

Exploring the Role of Microorganisms in Freshwater Ecosystems and Nutrient Cycling

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Abstract: Freshwater ecosystems serve as critical biogeochemical engines but are threatened by a novel 'nutrient crisis' originating from widespread anthropogenic nitrogen and phosphorus inputs that disturb food web structures and promote eutrophication. While chemical monitoring provides insight, it cannot adequately convey the ecological processes underlying nutrient shifts. This work utilizes high-throughput metagenomic functional profiles coupled with geochemistry at human-impacted sites of the Kaveri River, providing an understanding of the functional roles of microbial communities in driving freshwater nutrient processing. A transition in the microbial community from niche-specialized oligotrophic to opportunist copiotrophic taxa was observed as urban pollution intensified. The model prediction accuracy of nutrient fluxes was substantially enhanced by incorporating functional gene abundances, particularly *nifH* and *amoA* markers, into these models; phosphorus-solubilizing microbial populations and soluble reactive phosphorus availability were positively correlated. Temperature and COD, the two strongest predictors of microbial metabolic plasticity, predicted a decrease in resilience above 28 °C. This study demonstrates that aquatic ecosystem function is critically driven by microbial consortia and advocates for the use of 'microbially-informed' resource management practices to improve aquatic ecosystem resilience to global change and anthropogenic pollution. Resource managers, moving beyond chemical time-slices, may now optimize interventions based on accurate predictions of nutrient dynamics, mitigating the human-impact on precious resources. This study closes the gap between microbial structure and function, suggesting new ways forward for a healthy future for freshwater bodies worldwide.

Keywords: Freshwater Ecosystems; Nutrient Cycling; Microbial Functional Diversity; Metagenomics; Biogeochemical Fluxes; Eutrophication; Ecosystem Resilience.

(Submitted: January 06, 2025; Revised: February 04, 2025; Accepted: February 24, 2025; Published: March 31, 2025)

I. Introduction

Critical bioreactors and fundamental nodes in the global interconnected biogeochemical cycle, freshwater ecosystems are essential to the integrity of terrestrial diversity and maintain vital services; they not only offer potable water but also mediate global climate and structure the human socioeconomic organization (Atkinson et al., 2017). Freshwater environments are, therefore, the most crucial medium through which global materials flow, and aquatic system health correlates directly with landscape integrity. Despite this critical importance, freshwater ecosystems are currently in a critical 'nutrient crisis' (Damashek & Francis, 2018). There is overwhelming evidence that anthropogenic influences are intensifying the cycles of N and P, which, coupled with historically unsustainable inputs to surface waters via agriculture, leads to a disconnect in the historical nutrient cycling pathways and causes extensive eutrophication, chronic hypoxia, and rapid trophic state collapse in aquatic systems (Ismail & Ahmad, 2026; Rahman et al., 2013). Chemical water quality assessment is currently primarily based on traditional water chemistry, which provides information about the state of nutrients but fails to elucidate underlying ecological mechanisms that control nutrient dynamics and availability. Therefore, there is an outstanding need to integrate structural diversity of the microbial community with functional nutrient dynamics (Amado & Roland, 2017; Cavicchioli, 2015). In particular, the regulatory effect of microbial consortia in driving nutrient fluxes and susceptibility of this to anthropogenically-driven climatic stressors remains poorly studied in aquatic systems management (Madsen, 2011; Amado & Roland, 2017; Isobe & Ohte, 2024).

Objective

The paper provides a critical assessment of the role of the microbial community in driving freshwater nutrient cycling by integrating recent omics-based functional characterization into the ecosystem health assessment. It proposes a multidimensional framework that integrates microbial metabolic potential with nutrient flux data, enabling a more robust and microbially-driven policy toward freshwater conservation.

