Linking Fungal Dynamics and Functional Genes to Environmental Factors

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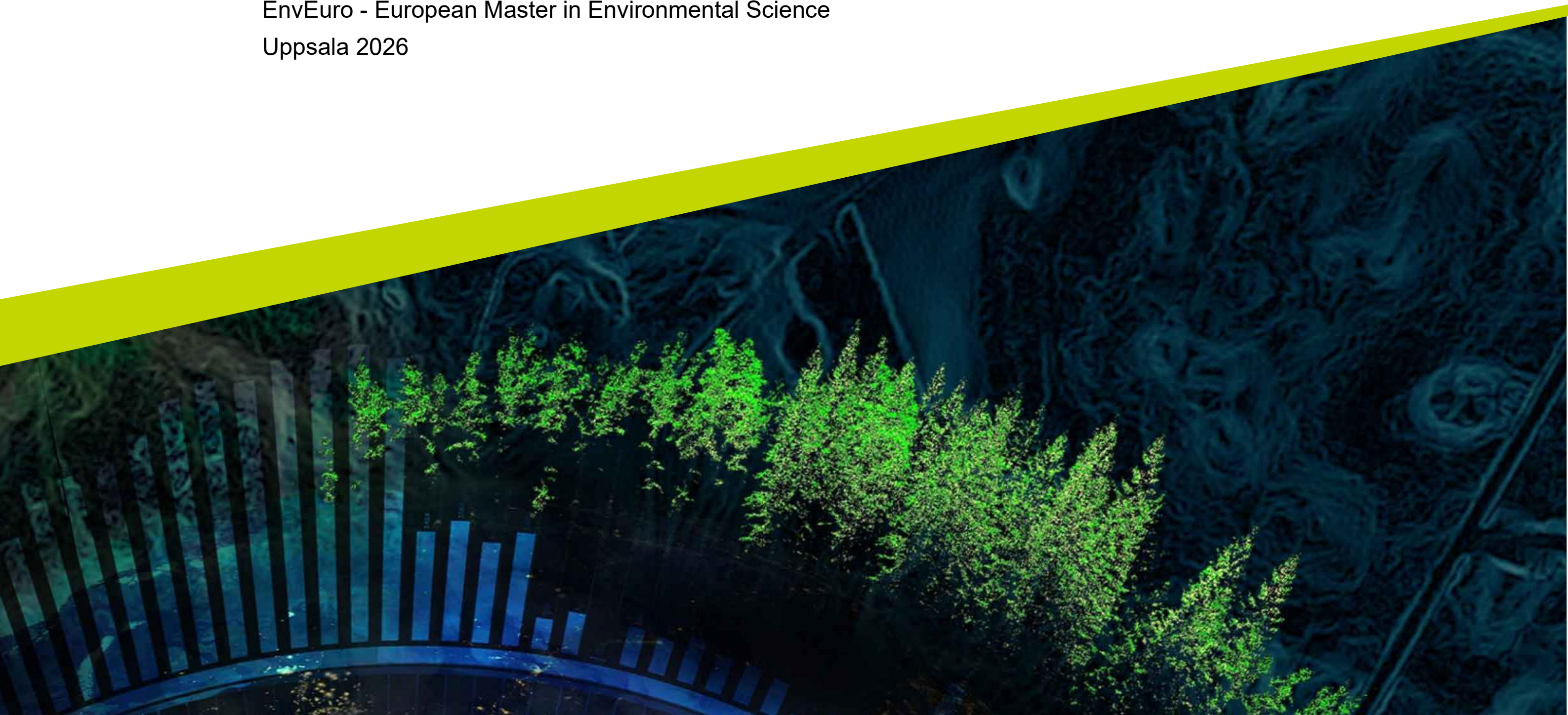

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Abstract

Decomposition is a key ecosystem function and one of the fundamental processes in freshwater ecosystems, driving nutrient cycling and energy flow within aquatic food webs. However, decomposition processes can be strongly influenced by nutrient enrichment coming from agriculture. Aquatic hyphomycetes (subgroup of aquatic fungi) are among the most important organisms driving decomposition in streams, making their response to environmental change a pivotal factor in overall ecosystem health. This study investigates whether fungal decomposition-related genes can help us understand how environmental conditions affect decomposition processes. A laboratory decomposition experiment was conducted using three species and a mixed species community grown in a mineral solution with three different N:P ratios and a carbon source of cellulose substrate for decomposition. Decomposition was assessed as cellulose mass loss, DNA abundance was quantified and qPCR was performed with primers for cellobiohydrolase (CBHI, a functional gene) to determine gene copy number. For all groups an increase in P (lower N:P ratio) resulted in an increase in mass loss (decomposition). DNA abundance was significantly positively correlated with decomposition for all the groups and increased P-levels generally resulted in higher DNA concentrations for most groups. In contrast, qPCR analysis revealed CBHI copy numbers did not differ significantly between nutrient treatments and showed no significant relationship with decomposition. The positive association between DNA abundance and decomposition within species groups indicates that molecular measurements have potential to be used as proxies for fungal activity. However, the absence of a predictable correlation between CBHI copy numbers and decomposition indicates that CBHI abundance alone cannot yet predict ecosystem functioning. Further research is needed to evaluate the applicability of aquatic fungal functional gene-based approaches for monitoring decomposition processes in freshwater ecosystems.

Keywords: aquatic fungi, aquatic hyphomycetes, decomposition, eDNA, monitoring, freshwater, N:P ratio

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Abbreviations

Abbreviation	Description
AF	Aquatic Fungi
CBHI	Cellobiohydrolase
CTRL	Control Group
Cq	Quantification Cycle
dNTP	Deoxynucleotide Triphosphate
FISP	*Filosporella sp*.
IC	Ion chromatography
MIX	Mixture of TEMA, FISP and NELU
NELU	*Neonectria lugdunensis* (old name *Heliscus lugdunensis*, HELU).
NTC	No template control
OC	Organic carbon
PDA	Potato Dextrose Agar
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
RFU	Relative Fluorescence Units
TEMA	*Tetracladium marchalianum*
Tm	Melting temperature
WFD	Water Framework Directive

1. Introduction

Freshwater ecosystems support a disproportionately high level of global biodiversity (over 10% of all known species on less than 1% of the Earth's surface), yet approximately 24% of assessed freshwater fauna species are threatened with extinction in part due to agricultural impacts (Sayer et al. 2025). Reductions in freshwater quality through pollution, nutrient enrichment, and habitat degradation modify habitat structure and community composition, reshaping trophic interactions and leading to measurable consequences for key ecosystem functions (Dudgeon et al. 2006). Ecosystem functions refer to the natural biological, chemical, and physical processes that occur within ecosystems, such as primary production, nutrient cycling, decomposition, and energy flow. Healthy ecosystem functioning enables ecosystems to provide benefits for humans, known as ecosystem services, including provisioning (e.g. food and freshwater), regulating (e.g. climate and water regulation), cultural (e.g. recreation), and supporting services (Schröter et al. 2019). Among these, decomposition — the biological breakdown of organic matter such as leaf litter — is one of the most fundamental processes in stream ecosystems, driving nutrient cycling and energy flow within aquatic food webs (Gessner & Chauvet 2002). Aquatic fungi are among the most important organisms driving decomposition in streams (Bärlocher & Kendrick 1974; Gessner et al. 2007), making their response to environmental change a pivotal factor in overall ecosystem health. Despite their ecological importance, our ability to predict or protect ecosystem functions and fungal biodiversity from the impacts of nutrient additions remains highly limited. To safeguard these ecosystems, it is therefore critical that future biomonitoring programmes explicitly incorporate aquatic fungi and functional process metrics.

