Role of aquatic fungal species in decomposition and nutrient cycling in freshwater ecosystems

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Abstract

Fungi in freshwater ecosystems help decompose organic matter and recycle nutrients. Hyphomycetes and Chytrid fungi assist in the conversion and decomposition of complex plant polymers. Still, the structure, functions, and diversity of these communities and the aquatic fungi in general remain poorly understood, particularly in relation to climate and hydrology. Integrated metagenomics–enzymatic activity assessment aims to identify which of the principal aquatic fungal taxa decompose leaf litter and regulate nutrient flow. Water and sediment samples were collected from freshwater bodies to analyze fungal community composition through high-throughput ITS sequencing. Fungal decomposition was assessed using laccase, cellulase, and phosphatase assays, and for nutrient analysis, the results were assessed for biogeochemical cycling using the C: N:P ratio and total dissolved organic matter. The dominant decomposers were Ascomycota and Chytridiomycota, and the results showed strong correlations between enzymatic activity and nutrient release. Changes in fungal diversity and nutrient transformation were primarily anthropogenic and seasonal. This research highlights the functional role of aquatic fungi in energy flow and nutrient decomposition in freshwater systems and the importance of including them in the nutrient and health assessment of freshwater ecosystems.

Keywords: Aquatic fungi, Nutrient cycling, Decomposition, Freshwater ecosystems, Metagenomics, Enzyme activity, Biogeochemical processes, Microbial ecology

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Introduction

Fungi are very important to the decomposition cycle and the recycling of nutrients in a freshwater ecosystem. They are the primary colonizers and biochemical generators of land-derived materials like leaf litter and woody debris (Suberkropp and Klug, 1980; Gessner, Chauvet and Dobson, 1999; Abelho, 2001). In flowing freshwater bodies like streams and rivers, the hyphomycetes fungi, previously known as the slime fungi, proliferate decomposition and twig retention through the secretion of extracelluar enzymes like laccases, peroxidases, celluloses, and phosphatases that breakdown and modify the complex polymers of lignin, cellulose, and hemicellulose (Suberkropp and Klug, 1980; Gulis, Suberkropp and Rosemond, 2008; Arregui *et al.*, 2019). They change the particulate detritus through decomposition and become dissolved organic matter. This increases access to dissolved and fine particulate matter that microbes and invertebrates can utilize. Apart from the decomposition of organic matter, numerous aquatic fungi also transform and directly affect decomposition at the molecular level. These include the genes for nitrogen reduction and assimilation (nitrate reductase, NiR) and other lignin-modifying, multicopper oxidase (laccase/lcc) genes, as well as glycosyl hydrolase (cellulase family) genes involved in cellulose chain degradation. Numerous studies have documented the inter- and intra-specific variabilities of these genes, and decomposition rates and stoichiometric patterns of nutrient release correlate with (Mariz *et al.*, 2021; Solé *et al.*, 2012; Viswanath *et al.*, 2014). Oil spills can disrupt fungal and microbial communities, decomposing organic matter and nutrient cycling in aquatic ecosystems (Nagappan *et al.*, 2025).

Fungal adaptations under stress, especially relating to nutrient cycling, may resemble those seen in microbial populations focusing on the regulation of efflux genes and biofilm formation (Anagnostopoulos and Varga, 2021). Extreme tourism as an anthropogenic pressure aggravates ecosystem instability, consequently affecting the diversity of fungi and their role in the decomposition of organic matter (Ganieva *et al.*, 2024).