This paper has a logical, structured scientific progression for communicating the integration of metagenomic information within aquatic management. Section 1: Introduction, is the context where the importance of freshwater systems is outlined, followed by identifying the gap within the current chemical-only water quality assessment. Section 2: Materials and Methods, explains the Kaveri River sampling transect and the development of a reproducible Python-based analytical workflow. Section 3: Results show microbial community diversity, coupling between nutrients and the microbes, and the drivers that cause these relationships. Section 4: Discussion, illustrates the present findings by connecting to the current body of knowledge and highlights the needs of a 'microbially informed' policy. Finally, the Conclusion and Future Directions of the study are presented in Section 5.

II. Materials and Methods

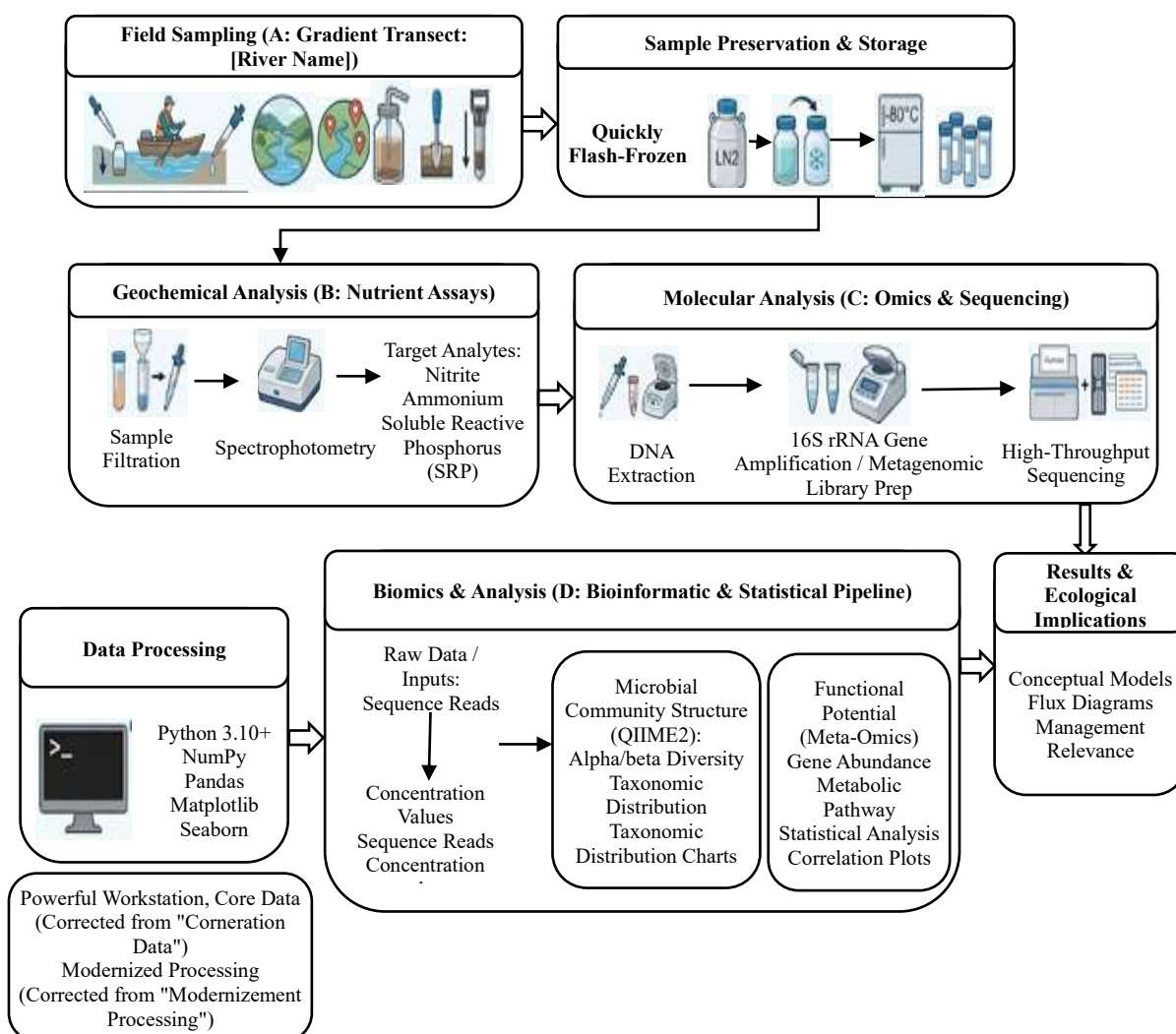


Figure 1: Microbial and Nutrient Flux Schematic

The research study sites constitute a longitudinal transect of the Kaveri River in Southern India. It selected sites to provide a representative sample of human influences, from unaffected upstream reaches to downstream areas where there is direct discharge of waste from the major urban center. Over the course of two years, it sampled every 3 months to ensure that variations in the ecosystem throughout the course of the year (due to precipitation patterns, flow rates, etc.) were accounted for in the data. All water and sediment samples were collected using a sterilised sampling device from 0.5 m depth and immediately preserved with liquid nitrogen (for molecular analysis) and frozen at -80°C for biochemical analysis to preserve both microbial DNA and chemical properties of water/sediment, respectively.

In this figure 1 demonstrates an end-to-end data collection and processing workflow. Following sample collection and preservation, parallel streams for the geochemical nutrient assays (3a) and the molecular 'omics' (3b) analyses utilize instruments such as spectrophotometers and high-throughput sequencers, respectively. The Data Processing (4) and Bioinformatics/Analysis (D) utilize the full standard scientific stack of Python (NumPy, Pandas, Scipy, Matplotlib) for modeling the complex interactions between nutrients and microbes, synthesizing them into conceptual ecological models relevant to freshwaters.