Decomposition & Aquatic Hyphomycetes

Leaf litter decomposition in streams is driven by the interaction of physical fragmentation, microbial conditioning, and consumption by invertebrate detritivores (Cummins 1974). Among microbial decomposers, aquatic fungi play a particularly important role by breaking down complex plant materials such as cellulose and lignin (Bärlocher & Kendrick 1974). These fungi depend on aquatic habitats for the whole or part of their life cycle (Grossart et al. 2019). They come from across the fungal tree of life (Shearer et al. 2007) and play important roles in ecosystems through their contributions as decomposers, parasites, symbionts, and food resources for consumers (Grossart et al. 2019). Aquatic hyphomycetes are a subgroup of aquatic fungi, comprising around 300 described species and reproducing asexually by producing spores (conidia) while submerged (Bärlocher 2009). They are commonly found on decaying deciduous leaves in running freshwaters (Ingold 1942). They are particularly important in stream

decomposition processes because they fragment leaf litter, release nutrients, and increase the palatability and digestibility of organic matter for detritivores and other organisms within the food web by releasing extracellular enzymes (Bärlocher 1985; Pérez et al. 2018; Camelo et al. 2022).
Changes in species composition and diversity of leaf litter can strongly influence the dynamics and rates of decomposition (Gessner et al. 2010). Graça et al. (2024) found that fungal biodiversity largely buffered the effects of different environmental stressors, including increased nutrients and temperature, by retaining community-level decomposition function. They showed that fungal species differ in their sensitivities to environmental variability and that this variability of responses underpins community stability. Together, these findings highlight that both the diversity and the identity of aquatic hyphomycete communities matter for decomposition, and that environmental pressures capable of shifting community composition may therefore have cascading consequences for this ecosystem function.

Including Aquatic Fungi and Their Functions in Monitoring

Considering these dynamics, tracking changes in aquatic fungal communities and their functional contributions over time is essential for understanding the ecological consequences of environmental change. Widespread and recurring monitoring is therefore necessary to observe trends in decomposition as an ecosystem function and to detect shifts in the composition of decomposer communities. Stream decomposition studies using leaf litter bags — the current standard approach — are effective at local scales but reach their practical limits when applied to large-scale and continuous monitoring. Conventional monitoring techniques, although useful, are often constrained by high costs, intensive labour requirements, the need for expert knowledge and specialised training, and limited capacity to detect comprehensive biodiversity patterns, especially for elusive or rare species (Pochon et al. 2025). Biomass measurements using ergosterol as a fungal biomarker are similarly time- and labour-intensive, costly, and can produce unreliable results due to considerable variations among fungal species, growth stages and environmental conditions (Gessner & Chauvet 1993), raising the need for alternative approaches to estimate fungal biomass and evaluate ecosystem health. To overcome these limitations, molecular methods such as environmental DNA (eDNA) have become valuable tools for improving the monitoring and management of aquatic ecosystems (Taberlet et al. 2012).

Environmental DNA (eDNA)

eDNA refers to genetic material released by organisms into their surrounding environment through processes such as excretion, reproduction, and decomposition. It can be collected directly from environmental samples including

water, soil, and air without the need to isolate target organisms beforehand (Taberlet et al. 2012; Pochon et al. 2025). By using group-specific primers to amplify informative genetic regions from environmental samples (eDNA metabarcoding), multiple taxa can be simultaneously detected in a rapid, scalable, and cost-effective manner (Ji et al. 2013; Fernandez Nuñez et al. 2021). eDNA metabarcoding has increasingly been used alongside conventional monitoring approaches to support freshwater biodiversity assessments, particularly for fish and aquatic invertebrates (Harper et al. 2019). Aquatic fungi, however, have not been included in any long-term monitoring efforts so far, despite playing an important role in ecosystem functioning. FUNACTION and MoSTFun, two projects jointly funded by Biodiversa+ and the European Union, are currently working to develop approaches to assess aquatic fungal diversity and function using eDNA, with the goal of incorporating these organisms into conservation and biodiversity monitoring programmes. Part of this process involves investigating fungal functional dynamics and how changes in environmental conditions affect them. Important unanswered questions remain, including whether eDNA can provide insights into the status of fungal functions in response to environmental conditions and changes, in addition to information on distributions and diversity. To begin to address these questions, this study focuses on nutrient enrichment from agricultural land use, a well-known and ecologically important environmental pressure.

Agricultural Impacts on Stream Nutrient Levels

Agriculture and land use intensification have increased worldwide over recent decades, resulting in elevated nutrient inputs into streams and freshwater systems through fertilisation and agricultural runoff. Agricultural streams, in particular, experience high loads of reactive phosphorus (P) and nitrogen (N), influencing water quality and the ecological state of these and downstream water bodies (Chambers et al. 2006). The consequences are often manifested as eutrophication, algal growth, oxygen depletion, acidification, or reduced nutrient cycling efficiency (Pinckney et al. 2001; Göthe et al. 2015). As agricultural practices intensify, riparian forests and wetlands have also become fewer and less connected to streams, resulting in reduced inputs of organic carbon (OC) of high complexity — such as leaf litter, woody debris, and other allochthonous dissolved organic matter — into stream channels, ultimately disrupting nutrient cycling. The combination of increased inorganic nutrient loading and reduced organic carbon input has resulted in a decrease in the in-stream stoichiometric ratio of OC relative to N (C:N) and P (C:P) (Elser et al. 2000), which influences natural self-purification processes and the nutrient retention capacity of aquatic systems. Both the availability and stoichiometric balance of nutrients can disrupt aquatic fungal

and microbial functioning by altering the nutrient ratios that regulate growth, metabolism, and interactions within the ecosystem (Falkowski et al. 2000). Nutrient availability can strongly influence decomposition in freshwater ecosystems by stimulating microbial and fungal activity on leaf litter. Aquatic fungi play a key role in this process because nutrient enrichment, particularly nitrogen (N) and phosphorus (P), can enhance fungal growth, increase litter breakdown rates, and promote the conversion of coarse particulate organic matter into fine particulate organic matter (Tant et al. 2015). Similarly, Pérez et al. (2018) found that warmer and nutrient-richer stream conditions accelerated leaf-litter decomposition and increased the diversity of aquatic hyphomycetes. However, responses are not uniform: some studies have reported that moderate nutrient enrichment did not further accelerate decomposition and could even reduce fungal biomass production, depending on fungal species identity and ecosystem context (Camelo et al. 2022). While most existing studies assess whether decomposition as a bulk function is altered, less is known about how nutrient enrichment shapes individual species-level responses and the mechanistic links between fungal activity, gene expression, and overall decomposition rates. Addressing this gap is essential for developing molecular tools capable of capturing functional change, not merely the presence or absence of decomposer taxa.

Research Question and Objectives

This study aims to answer the question: Can aquatic fungal decomposition-related genes help us understand how environmental conditions affect decomposition processes?

Therefore, the following objectives are addressed:

1. Determine the effects of nutrient ratios on decomposition
2. Investigate the relationship between decomposition and DNA/gene copies
3. Assess the potential for fungal monitoring using eDNA approaches

In this study I measured cellulose decomposition under different nutrient ratios of N:P to test whether the resulting differences vary predictably with changes in the relative abundance of a decomposition-related gene as the first step toward identifying an eDNA-based tool for monitoring this key ecosystem function. I investigate three aquatic hyphomycete species separately to understand intraspecific variation in their responses to nutrient availability and together as an artificial community to also represent part of the complexity found in nature. As described in previous literature results, decomposition rates are expected to increase with higher nutrient levels, in particular higher phosphate concentrations (Costello et al. 2022). This increase may reflect an increase in fungal biomass/growth and corresponding increase in the abundance of gene copies for functional genes in resulting DNA samples, providing an indirect, but scalable,

window into fungal function and freshwater ecosystem health using eDNA. Given a positive correlation, expanding eDNA monitoring programs across Europe could be used to also describe decomposition and environmental interactions without requiring substantial additional sampling efforts.

