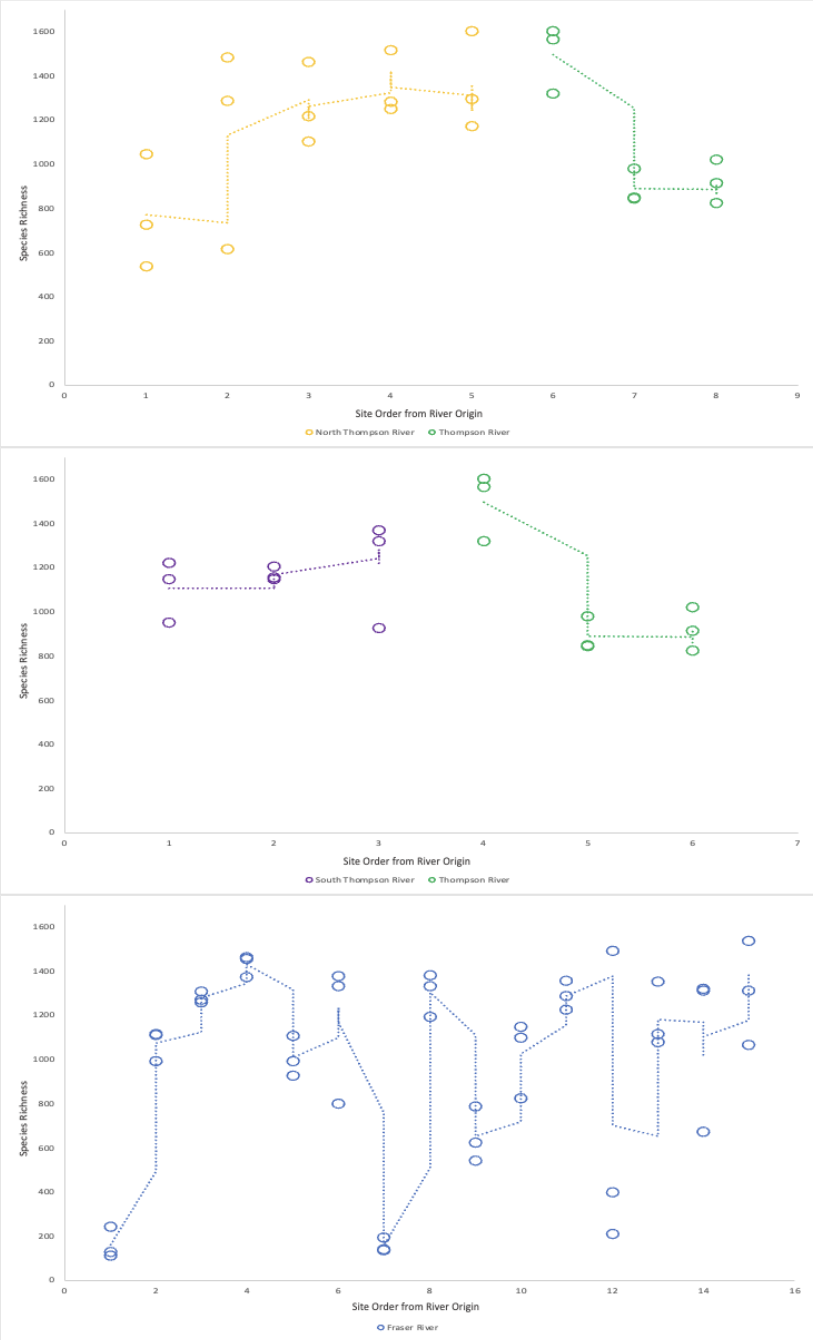

Figure 3. Species richness of sediment samples arranged from upstream to downstream.

Chemical Composition

PCA was used to reduce the dimensionality of the chemical data set, allowing us to visualize the most important patterns and correlations od the chemical composition of the sediment and water samples between each site. The Scores Plots were visualized as shown in *Figure 4* to observe which sites have similar and different chemical compositions.

Figure 4. Scores plots illustrating the PCA results of the chemical composition of all samples with water samples outlined in grey (top), sediment samples (bottom left), and water samples (bottom right).

Microbial Composition Drivers

Variance partitioning based on redundancy analysis was used to determine how much variation in microbial community composition could be explained by spatial variables (PCNM scores derived from site locations) and chemical variables (PCA scores derived from element concentrations).

As shown in *Figure 5,* 28% of the variation in community data could be explained by spatial and chemical variables: 10% explained by spatial data alone, 14% explained by chemical data alone, and 4% explained by both datasets simultaneously.