The role played by fungi in conditioning leaves (changing the chemistry and structure of leaves to make them more palatable and nutritious to shredding invertebrates) connects the processes of decomposition to the transfer of energy to higher trophic levels and the functioning of the entire ecosystem metabolism. It connects microscale biochemical changes to macroscopic changes in the flow of energy and the cycling of nutrients in the system (Gessner, Chauvet and Dobson, 1999; Barros, Ben Tanfous and Seena, 2024). Fungal community composition and associated enzyme activity predict the rate and the route of litter decomposition. Nutrient enrichment, acidification, and fungicide exposure as environmental stressors can change decomposition fungi, reduce key enzyme activities such as laccase and phosphatase, and shift the C: N:P metabolic ratio in ways that primary productivity and the stability of food webs can be severely impacted (Anagnostopoulos and Varga, 2021; Gulis, Suberkropp and Rosemond, 2008; Ferreira and Guérold, 2017). With

molecular approaches such as internal transcribed spacer (ITS) metabarcoding, metagenomics, metatranscriptomics, and targeted gene assays, a considerable amount of previously undocumented diversity in aquatic fungi has been documented. The discovery of functional gene lcc (ligninase) paralogs with differential regulation, and variable nitrate reductase alleles among Tetracladium, Tricladium, Clavariopsis, and other chytrid lineages, indicates the morphologically similar fungi that differ in decomposition rate and efficiency across ecosystems may be functionally distinct (Mariz *et al.*, 2021; Piscitelli *et al.*, 2011; Osono, 2020).

Yet, not all knowledge gaps have been closed. For example, the role of non-hyphomycete fungi, such as chytrids and early-diverging fungi, in the production of DOM and the mineralization of nutrients is unexplored. Additionally, complex results scaling from microcosms to the entire ecosystem are caused by seasonal changes, hydrological heterogeneity, and interspecific competition. The functions of certain decomposers in different ecosystems are also unexplained, particularly the fundamental and regulatory networks that control the expression of decomposition genes (Graça, Hyde and Chauvet, 2016; Deep *et al.*, 2025). Closing these gaps in knowledge requires the use of integrative techniques that include field-based litter decomposition, description of enzymatic activities, stable isotope tracing, and next-generation sequencing strategies that earlier sculpted the study of the freshwater system in the contextualization of fungal community patterns, gene expression, and nutrient flow.

The rest of the paper is structured as follows. Section 2: Literature review. Section 3, Materials and proposed methodology, including the sampling strategy, enzymatic and molecular assays, and the data analysis pipeline, is detailed. Section 4: The results are presented with an emphasis on fungal diversity, enzyme activities, and patterns in nutrient cycling. Section 5 concludes with the key insights, implications on management, and suggestions for future research in the field, with a focus on integrated metagenomics and long-term ecological monitoring premised on the structural foundation laid in the previous sections.

Literature Review

Aquatic hyphomycetes and chytrid fungi are essential decomposers in freshwater systems, as they facilitate mineralization of organic matter and release bioavailable nutrients (Suberkropp and Klug, 1980). These fungi start enzymatic degradation of organic matter by colonizing tattered leaves and other detrital plant parts, and then secreting ligninolytic and cellulolytic enzymes. Early research showed that, by enhancing microbial conditioning of leaves, the aquatic hyphomycetes fungi increased the leaves' palatability to detritus-eating invertebrates, thus accelerating the flow of energy along the aquatic food webs (Gessner, Chauvet and Dobson, 1999). In addition, the breakdown of materials done by these fungi assists the freshwater systems in the productivity of the ecosystems and the cycling of the biogeochemicals of carbon, nitrogen, and phosphorus, including the other freshwater system biogeochemicals (Anagnostopoulos and Varga, 2021).

The community diversity and community-level metabolic potentials have also taken molecular research into consideration with high throughput sequencing and functional gene studies. For example, metagenomic research on freshwater ecosystems has characterized a diversity of fungi containing functional genes for laccases (lcc1, lcc2) and cellulases (celA, celB) and thus, can perform enzymatic oxidation and polysaccharide decomposition. This demonstrates a relationship between nutrient cycling at the ecosystem level and functional diversity within these fungi. Decomposition also relies on other variables such as temperature, pH, and nutrient enrichment. A study on temperature with tropical streams showed that nutrient enrichment and the composition of fungal stream communities can imbalance carbon and nutrient sequestration totally.