2. Material and Methods

2.1 Literature research

To assess the current knowledge base and understand the bigger context a list of literature was compiled and reviewed. Several search engines were used for identifying relevant literature including SLU Online Library, Science Direct, Google Scholar, Google search, Ecosia and the AI tool Consensus. The reference directory of existing literature also provided substantial material. To narrow down the search keywords and various combinations thereof were used.

Keywords: aquatic fungi saprotrophic, (aquatic) fungi, qPCR, ecology fungi, (aquatic) hyphomycetes, eDNA (fungi), (environmental) monitoring, gene copies + fungi, fungal diversity, aquatic ecosystems, …

2.2 Fungal species and pre-cultures

Four species were used in this project: *Tetracladium marchalianum* (TEMA), *Tetracladium setigerum* (TESE), *Filosporella sp.* (FISP) and *Neonectria lugdunensis* (NELU, old name *Heliscus lugdunensis*). TEMA, TESE, and FISP were isolated by Jennifer Anderson from Sweden (Table 1). NELU was obtained from Andreas Bruder (Switzerland, SUPSI) (Table 1).

Table 1: Aquatic hyphomycete species used and their origin

Table 1

Species	Abbreviation	Strain ID	Origin	Isolated by	Collection/isolation details
Filosporella sp.	FI *sp.*	F4	Sweden	Jennifer Anderson	Isolated from decomposing Nymphoides peltata in Lake Mälaren, Anderson and Wang 2025
Neonectria lugdunensis	NELU	AH 2 TICINO 2016	France	Andreas Bruder, SUPSI	isolated from foam January 2016
Tetracladium marchalianum	TEMA	JA 0051	Sweden	Jennifer Anderson	Anderson and Marvanová 2020
Tetracladium setigerum	TESE	JA001	Sweden	Jennifer Anderson	Anderson and Marvanová 2020

The fungi were maintained in culture on Potato Dextrose Agar (PDA, Eur. Pharm., VWR Chemicals, 84651.0500) in 90 mm Petri dishes at room temperature with ambient light. To have a sufficient amount of biomass for the experiment and to acclimate them to the liquid growth condition, approximately 2 × 0.5 cm plugs were cut from agar grown fungal cultures. These were broken down into smaller pieces and suspended in approximately 200 ml of GMS (Glucose Mineral Solution), described by Gessner & Chauvet (1993) with some modifications and consisted of 10 ml of $NaNO_3$ (0.1M), 47 µl of K_2HPO_4 (1M) and 30 µl of KH_2PO_4 (1M), 0.5 g of $MgSO_4 \bullet 7H_2O$, 0.15 g of $CaCl_2 \bullet 2H_2O$,

3.33 mg of $FeCl_3 \cdot 6H_2O$, 1 mg of $MnSO_4 \cdot H_2O$, 1 mg of $ZnSO_4 \cdot 7H_2O$, 1 mg of H_3BO_3, 0.074 mg of $KAl(SO_4)_2 \cdot 18H_2O$, 0.1 mg of KI, 0.1 mg of $Na_2MoO_4 \cdot 2H_2O$, 0.04 mg of $CoCl_2 \cdot 6H_2O$, 0.025 mg of $NiCl_2 \cdot 6H_2O$, and 5 g of glucose in 1 L of H_2O. Two 500 mL flasks of each species were prepared and mounted onto a shaker at constant speed of 100 rpm for 2 weeks.

2.3 Preliminary test (Pre-Test)

To measure decomposition rate responses to nutrient levels and test their associations with functional gene abundance, it was first necessary to develop a suitable experiment design with emphasis on determining effective experiment duration and experimental protocols.

The Pre-Test was set up under the laminar flow using sterile technique. TESE did not show enough growth during the pre-culture phase and was therefore excluded from the Pre-Test and substituted with NELU for the main experiment. The species TEMA, and FISP, as well as a mixture (MIX) of the two were used for inoculation. Only one nutrient ratio at 13:1 for N:P was used in the Pre-Test. Triplicates of each group (TEMA, FISP, and MIX), as well as one control without fungi were prepared according to the following steps, for a total of 10 microcosms in 50 mL Falcon tubes. As a proxy for leaf litter and as a carbon source, cellulose filters (Ø 150 mm Filter Paper Circles, CAT no. 1001-150, Whatman™, Cytiva) were cut into 16 segments per filter to have a weight of 0.09 mg each. One sterile filter segment was added to each tube with 25 mL GMS, 14 mg L^{-1} of N-NO_3^- and 2.4 mg L^{-1} of P-PO_4^{3-} to generate the 13:1 N:P ratio.
To start the Pre-Test, fungal inocula were prepared from the liquid cultures as follows. After removing the majority of the liquid growing media from the fungi cultures in the flasks, it was attempted to break down fungi into smaller pieces (disrupting) using a small stick blender with an approx. Ø 0.5 cm blade. The viability of the fragmented mycelium was confirmed by growth on PDA. Inoculation amounts of 1 mL for FISP, 1ml for MIX and 0.5 mL for TEMA (more biomass) were used. The MIX, acting as an artificial community, was created by combining approximately the same amounts of TEMA and FISP using 1 mL as inoculum.
After inoculation the lids were loosely screwed onto the tubes and sealed with parafilm to ensure gas exchange with the surrounding air. The samples were incubated in darkness at 20°C in a temperature-regulated room. A continuous and consistent movement of liquid media in the microcosms was created by fixing the experiment on two shakers and keeping them at a constant speed of 250 rpm to ensure oxygenation. After 30 days, the test was terminated and the samples were

prepared for the measurement of decomposition and DNA extraction as described below.

2.4 Experiment design and setup

Based on the outcome of the preliminary test the experimental design was defined as follows. The main experiment was set up in the same manner as the Pre-Test with some alterations.

To determine growth and decomposition under varying nutrient ratios 3 different treatments were prepared. "Group" refers to the different strains/mix and control, while "Ratio" is used to describe the nutrient treatment. Biological Groups were TEMA, FISP, NELU, MIX and Control (CTRL) with five replicates each, across the three Ratios (N:P) of 130:1, 13:1 and 1.3:1, adding up to a total of 75 Falcon tubes (microcosms, Figure 1).

75 microcosms		3 Nutrient Ratios N:P		
		P-poor = 130:1	Balanced = 13:1	P-rich = 1.3:1
5 Groups	TEMA	1 2 3 4 5	26 27 28 29 30	51 52 53 54 55
	Fl sp.	6 7 8 9 10	31 32 33 34 35	56 57 58 59 60
	NELU	11 12 13 14 15	36 37 38 39 40	61 62 63 64 65
	Mix of 3	16 17 18 19 20	41 42 43 44 45	66 67 68 69 70
	Control	21 22 23 24 25	46 47 48 49 50	71 72 73 74 75

Figure 1: Schematic graph of the Experiment Design with the 5 groups (TEMA, FISP., NELU, MIX and CTRL) for the 3 nutrient ratios N:P (P-poor = 130:1, balanced = 13:1 and P-rich = 1.3:1), and 5 replicates for each combination, adding up to a total of 75 microcosms

For a more equally distributed mass of filter paper three small cellulose filter discs (Ø 12.8 mm Filter Paper Circles ashless/Blue ribbon, product no. 10300263, Whatman™, Cytiva) were used. Cellulose discs were weighed to an average mass of 0.011 g per disc with a standard deviation of 0,54 mg, resulting in an average of 0.032 g starting mass for the 3 discs per tube.
The previously prepared and autoclaved GMS solution was used, 25 mL per tube, and 14 mg L^{-1} of $N\text{-}NO_3^-$ added for all ratios (N constant). Furthermore, the growth media contained $P\text{-}PO_4^{3-}$ of either 0.24 mg L^{-1} for N:P of 130:1 (phosphorus-poor), 2.4 mg L^{-1} for 13:1 (balanced) or 24 mg L^{-1} for 1.3:1 (phosphorus-rich). Ratios were calculated as molar ratios. The nominal ratios for each treatment were verified with ion chromatography (IC), see Table 2.