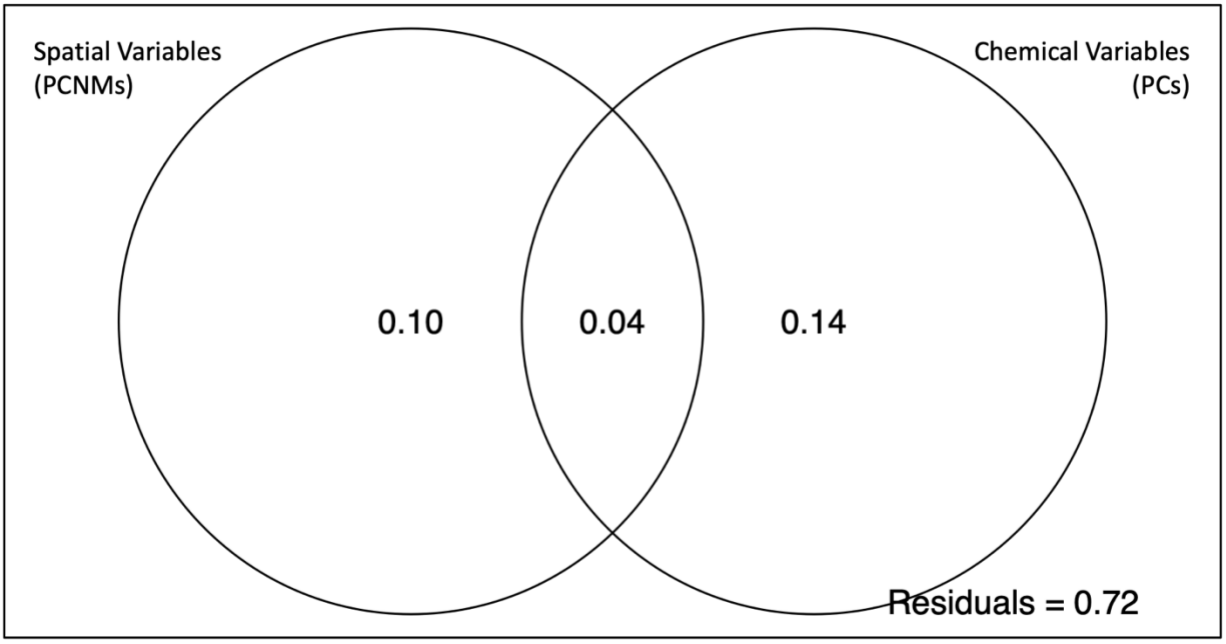

Figure 5. Variance partitioning based on redundancy analysis showing the influence of spatial and chemical factors on microbial community composition.

Discussion

This study highlights the critical role of chemical composition, especially sediment chemistry, in shaping microbial community structure across river ecosystems in British Columbia. Our results support the hypothesis that chemical similarity, rather than spatial proximity alone, is a primary driver of microbial community composition.

The NMDS and species richness analyses confirmed distinct microbial communities between sediment and water, which is consistent with prior work noting that sediment habitats accommodate more stable and diverse microbial populations compared to the often more transient and homogenous communities in the overlying water (Zeglin, 2015; Newton et al., 2011). Sediment environments provide physical structure and sustained nutrient availability, which can support more complex communities, as seen in the wide range of species richness observed in the sediment samples.

Interestingly, two sites on the Fraser River, Moose and William's Lake, showed both low species richness and high similarity in microbial community composition, despite being geographically distinct. This finding suggests that environmental factors can outweigh spatial distance in determining microbial structure (Fierer et al., 2007). It also raises the possibility that these sites are less impacted by anthropogenic disturbances or share similar chemical baselines due to geology or land use.

Our PCA results further demonstrate that water and sediment samples exhibit distinct chemical profiles. Sediment chemistry is influenced by both upstream geological inputs and the accumulation of metals and nutrients from surrounding land use. Water chemistry; however, is more dynamic and reflects short-term environmental changes. These chemical differences likely contribute to the observed difference in microbial communities between sample types.

Variance partitioning analysis confirmed that chemical variables explained more variation in microbial community structure than spatial variables. This aligns with prior studies indicating that river microbial communities are shaped by complex environmental interactions rather than dispersal limitations alone (Ruiz-González et al., 2015; Crump et al., 2012).

Implications

Understanding the chemical drivers of microbial community structure has important implications for watershed management. Microbial communities are essential for functions like organic matter decomposition, nitrogen cycling, and contaminant degradation. By identifying chemical composition as a key determinant of microbial community compositions, this study offers a potential method for predicting ecosystem function based on chemical monitoring.

Future Work

There are several limitations in this study. First, this study relied solely on 16S rRNA gene sequencing, which provides information about community composition, but not function. Future research using metagenomic or metatranscriptomic analyses could provide a more detailed understanding of the metabolic capacities of these communities.

Second, while ICP-MS enabled broad elemental analysis, key biogeochemical elements such as nitrogen, phosphorus, and sulfur were not included. These nutrients are essential to microbial growth and could further clarify the drivers of community variation.

Third, the cross-sectional sampling protocol limits insight into seasonal or event-driven variability that could significantly influence both chemical composition and microbial populations.

Finally, spatial analyses did not account for the possibility that two sites may be geographically close but located on different rivers. By considering the differences in river type and spatial distribution, future analyses could more effectively compare the datasets to identify potential regional or river-specific patterns.

Conclusion

Our results suggest that chemical composition, particularly in sediment, is a stronger predictor of microbial community composition than spatial proximity alone. Microbial communities were more similar between chemically analogous sites, even if separated by large distances. This finding supports our hypothesis and highlights the importance of environmental chemistry in freshwater microbial ecology, offering a potential method for predictive ecosystem monitoring.

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