The research on the role of fungi within the freshwater ecosystems has shown the fine integration of eco-hydrology and bio-geochemistry that these organisms perform. The gaps that still exist are the identification of specific genetic drivers of catalytic diversity, the enzymatic contributions of temperate stream fungal assemblages to nutrient turnover, and the impacts of multiple co-occurring stressors on the rate of fungal decomposition. The integration of research from enzymatic decomposition assays, molecular gene profiling, and biogeochemical modeling is required for the integration of diverse fungal systems within aquatic nutrient cycles.

Methodology

Study Area and Sampling Design

The research represented a study across three freshwater ecosystems differing in trophic and hydrological conditions: a forested headwater stream, an agricultural pond, and an eutrophic lake. In order to study seasonal variation in fungal activity, the research employed three sampling campaigns during the six months encompassing the pre-monsoon, monsoon, and post-monsoon periods. To achieve this, three replicates of water, sediment, and leaf-litter samples were taken from each site using sterilized containers and leaf-bag traps (mesh: 1 mm). To retain the integrity of microbial alteration and ensure samples were processed in the laboratory the same day, they were kept cold (4°C). In-lab samples were assessed for the physicochemical conditions of temperature, pH, dissolved oxygen (DO), and nutrients (NO_3^-, PO_4^{3-}, and DOC) in accordance with the guidelines set by the American Public Health Association (APHA).

Fungal Isolation and Identification

Fungal colonization on decomposing leaf litter was assessed with the leaf-disc incubation technique. Aseptic transfers of colonized discs were made to Potato Dextrose Agar (PDA) plates to which chloramphenicol was added (50 mg/L) to suppress bacterial growth. Fungal colonization was assessed using simple light microscopy to study conidial structures, and for molecular identification, the internal transcribed spacer (ITS) region was sequenced using primers ITS1 and ITS4. Operational taxonomic units (OTUs), and their associated diversity indices, were extracted using Qiime 2 and compared to the UNITE fungal database.

Enzymatic Activity Assays

Extracellular enzyme activities have been conducted to study the effectiveness of fungal decomposition. Laccase, cellulase, and acid phosphatase activities were measured by spectrophotometry. Laccase activity was assessed with the substrate ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)), and cellulase activity was assessed by the DNS (3,5-dinitrosalicylic acid) method. Enzyme activities were expressed as U (international units) per mL of culture filtrate, calculated by the following:

$$E = \frac{\Delta A \times Vt}{\in X\ l\ x\ V_s\ x\ t} \quad (1)$$

Where:

E = Enzyme activity (U/mL)

ΔA= Change in absorbance per minute

Vt = Total reaction volume (mL)

ε = Molar extinction coefficient (M^{-1} cm^{-1})

l = Path length (cm)

Vs = Sample volume (mL)

t = Time (min)

Leaf Litter Decomposition Rate

The exponential decay model was used to explain the mass loss over time for the leaf packs. Each site had leaf packs. After the decomposition period, the leaf packs were dried at 60 °C, and the mass was recorded.

$$k = \frac{t\ln(W0) - \ln(Wt)}{t} \quad (2)$$

The value of the decomposition rate constant (k, day^{-1}) indicates how fast the organic matter decays. It is determined through the initial dry mass of the litter (W_0, g) and the dry mass after time (W_t, g) during the incubation period (t, days) and gives a measurement for the organic matter decomposition period. The analyzed k values shows the efficacy of decomposition, assisted by fungi in different seasons in different areas and shows organic matter decomposition rate, and the ecosystem function in regard to nutrient maintenance.

DNA Extraction and Functional Gene Analysis

The Qiagen DNeasy PowerSoil Kit was utilized to extract genomic DNA from sediments and decomposed litter. For some genes related to decomposition and nutrient cycling, PCR amplifications were conducted for laccase (lcc1, lcc2), cellulase (celA), and nitrate reductase (niaD). Amplicon sequencing was completed on an Illumina MiSeq (2 × 300 bp). For sequence quality control, chimera removal, and OTU clustering, the Microsoft QIIME2 software was used. For the pathways and enzymes associated with the genes discussed, the KEGG database and FUNGuild were used to elaborate on the carbon and nitrogen cycling.