Table 2: Nutrient contents of GMS
expected/calculated (column 2&3) and IC measured concentrations (column 4&5)

µ

Sample ID	expected N (µg L^{-1})	expected P (µg L^{-1})	Total N (µg L^{-1})	Total P (µg L^{-1})
R130	14000	240	14600	269
R13	14000	2400	14500	2350
R1.3	14000	24000	14500	23600

To homogenise the inoculum fungi were fragmented by drawing up mycelium through a needle (1,20 × 50 mm, 18G × 2", Sterican 4667123, B. Braun, Germany) into a syringe, or a 25 ml pipette for bigger chunks of fungi. This method was more successful compared to the Pre-Test and was used going forth. The viability of the fragmented mycelium was confirmed by growth on PDA. Standardization of the starting inoculum was performed by determining the mean mass per volume in 1 mL of each strain after pelleting the fungal material in triplicates by centrifugation (~2 minutes at 15 000 rpm) and decanting the liquid media. The volume of inoculum to add for each strain was then standardised to match the concentration of the lightest (FISP with an average of 55 mg ml^{-1}). The MIX was determined as the 3 combined with their respective inoculation amounts and also standardised to FISP.

All other steps were done as described in the Pre-Test and the experiment was terminated after 30 days of incubation.

2.5 Determination of decomposition

To estimate decomposition the mass loss of the cellulose discs was calculated. The mass lost should be the sum of material/carbon taken up by the fungi and converted into fungal biomass, and the carbon set free into the air through respiration. The cellulose filter discs were weighed before and after decomposition. Weight prior to decomposition was determined by weighing a representative number of discs and calculating an average. This average was used for further calculations of mass loss. After the experiment, the samples were freeze-dried for approximately 30h then weighed on an analytical scale. The scale measured at a readability of 4 decimal places.

2.6 DNA extraction

DNA extraction was done with the DNeasy® PowerSoil® Pro Kit (QIAGEN N.V., Venlo, NL). The sample comprised the freeze-dried filter discs which were loaded in their entirety per sample and from which DNA was extracted according to the protocol of the manufacturer. In the Pre-Test samples were extracted testing both methods, extraction from pre-lysed (overnight) and "dry" samples. For the main experiment pre-lysing was used due to better results. One further modification was made to the protocol. Instead of using the centrifuge in steps 8-14 a pump was used to draw the liquids through the column.

To verify extraction was successful and to have an approximation of DNA quantity, DNA samples were analysed in the Qubit 3.0 Fluorometer with the Qubit ™ 1X dsDNA HS Assay Kit and according to manufacturer protocol.

DNA was extracted for all 10 samples from the Pre-Test and three of the five replicates of each group for the main experiment (45 samples). To minimise the error of handling, and to reduce the biases of batch extractions, the sample orders were randomised for each series of extraction.

2.7 Development and validation of primers

The second part of this project was to design primers to be used in qPCR. Fungal enzymes involved in cellulose decomposition were identified from literature: endoglucanase, cellobiohydrolase, β-glucosidase and lytic polysaccharide monooxygenases (Barbi et al. 2020). Within the scope of this thesis, I worked on the protein β-glucosidase, but in parallel there was work being done on designing primers from cellobiohydrolase as well.

The process for designing primers was roughly based on the method used in Edwards et al. (2008) for designing degenerate fungal primers. Thirty sequences (see Table 3) of amino acids and the corresponding DNA for fungal β-glucosidase were downloaded from the NCBI database and uploaded to the programme Unipro UGENE v53.1. Due to a lack of sequences from aquatic fungi, terrestrial fungi were used and attention was given to a representation of both Ascomycota and Basidiomycota within the selection. The amino acid sequences were aligned using the MUSCLE module and the consensus (at 70-80% of similarity) examined for conserved regions to determine possible primer sites. Conserved regions in the amino acid sequences were then also inspected in the aligned DNA sequences to determine codon biases. The primers were synthesised by Integrated DNA Technologies (IDT), Belgium.

Table 3: Taxonomy and NCBI reference sequence number of fungal β-glucosidase genes used in primer design

Phylum and species	Order	Family	NCBI Reference sequence
Basidiomycota			
Volvariella volvacea	Agaricales	Pluteineae	AF329731.1
Rhizoctonia solani	Cantharellales	Ceratobasidiaceae	CUA68916.1
Antrodia serialis	Polyporales		XM_048029497.1
Epithele typhae	Polyporales	Epitheliaceae	XM_048027220.1
Ganoderma leucocontextum	Polyporales	Polyporaceae	KAI1787707
Phanerochaete sordida	Polyporales	Phanerochaetaceae	GJE85970.1
Rhodotorula toruloides	Sporidiobolales	Sporidiobolaceae	KAK4329688.1
Cryptococcus deuterogattii	Tremellales	Cryptococcaceae	XM_063027714.1
Ustilago esculenta	Ustilaginales	Ustilaginaceae	AB618734.1
Ascomycota			
Capronia coronata	Chaetothyriales	Herpotrichiellaceae	XM_007729207.1
Talaromyces islandicus	Eurotiales	Trichocomaceae	CRG86429.1
Aspergillus eucalypticola	Eurotiales	Aspergillaceae	XM_025530830.1
Aspergillus heteromorphus	Eurotiales	Aspergillaceae	XM_025546008.1
Penicillium psychrosexuale	Eurotiales	Aspergillaceae	XM_057185590.1
Penicillium verrucosum	Eurotiales	Aspergillaceae	XM_057220740.1
Penicillium canescens	Eurotiales	Aspergillaceae	XM_058519782.1
Colletotrichum tofieldiae	Glomerellales	Glomerellaceae	GKT55270.1
Lachnellula cervina	Helotiales	Lachnaceae	TVY56203.1
Trichoderma orientale	Hypocreales	Hypocreaceae	JQ904600.1
Pochonia chlamydosporia	Hypocreales	Clavicipitaceae	OAQ57427.2
Isaria fumosorosea	Hypocreales	Cordycipitaceae	XM_018850569.1
Fusarium subglutinans	Hypocreales	Nectriaceae	XM_036684445.1
Purpureocillium takamizusanense	Hypocreales	Ophiocordycipitaceae	XM_047983020.1
Fusarium albosuccineum	Hypocreales	Nectriaceae	KAF4471433.1
Brettanomyces bruxellensis	Pichiales	Pichiaceae	KR181960.1
Pyrenophora teres f. teres	Pleosporales	Pleosporineae	CAE7174324.1
Exserohilum turcicum	Pleosporales	Pleosporineae	KAL6166681.1
Setomelanomma holmii	Pleosporales	Pleosporineae	KAF2030523.1
Verticillium alfalfae	Sordariomycetes	Hypocreomycetidae	XM_003007713.1
Neocallimastigomycota			
Neocallimastix californiae	Neocallimastigales	Neocallimastigaceae	ORY72777.1

To validate the developed primer pairs target sequences were amplified with PCR in 20 µL reactions. The reactions contained 1 µL of extracted DNA from one sample of the groups TEMA, FISP and MIX, as well as pure TEMA from the preculture (all from the Pre-Test). Also included in the reaction mix were 2 µL of DreamTaq Buffer, 0.4 µL of deoxynucleoside triphosphate (dNTP, 10 mM), 0.1 µL of Taq polymerase (5 U μL^{-1}), 1 µL of each primer (10 µM) and 14.5 µL of nuclease-free water. Negative controls were included for each batch, containing primers but no DNA template. PCR was performed with the following protocol: Initial denaturation at 98°C for 3 min, followed by touchdown cycles of denaturation at 98°C for 15 s, annealing from 60°C to 51°C (-1°C/cycle) for 30 s and extension at 72°C for 30 s; thereafter 25 cycles of denaturation at 98°C for

15 s, annealing for 50°C for 30 s and extension at 72°C for 30 s; and to conclude one final extension at 72°C for 15 min.

PCR result was evaluated by running a 2%-agarose gel electrophoresis with GelRed® nucleic acid stain (Biotium, Cat: 41003, Lot: 24G0926).