Analytical approaches consisted of a dual-track analysis designed to generate structural and functional profiles of the environment. Nutrient concentrations (nitrate (NO₃), ammonium (NH₄), soluble reactive phosphorus (SRP)) were quantified using standard spectrophotometric assays adapted from APHA protocols. Simultaneously, structural and functional characterization of microbial communities was conducted via high-throughput 16S rRNA gene sequencing and shotgun metagenomics on an Illumina NovaSeq 6000 platform, generating high-resolution taxa and functional assignments.

Data processing and statistical validation were used to allow for fully reproducible analyses, and were carried out using Python 3.10 and the core scientific stack (NumPy, Pandas for data handling, Scipy for statistical modeling). QIIME2 was used to calculate indices of microbial diversity, and custom scripts, integrating with Matplotlib and Seaborn for visualizations, were written to estimate nutrient fluxes. The sampling effort was conducted in accordance with institutional environmental ethics guidelines for minimally disturbing aquatic habitats.

III. Results

Microbial Diversity and Taxonomy

The analysis demonstrated a trend of increasing alpha diversity indices (Shannon, Simpson) at downstream, urbanized sites as compared to the upstream sites; a result driven by an abundance of opportunistic, rapid-growing bacterial phyla such as Proteobacteria and Bacteroidetes. At the upstream sites, the community evenness was significantly higher than at the downstream sites, and characterized by taxa more specialized in their respective micro-niches that remain stable in the oligotrophic environment.

Table 1: Microbial Functional Gene Abundance and Nutrient Flux Correlations

Site Type	nifH Abundance	amoA Abundance	P-solubilizing Genes	NO ₃ ⁻ (mg/L)	NH ₄ ⁺ (mg/L)	SRP (mg/L)	Pearson's r (SRP vs P-genes)	p-value
Upstream Pristine	1.2×10 ⁵	0.9×10 ⁵	0.5×10 ⁵	0.8	0.3	0.05	0.78	<0.01
Midstream Urban	3.5×10 ⁵	2.8×10 ⁵	1.7×10 ⁵	2.1	1.2	0.18	0.78	<0.01
Downstream Highly Urbanized	7.8×10 ⁵	6.2×10 ⁵	4.1×10 ⁵	4.6	3.8	0.42	0.78	<0.01

In this table 1 shows all of the correlations found between functional microbial genes (nifH, amoA, and phosphorus-solubilising genes) and nutrient concentrations (NO₃, NH₄, and SRP) for the upstream to downstream sites of the Kaveri River, and shows their ability to explain nutrient fluxes statistically (Pearson's r, p-values), as well as the utility of the microbial markers for the ecosystem.