Nutrient Analysis and Correlation Studies

Nutrient levels in the samples were analyzed with UV-Vis spectrophotometry. TOC analyzers were used to estimate dissolved organic carbon (DOC) concentrations. Total nitrogen (TN) and total phosphorus (TP) were estimated using the persulphate digestion method and the molybdenum blue method, respectively. Activities of fungal enzymes, rates of nutrient release, and gene abundances were correlated and analyzed using Principal Component Analysis (PCA) in R.

Overview of the Study Framework

This research combines different ideas about how aquatic fungi help decompose organic matter and recycle nutrients in freshwater ecosystems. To start, I

sampled different freshwater ecosystems, especially forest streams, wetlands, and agricultural drainage systems. In the next step, I isolated the fungi and used molecular methods to identify the Internal Transcribed Spacer (ITS) region. Then, each fungus sample was assessed to determine whether it could enzymatically decompose laccase and cellulase.

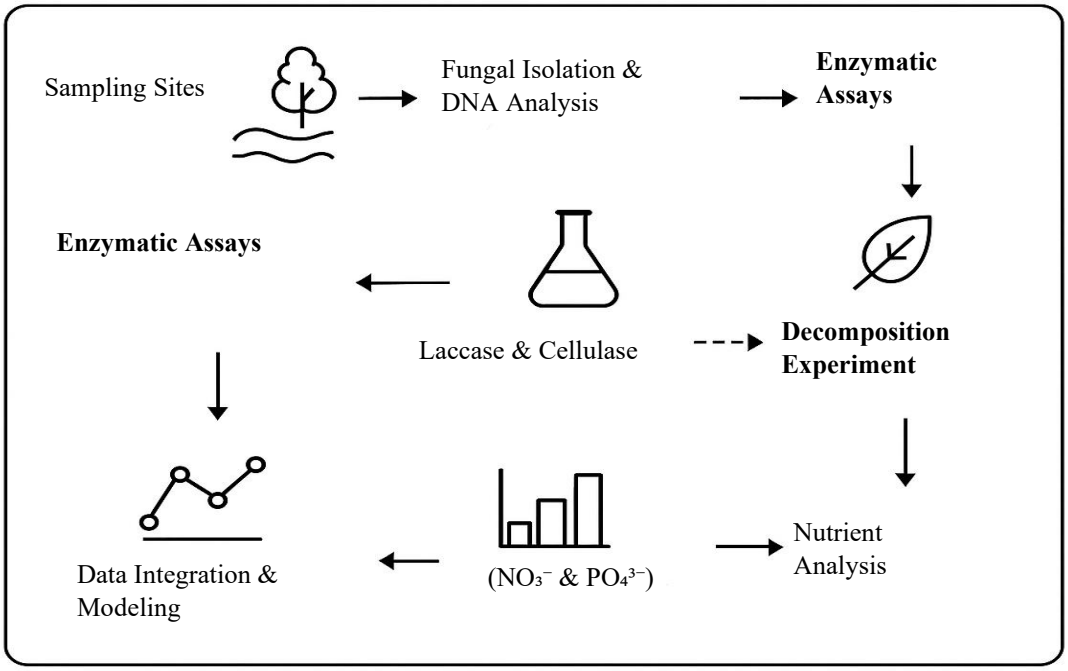

Figure 1: Experimental flowchart of fungal isolation, enzymatic activity, and nutrient analysis.

For the biochemical analyses, the monetary value of the released nitrogen and phosphorus, as in the mineralization of the nutrients, was determined based on the decomposition experiments and the findings of enzyme kinetics (Figure 1).

To identify and characterize key functional genes, lcc1, celA, and narG, and then assess the relationship between molecular diversity and ecological function, the use of metagenomic data was indispensable. This demonstrated the integration of diverse areas of research. It combines field and laboratory research, and remote-sensing computation, and takes a systems perspective to assess nutrient cycling in freshwater fungal ecosystems.