2.8 qPCR

Quantitative PCR was used to determine relative copy numbers of cellobiohydrolase (CBHI), for the extracted samples (3 replicates) of all groups of the nutrient ratios of 130:1 and 1.3:1 (N:P). Each sample in turn was loaded onto the plate in technical triplicates to assess the reproducibility of qPCR measurements and minimize the impact of technical variation. Each reaction consisted of 5 µL of DNA sample (template), 10 µL of SsoAdvanced™ Universal SYBR® Green Supermix, 1 µL of each primer (10 µM) and 3 µL of nuclease-free water, adding up to 20 µL per reaction. For the no template control (NTC) the 5 µL of the sample were substituted by 5 µL of nuclease-free water. The recently designed and validated primers for CBHI were used for this qPCR. The following protocol was followed: Initial denaturation and polymerase activation at 98°C for 3 min, denaturation at 98°C for 30 s, annealing at 57°C for 30 s and extension at 72°C for 1 min. After the first plate read the previous three steps were repeated for 44 more cycles (45 cycles in total), before ending with a final extension at 72°C for 10 min and a melting curve from 60°C to 95°C with an increment of 0.5°C every 5 s. A CFX Duet Real-time PCR System (BIO RAD) was used for performing the qPCR.

2.9 Data analysis

The data was arranged in Microsoft Excel and then processed in R (version 4.6.0) and visualised in R Studio (version 2026.04.0+526). The following packages were used: agricolae (1.3.7), dplyr (1.2.1), ggplot2 (4.0.3), ggpubr (0.6.3), readxl (1.5.0), writexl (1.5.4). Two-way ANOVAs with interaction were performed for testing the effect of group, ratio and group-ratio interaction on the mass loss (decomposition) data. To verify normality the Shapiro-Wilk Test was used. The same process was followed for DNA amount in relation to ratio, group and group-ratio interaction. Additionally, DNA amount, relative abundance of CBHI, and mass loss were tested for correlation using the Pearson test. For all statistical calculations the threshold for significance was set to $p \leq 0.05$. The R scripts can be found in the Appendix.

3. Results

3.1 Results and learning from Pre-Test

The Pre-Test provided some valuable information for setting up the main experiment. Factors included were experiment running time, amount of cellulose for decomposition, method of fungal tissue fragmentation for inoculum, and growth developments of the selected fungi species. Four weeks (~30 days) was a sufficient amount of time for selected fungi to grow, attach and start decomposition of the cellulose material. The cellulose discs were not completely decomposed during this time, indicating that the amount used for the experiment was sufficient.

TESE showed poor growth under the experimental conditions and was thus excluded from further study.

Different ways of disrupting/fragmenting the fungi after initial growth were tested to avoid variation in inoculation amounts for the decomposition experiment. The best results were acquired by disruption with a needle and syringe.
Due to concerns about DNA extraction from dry samples after freeze-drying, DNA extraction results (quantity measured with Qubit) of dry samples were compared with pre-lysed samples (overnight). Due to a substantial increase in the amount of DNA extracted from the pre-lysed samples, this method was used going forth.

3.2 Primer design

Two pairs of primers targeting conserved gene regions for β-glucosidase were designed:

- Fun_BG1_F (5'- TTT GTC ATG TCG ACT GGT GT -3')
- Fun_BG1_R (5'- GTC GTT CTT GAG CAG GAC AT -3')
- Fun_BG2_F (5'- GGC TCT GCC TCC AAG ATG -3')
- Fun_BG2_R (5'- ACA CCA GTC GAC ATG ACA AA -3')

Amplification was unsuccessful for both pairs under the tested amplification condition (Figure 2).

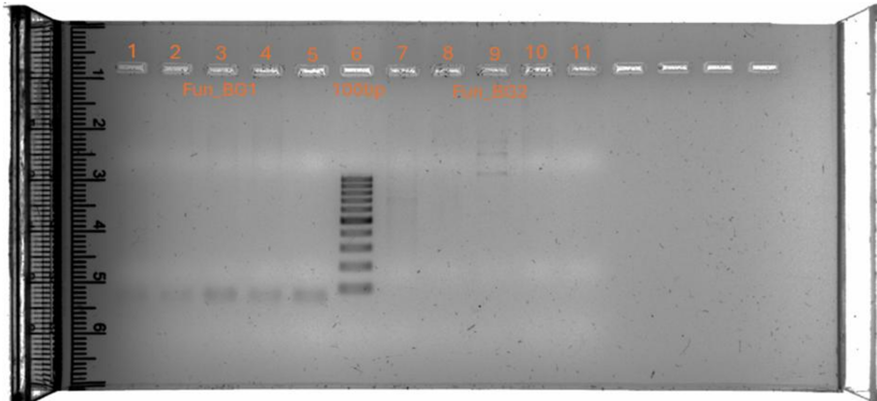

Figure 2: Electrophoresis gel with β-glucosidase primer pairs FUN_BG1 and FUN_BG2
Allocation of samples to wells (all samples from Pre-Test) 1: pure TEMA from MEP, 2: prelysed N:P (13:1) TEMA from cellulose, 3: prelysed N:P (13:1) FISP from cellulose, 4: prelysed N:P (13:1) mixture of TEMA and FISP from cellulose, 5: no template control

Another primer set designed by Ziming Wang for a different gene, cellobiohydrolase (CBHI), was then validated with PCR using the same procedure and these primers were successful (Figure 3). This suggests that the issue with the β-glucosidase primer pairs is most probably not with the samples used (e.g. too little or low quality DNA) but with the design of the primers themselves. Reasons for primer failure are discussed in the discussion.

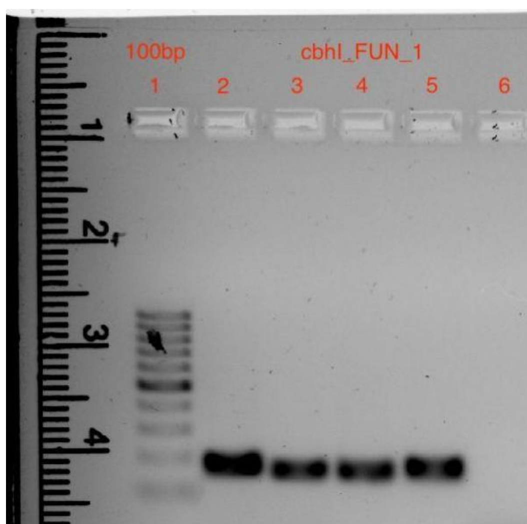

Figure 3: Electrophoresis gel with cellobiohydrolase primer pair cbhI_FUN_1
Allocation of samples to wells (all samples from Pre-Test) 1: ladder (100bp), 2: pure TEMA from MEP, 3: prelysed N:P (13:1) TEMA from cellulose, 4: prelysed N:P (13:1) FISP from cellulose, 5: prelysed N:P (13:1) mixture of TEMA and FISP from cellulose

3.3 Decomposition

The cellulose filter discs used in the experiment had an average mass of 0.011 g per single disc. Three discs were used per microcosm for an average of 0.032 g per replicate. Mass loss was determined by calculating the difference between mean starting mass and measured mass after 30 days of incubation. Mass loss varied among groups and by treatment (N:P ratios) with the lowest mass loss observed for NELU (4.7 %) in the P-poor treatment and the highest in TEMA (42.35 %) under the P-rich treatment. NELU had the lowest decomposition rates across the treatments (10.84 %) and TEMA (42.35 %) the highest (27.51 %).