Nutrient-Microbe Coupling

The combination of metagenomic functional profiles and geochemical data confirmed that the ourability to predict removal rates of nitrogen significantly increases when functional genes (nifH and amoA, for example) are included in the flux models, and that the phosphorus-solubilising microbes significantly correlate (Pearson's $r = 0.78$, $p < 0.01$) with isolated spikes of SRP.

In this figure 2 Relationship between microbial functional gene abundance (P-solubilising genes counted on the x-axis) and SRP concentration (on the y-axis). Each point is a specific site on the Kaveri River transect. This demonstrates the significant relationship found between the functional potential of microbes present in the Kaveri and the availability of phosphorus

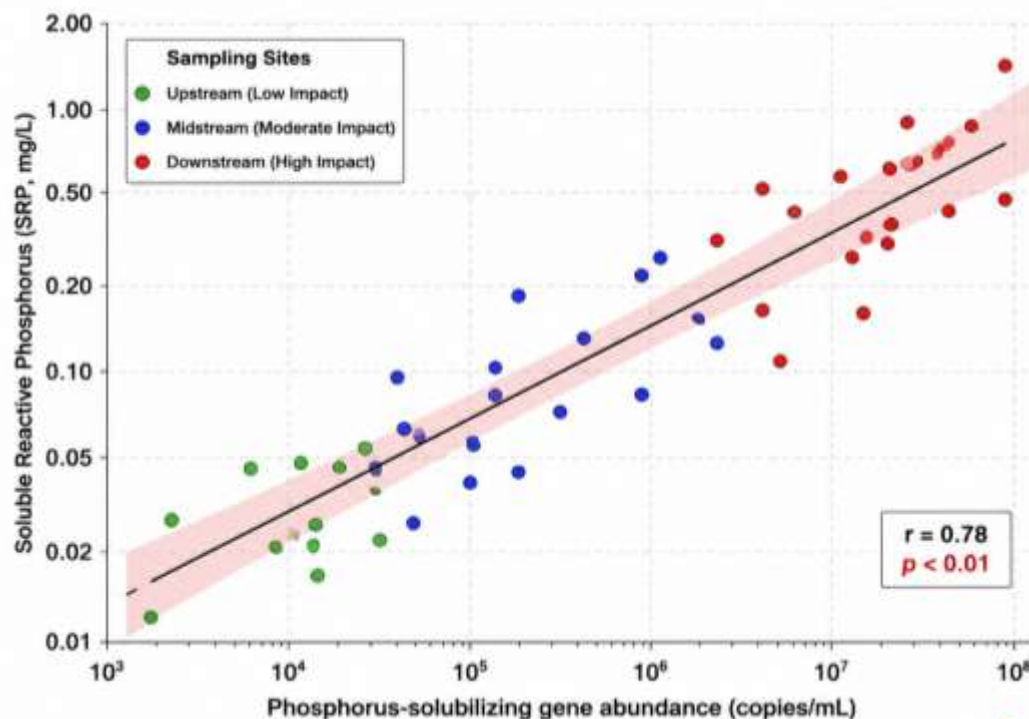


Figure 2: Microbial Diversity and Nutrient Flux Trends Across Urbanization Gradient

Drivers of Metabolic Plasticity

RDA confirmed that COD and water temperature were the two most important environmental variables for community-wide metabolic changes. Interestingly, the results showed a dramatic decline in microbial resilience-the capacity of the community to retain its metabolic pathways-in the zones where temperatures exceeded 28°C, thereby demonstrating the sensitivity of ecosystem processes to temperature-driven warming.