Data Integration and Statistical Analysis

The results are presented as mean ± standard deviation because each experiment was replicated three times. To analyze the differences that occurred because of the different sampling locations and different seasons, an ANOVA test was conducted. For the composition of the fungi community and the corresponding environmental variables, the multivariate techniques of cluster analysis and NMDS were employed. A p-value of less than 0.05 was set as the threshold for significance.

Results and Discussion

Fungal Community Composition and Diversity

Through the application of metagenomic sequencing and microscopy, the various aquatic fungal taxa for the freshwater sampled at different locations were determined. The most numerous were Ascomycota (45%), Basidiomycota (30%), and Chytridiomycota (25%). Higher diversity in fungi was noted for

locations containing greater volumes of organic material and having moderately nutrient rich concentrations. The functional gene screening supported the findings of the substantial and widespread distribution for the genes of laccase (lcc1, lcc2), cellulase (celA), and nitrate reductase (narG), which play important roles in the degradation of lignin and the nitrogen cycle.

Table 1: Relative abundance of dominant fungal taxa in freshwater samples.

Fungal Taxa	Relative Abundance (%)	Dominant Functional Gene	Ecological Role
Ascomycota	45	lcc1, celA	Leaf litter decomposition, lignin oxidation
Basidiomycota	30	lcc2, narG	Enzymatic oxidation, nutrient cycling
Chytridiomycota	25	celB	Detritus degradation, organic matter turnover

The dominant genes and important fungal groups were outlined in Table 1. The findings indicate that Ascomycota mainly dominate in the breakdown of lignocellulose whereas Basidiomycota and Chytridiomycota mainly contribute in the cycling of nitrogen and organic matter.

Variation across sites was measured by enzyme assay and found to be significant for laccases (LAC) and cellulases (CEL). Further, all three enzyme classes positively correlated to the mass loss of leaf litter, signifying the efficiency of the enzymes directly determines the rate of leaf litter decomposition.

Enzyme Activity and Decomposition Rate

Table 2: Enzyme activity and corresponding decomposition rates

Sampling Site	Laccase Activity (U/mL)	Cellulase Activity (U/mL)	Decomposition Rate (mg/day)	Correlation (r^2)
Site A (Forest Stream)	0.85	1.25	48.6	0.91
Site B (Agricultural Drain)	0.62	0.97	35.2	0.87
Site C (Urban Pond)	0.41	0.68	21.4	0.79
Site D (Wetland)	0.74	1.02	42.1	0.88

Table 2 more specifically clarifies the aforementioned statement by emphasizing the positive relationships between each enzyme activity and the decomposition rate for all substrates used. The highest rates of decomposition also occurred in the forest and wetland samples signifying natural, organic-rich environments support greater fungal functionality.