To determine the effect of group and ratio on decomposition mass loss a two-way ANOVA was performed for the two variables, as well as interactions of the two. The results showed a significant effect of all three (Group $p = 1.92e^{-15}$, Ratio $p = 1.10e^{-11}$, Group:Ratio $p = 1.88e^{-05}$) indicating that decomposition was influenced by different N:P ratios and groups responded differently to varying ratios (Figure 4). The Shapiro-Wilk test confirmed normality of the data ($W = 0.98025$, $p = 0.2916$), legitimising the use of ANOVA. CTRL showed no difference between ratios, and significantly differed from all other groups, as would be expected. NELU showed a significantly different mass loss to all other groups, while differences between TEMA, as well as FISP and MIX were not significant. For each group, the mass losses of one ratio are significantly different from the two other ratios.
The effect of ratio depended on the group, N:P ratio did not affect all groups equally. The TEMA group under low N:P ratio (imbalanced, P-rich) had the highest decomposition rates in the experiment and the average for TEMA with balanced ratio was also higher than all other groups for balanced ratio, but lower than the average of FISP and MIX in the P-poor ratio (“high”). MIX under P-rich ratio also significantly enhanced cellulose mass loss compared with several P-poor treatments. The intra-group ratio effects showed different behaviour: TEMA strongly responded to the ratios, FISP and MIX had moderate responses and NELU showed a weaker ratio response. There was a clear trend describing higher decomposition rate with low N:P ratio treatments (P-rich), especially for TEMA, MIX and FISP, and decreasing with increasing N:P ratio. Under high N:P ratios (P-poor), decomposition rates were relatively similar across groups, but differences increased towards lower ratios.

Worth noting are several outliers, indicating variation among replicates. Particularly the lower outlier (below 0) is most likely due to slight variances in starting mass of cellulose filter discs, which was not directly accounted for in calculations of mass loss (average mass of cellulose discs before treatment was used).

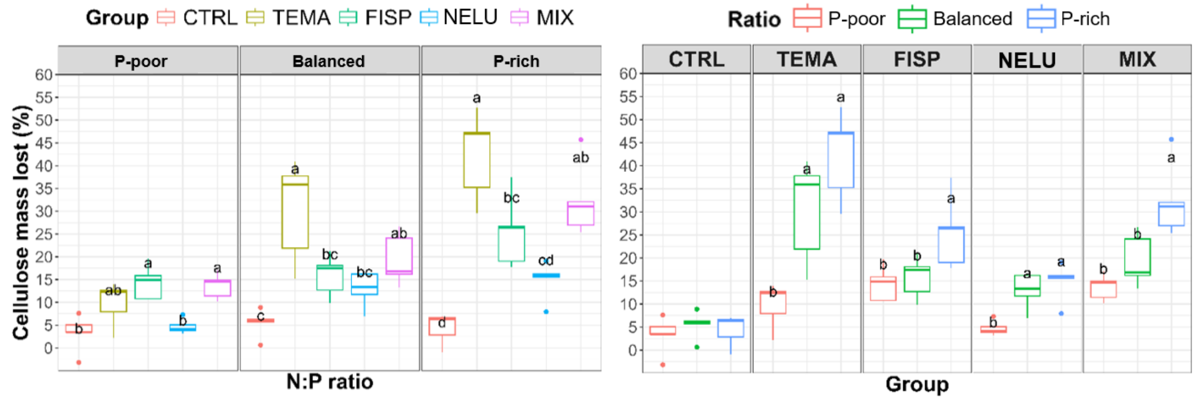

Figure 4: Cellulose mass loss (%) by ratio (left) and group (right)

Nutrient levels after decomposition were measured with ion chromatography (IC) for an average of all replicates of each group for the N:P ratio of 130:1 (P-poor) and showed a decrease for N (within a few 1000 ng μL^{-1}) and for P (around 200 ng μL^{-1}), with minor differences between the groups (Table 4: Nutrient contents of GMS (130:1) before and after decomposition experimentTable 4). The CTRL exhibited similar concentrations of N and P before and after the experiment, as would be expected.

Table 4: Nutrient contents of GMS (130:1) before and after decomposition experiment Original/prepared concentrations before decomposition (column 2&3) and IC measured concentrations after decomposition experiment (column 4&5)

Sample ID	original N ($\mu g\ L^{-1}$)	original P ($\mu g\ L^{-1}$)	Total N ($\mu g\ L^{-1}$)	Total P ($\mu g\ L^{-1}$)
TEMA130	14000	240	11800	8,5
FISP130	14000	240	10700	9,6
NELU130	14000	240	12700	14,9
MIX130	14000	240	12000	9,2
CTRL130	14000	240	14600	217

3.4 DNA quantity

To determine a possible effect of group and ratio on the amount of extracted DNA a two-way ANOVA was performed for the two variables, as well as interactions of the two compared to the Qubit measurements of extracted DNA (in ng μL^{-1}).

The CTRL was removed for this dataset as the measured DNA fell below the detection threshold of the Qubit Reader, which is the desired result. Furthermore, the dataset only contains 45 samples, as DNA was only extracted from three replicates of each group.

The Shapiro-Wilk test showed a normally distributed dataset. The effect of Group, Ratio and their interaction on DNA amount was significant for each factor (Group $p = 4.22e^{-07}$, Ratio $p = 2.97e^{-08}$, Group:Ratio $p = 1.90e^{-06}$). All groups significantly differed from each other apart from NELU and MIX. DNA amount showed significant differences between P-rich and P-poor treatment, as well as P-rich and balanced, but was insignificant for balanced to P-low.

mN:P ratio did not affect all groups equally (Figure 5): FISP P-rich vs FISP P-poor differ by more than 50 ng, while TEMA P-rich vs TEMA P-poor differed by less than 10 ng and this difference was non-significant. The FISP group under low N:P ratio exhibited the highest DNA concentrations (up to 99,2 ng) and differed significantly from most other treatment combinations, particularly TEMA treatments, while some comparisons with NELU and MIX treatments were not significant after Tukey adjustment. Low N:P ratio generally promoted higher DNA amounts in several groups, particularly NELU and FISP. However, changes between TEMA P-poor and TEMA balanced were very low and FISP balanced showed lower DNA amount then FISP P-poor.

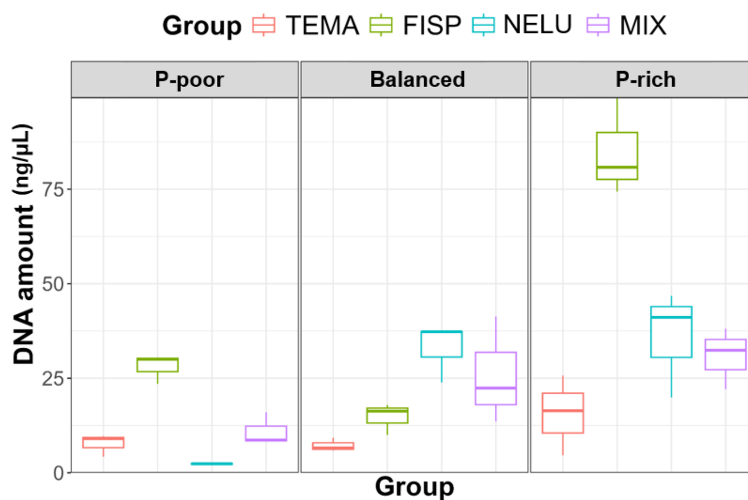

Figure 5: DNA amount (ng μL^{-1}) across groups and ratios

When all groups and ratios analysed together, Pearson's Correlation test showed a weak positive correlation between DNA amount and cellulose mass loss ($r = 0.27$), the relationship was not statistically significant ($p = 0.11$). When visualising the Decomposition - DNA relationship by groups (see Figure 6) a

strong correlation is seen with values for r of 0.69 (TEMA), 0.85 (FISP), 0.97 (NELU) and 0.96 (MIX) respectively, all of them being significant. DNA amount strongly correlates positively with decomposition rate within the groups but only shows a slight positive correlation for over all groups.