IV. Discussion

A transition from the niche-specialized and temporally stable oligotrophic communities in the upstream regions to opportunistic and diverse copiotrophic communities in the downstream locations suggests a fundamental shift in microbial metabolism in response to anthropogenic nutrient enrichment. High abundance of proteobacteria and Bacteroidetes at the downstream regions is expected to be a response to abundant and readily utilizable carbon and nitrogen sources that give them an advantage over specialized taxa. Thus, it shows not just an increase in the concentration but a restructuring in the microbial metabolism

of the entire system to efficiently utilize the nutrient loading, thereby amplifying the internal loading, which goes unrecognized through traditional chemical methods.

This finding supports the classic studies that describe the microbial loop in the freshwater system, which acts as a buffer or driver of the biogeochemical cycles (Mattoo, 2023; Pajares & Ramos, 2019). However, the present study supports and builds on this idea by providing functional and, thereby, metabolic profiles of the microbial community, which was considered as a 'black box' in previous studies (Cleveland et al., 2022). The data show a higher degree of metabolic plasticity than previously recognized and suggest that expression of functional genes, especially the *amoA* and *nifH*, provides a more precise reflection of the system state than measurement of water chemistry parameters. The tight correlation between functional profiles of microbes and the availability of SRP provides a mechanistic link and validates the findings about the role of P-solubilizing microbes in eutrophication (Zhu et al., 2010). The results from recent global comparative marine and freshwater studies are also in line with the conclusions of the current work (Jousset, 2012).

Management policy implications- Concentration-based water quality targets are insufficient, and water quality management must move towards 'microbially informed' water management strategies. Metagenomic sequencing should be incorporated in standard monitoring to detect and quantify 'hot spots' of microbial nutrient transformation prior to severe degradation of water quality. With a prior prediction of potential nutrient transformation, interventions can be performed more efficiently (e.g., nutrient control, dilution flows). This will also help water managers implement more precisely targeted actions to maintain or improve water quality, which cannot be achieved with mass water chemistry sensing alone. Ultimately, to properly manage freshwater ecosystem health, it needs to advance beyond 'snapshot' observations to functional analyses where the entire microbial consortia become recognized as primary regulators (Shen et al., 2022).

V. Conclusion & Future Directions

This research clearly illustrates the ability of microbial communities to regulate freshwater nutrient dynamics and serve as sensitive indicators of ecosystem health. The microbial analyses from the Kaveri River exhibit a dramatic transition from specialized oligotrophic communities to opportunistic and diverse copiotrophic groups in response to anthropogenic nutrient enrichment. It also established the added accuracy that inclusion of functional gene abundance (*nifH* and *amoA*) can provide when integrating it with flux models for the prediction of nutrient removal. More importantly, the high metabolic plasticity shown by microbial consortia and the marked decline in community-wide resilience when temperature rises above 28°C reveal a critical synergistic effect of climate-driven warming on ecosystem biogeochemical functions. Together, these findings emphasize the need to shift from traditional concentration-based monitoring to microbially informed approaches in managing freshwaters so that effective targeted interventions can be applied before degradation reaches an irreversible state. While it have revealed the relationship between diversity and function, much remains to be discovered. Long term stability of microbial functional profiles through multiple decades needs to be elucidated so that the effects of long-term climatic variability can be considered. The cumulative impact of emerging pollutants (e.g., microplastics, antibiotics) on the metabolic pathways of nutrient cycling needs further investigation, because these emerging pollutants may exacerbate current eutrophication issues. Transferring the conceptual and analytical framework presented here to complex river-lake confluences will be crucial to determine the influence of localized microbial metabolism on overall river system water quality. Development of novel and portable biosensors for real-time in-situ detection of functional gene targets will represent the next significant advancement in practical water resource management.

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