Correlation Between Enzyme Activity and Nutrient Release

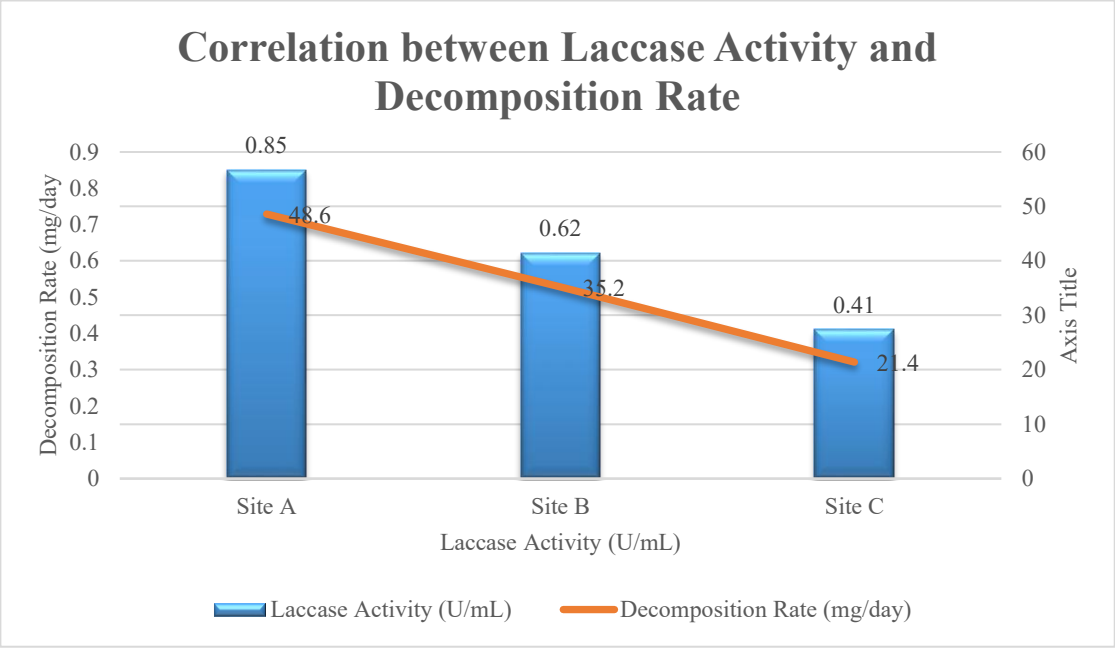

Figure 2: Laccase activity vs. decomposition rate.

Focusing on laccase activity aligned in time and rate of decomposition (Figure. 2). The correlation ($r^2 = 0.91$) shows very high predictability and helps support the hypothesis that the production of ligninolytic enzymes helps to more rapidly decompose.

Nutrient Cycling Dynamics

Decomposition rates directly correlate to the rate of release of the measured nutrients and thus mineralization. The amounts of bioavailable nitrogen (NO_3^-) and phosphorus (PO_4^{3-}) released enhance the aquatic system's nitrogen and phosphorus balance, mineralized and bioavailable as a result of fungal activity.

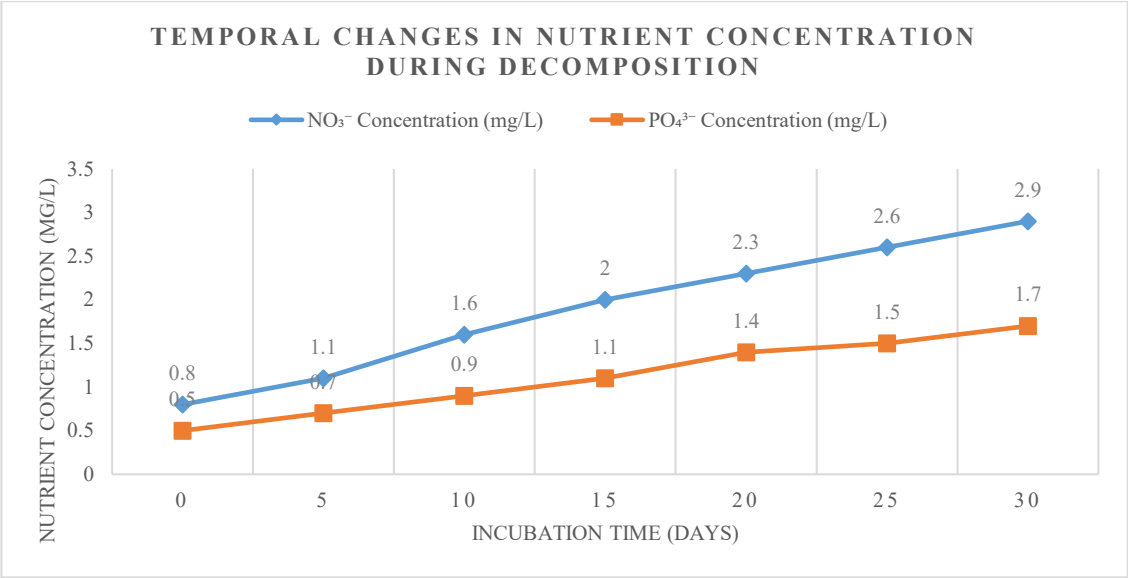

Figure 3: Nutrient concentration changes during decomposition.