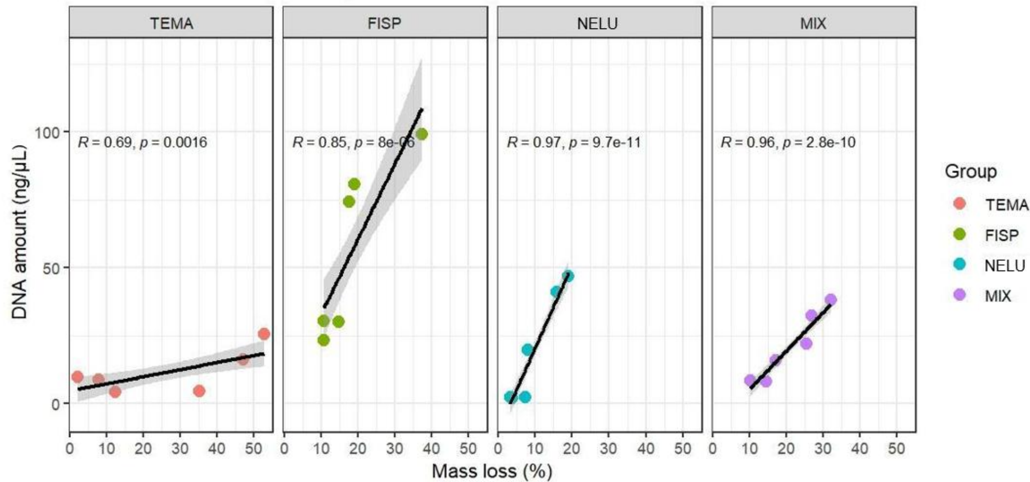

Figure 6: Correlation between DNA amount (ng µL^{-1}) and mass loss (%) for all groups, excluding CTRL

3.5 qPCR

Without having a standard with known CBHI copy numbers, the absolute copy number represented by each quantification cycle (Cq) could not be determined. Thus, a single plate qPCR was performed with only the two ratios for N:P of 130:1 and 1.3:1, representing the extremes of the nutrient treatments. This allowed us to use Cq directly in the result analyses as an approximation of CBHI copy numbers.

The amplification was successful (Figure 7) and the threshold was kept as a single threshold at default setting (approximately 1.9 RFU). Groups showed similar melting behaviour and temperature within the group. There was one exception for one technical replicate of TEMA at P-rich treatment, featuring a Cq of 43.7 compared to the other two technical replicates for this sample with around 15.4 cycles. This high Cq resembles the Cq from NTC and CTRL, suggesting a missing DNA template in this reaction during preparation.

CTRL and NTC (no template control) show different levels of amplification, but all at distinctively higher Cq (above 35) than target sequences (between 15 and 26), suggesting possible primer dimers (primers binding to themselves), non-

target amplification or small-scale contamination. This is the normal result to be expected.

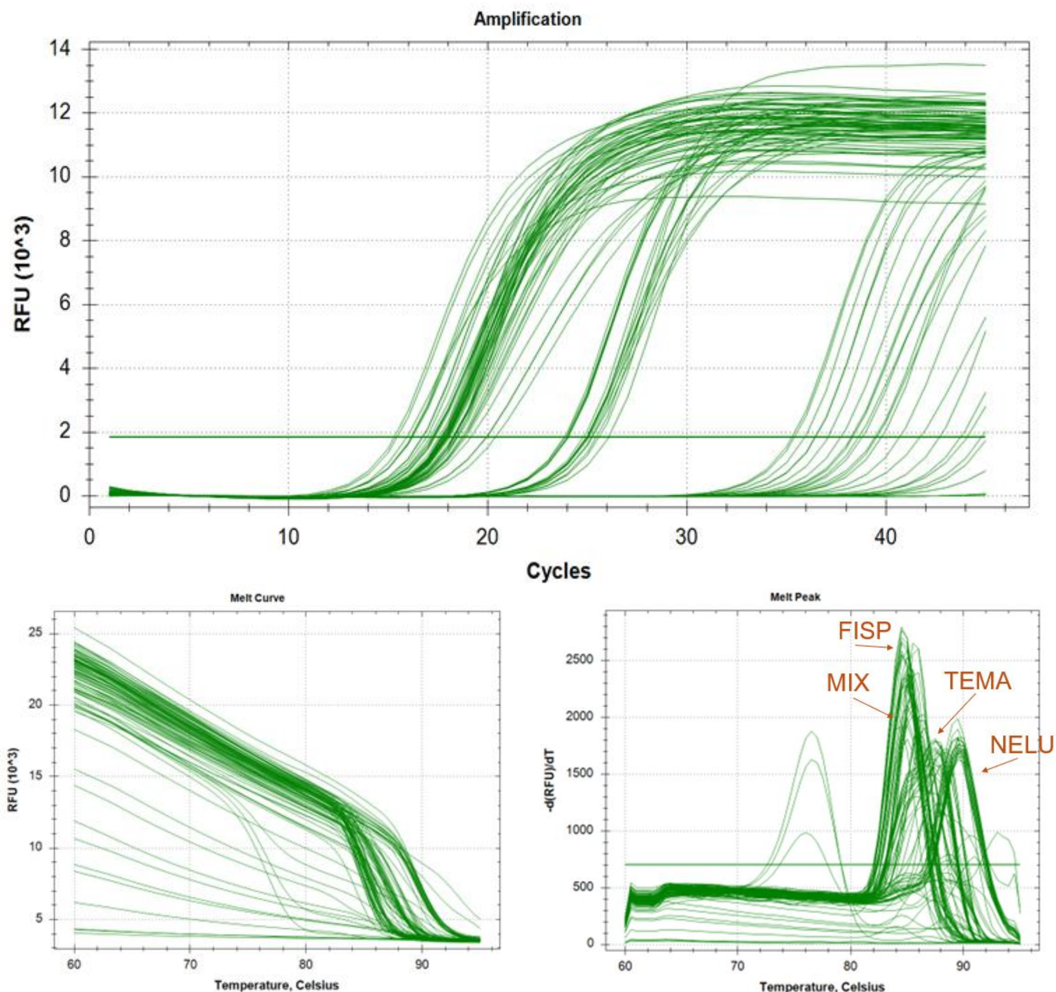

Figure 7: qPCR result with cbhI_FUN_1 primer pair
Amplification curve (top), Melting curve (left) and Melting peak (right)

No significant difference (p = 0.454) was identified in Cq between the two ratios for all groups (Figure 8). The degree of correlation between Cq and mass loss varies greatly between groups (from R = 0.6 for MIX to R = 0.26 for TEMA). Noteworthy is that TEMA data points would be better suited with a non-linear correlation and that the outlier greatly affects correlation for TEMA (Figure 9). Following the assumption that more DNA results in a lower cycle number, the Cq value was inspected for relationship and showed a clear trend (Figure 10).