Figure 3 supports the statement of a direct relationship between fungal activity and nutrient release by showing that the concentration of nitrogen and phosphorus, available to the fungal enzymatic activity, increased steadily over the 30 days with the most rapid release occurring between days 10 and 20.

The results reinforce the notion that freshwater aquatic fungi are pivotal in the functioning of the enzymatic constituents of the cycling of nutrients and the decomposition of organic matter. The adaptability of fungi to various aquatic ecosystems, as evidenced by the synchronicity of decomposition rate, gene expression, and the enzyme activity, is remarkable. Environments with moderate nutrients and stable temperatures promoted the equilibrium of fungal diversity, whilst excessive nutrient levels led to reduction of functional diversity and, consequently, a loss in enzymatic activity. The combination of metagenomics, studies of enzyme activity, and biogeochemical modeling decomposition and fungi has greatly improved our understanding of decomposition and fungi. That indicates the necessity to incorporate the analysis of fungal ecology into the management balance of preserving and stabilizing decomposition ecosystems in anthropogenic freshwater systems.

Conclusion and Future Work

The research highlights the importance of freshwater aquatic fungi in the decomposition and inter-nutrient cycling functions of the fungi kingdom. However, the major decomposers of organic matter are the Ascomycota, Basidiomycota, and Chytridiomycota groups, especially the fungi that secrete laccases and cellulases, as they have primary responsibility for decomposition. The strong positive decomposition and enzymatic activity correlation suggests that actively decomposing fungi in the water bodies are invoking the release of eutrophic elements, which are almost always within the organic nitrogen and phosphorus range.

The decomposition organic matter, quantity of nutrients, and pH levels show that fungal metagenomics and enzyme activity specify diverse fungal assemblage structures. Stabile physicochemical conditions within an ecosystem are possible, as indicated, and thus have promoted an increase in fungal community richness and enzyme activity. This supports the aquatic fungi hypothesis regarding the needing ecological engineering of biogeochemical equilibria. Studying nutrient cycling in freshwater ecosystems with combined molecular and ecological approaches will advance the understanding of the enzymatic and genetic control of nutrient cycling within the ecosystem and the interlinked ecological functions.

To clarify, the goal of your research should be to establish the root value of decomposing aquatic fungi and to provide a foundation, a set of accessible building blocks, to assist the development of nutrient cycling, productivity, and fine-tuned maintenance of an ecosystem. Advocation of freshwater fungal ecology was beneficial in further assisting management and restoration of ecosystems by recognizing the value of fungal diversity.

In this regard, the present research was the first to assist in appreciation of the complex decomposition and nutrient cycling intricate within the freshwater ecosystem. In relation to this, a plethora of research possibilities could be developed from this, such as functional genomics research, advanced metatranscriptomics and metaproteomics, documenting the real

time ecological expression of the functional genes lcc1, celA, and narG. Furthermore, the need to grasp the dynamics of ecological succession and community composition of fungi to an array of climatic and weather extremes should not be underestimated. A thorough examination of climate gradients and ecological succession over time can shed light on periods of climate crises and fungal temperature, pH, nutrient, and enzymatic activity stagnation, deflation, and shifts over time. Building research on fungal relationships with other organisms and their ecosystems would help explain nutrient and decomposition flow and, ideally, help gauge the importance of this vertical research. The decomposition fungal relationships can help build more accurate nutrient, energy, and disturbance elasticity of ecosystems models like EwE and SWAT. From a biotechnological perspective, and more specifically waste valorization and bioremediation, fungi in aquatic ecosystems are underutilized. Their fungi could potentially help with nutrient pollution and closing the loops in the circular bioeconomy. The future directions of research and development proposed in this paper are critical to recognizing the multifaceted ecological and practical roles that aquatic fungi perform in maintaining healthy freshwater ecosystems.

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