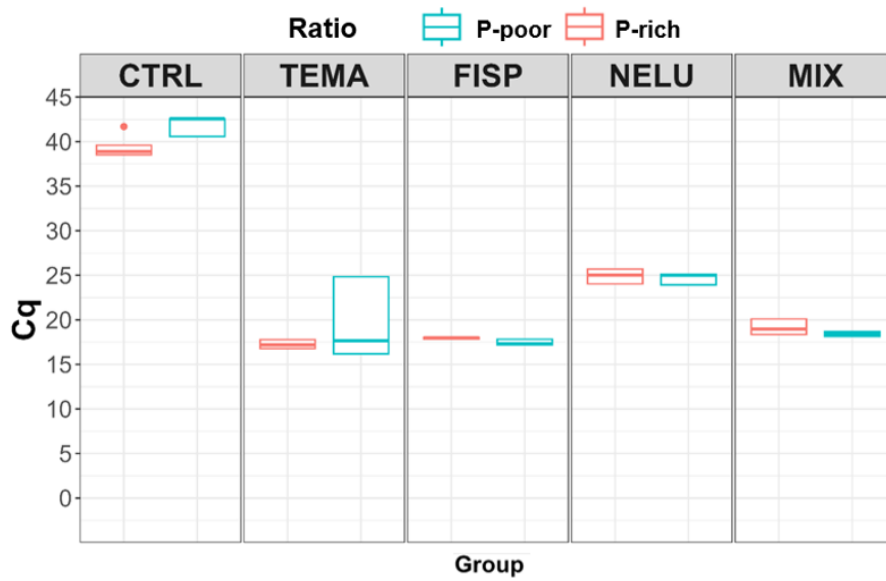

Figure 8: Cycle number (Cq) across the two N:P ratios

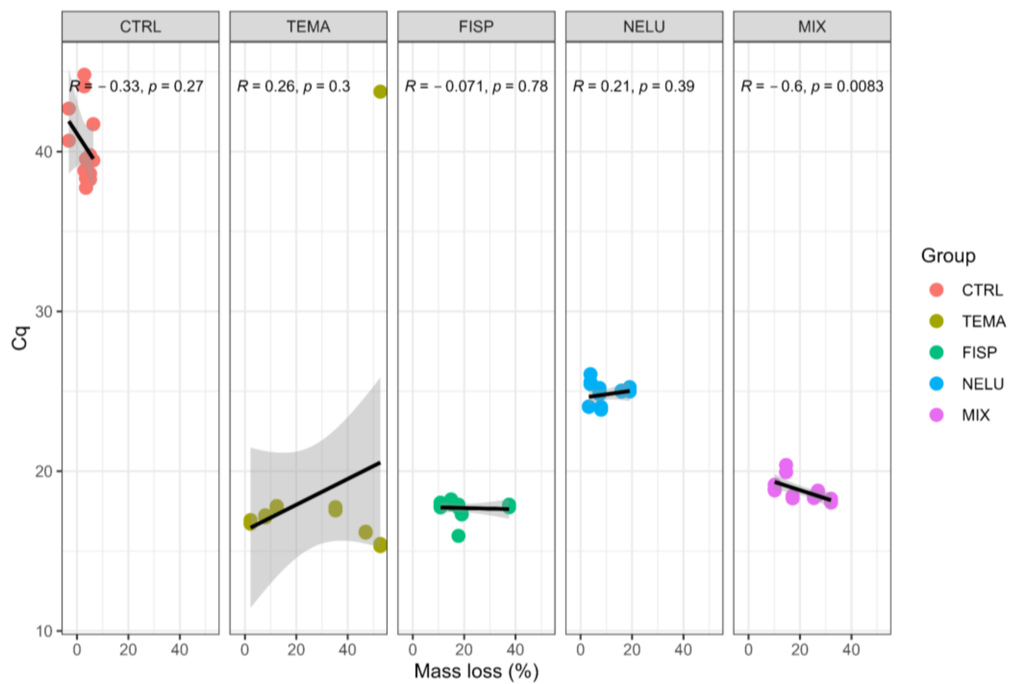

Figure 9: Pearson correlation of Cq and Decomposition

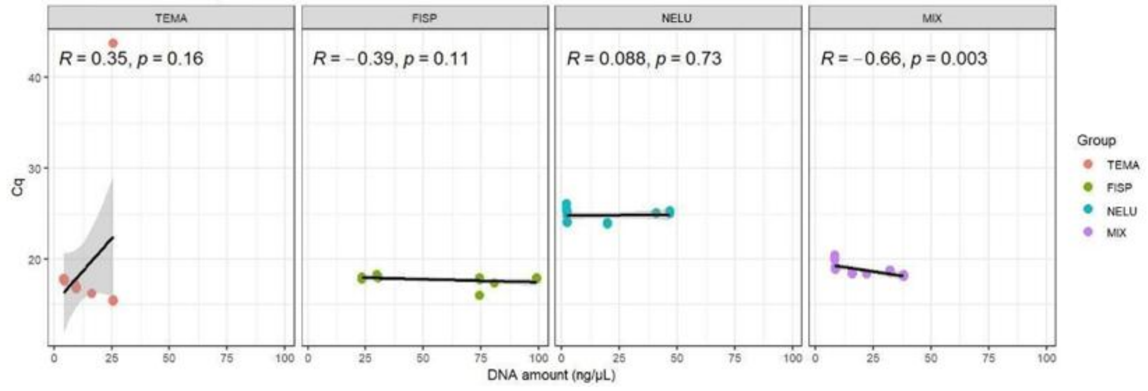

Figure 10: Pearson correlation of Cycle number (Cq) and DNA amount (ng µL^{-1})

4. Discussion

In this thesis work, I examined whether aquatic fungal decomposition-related gene abundance can help us understand how environmental conditions affect decomposition processes as a potential step towards identifying an eDNA-based tool for monitoring this key ecosystem function. I specifically focused on nutrient enrichments that are associated with intensive agriculture practices and the effects of the resulting stoichiometric disturbance on plant litter decomposition by aquatic hyphomycetes.

The underlying hypothesis of this study is that increased phosphorus availability stimulates fungal growth, which in turn drives decomposition. Higher growth is expected to be reflected in greater DNA abundance, and it is assumed that functional gene abundance is connected to decomposition rate through fungal biomass. The aim was therefore to examine a potential link between target gene abundance and ecosystem functioning. If such a link can be confirmed, this approach could serve as a proxy for evaluating decomposition function in stream ecosystems, though continued testing would be necessary to verify that the relationship holds consistently across conditions.

4.1 Determine the effects of N:P ratios on decomposition

Decomposition rate showed clear differences between the different ratios and groups. A clear trend of higher decomposition with lower N:P ratio (P-rich) was observed, particularly for TEMA, FISP and MIX, decreasing with increasing N:P ratio. This aligns with previous studies showing that nutrient enrichment can accelerate microbial decomposition of leaf litter, particularly where phosphorus availability is a limiting factor (Güsewell & Gessner 2009).
Phosphorus plays several important roles in cellular functions. It is a key structural component of genetic material (DNA and RNA), a key constituent of phospholipid cell membranes, and central to energy metabolism via ATP and other phosphorylated compounds. Phosphorus is therefore closely linked to microbial growth, enzyme activity, and overall cellular function (Danger et al. 2016), which formed part of the motivation for varying phosphorus concentrations in this experiment. Most species showed an increase in DNA concentration and decomposition with increasing P level, suggesting increased growth. Even though Gulis et al. (2017) found phosphorus not to be significantly related to fungal growth or production compared to a dissolved nitrate enriched treatment, the positive relationship between phosphorus, DNA abundance and decomposition

observed in this study suggests that phosphorus availability may still influence decomposition activity even if there might not be a direct correlation. The effect of ratio depended on the group, as N:P ratio did not affect all groups equally. TEMA strongly responded to ratio, FISP and MIX had moderate responses, and NELU showed a weaker ratio response. The differences of the individual species in their response to the N:P ratios underline the importance of studying individual species separately, complementing community-based decomposition studies.

Only one varying factor, P levels, was chosen for this project, but the reality shows that agricultural impacts are a complex combination of stressors, including pesticide and fungicide input, temperature, PFAS and of course fertilizer induced nutrient enrichment (Pérez et al. 2018). Nutrient enrichment in agricultural streams not only affects water quality but also degrades habitat complexity and structure (Feckler et al. 2023). Agricultural land use creates contrasting pressures on stream ecology. While increased nutrient availability can increase fungal growth and activity, this can also cause changes in habitat structure, water chemistry and community composition, thereby impairing the environmental conditions that fungi depend on. Complementary testing on different levels of N possibly in combination with ergosterol biomass measurements could give additional insight into functional changes and add a valuable perspective on the relationship between biomass, growth, decomposition, N availability and functional gene copies.

4.2 Investigate the relationship between decomposition and DNA/gene copies

The positive correlation of DNA amount and decomposition for each group supports the underlying hypothesis that increased phosphorus availability stimulates fungal growth, which is reflected in both greater DNA quantities and higher decomposition rates. The observed relationship is unlikely to result from changes in gene copy number per genome, but rather from an increase in the number of fungal cells and genomes present in the sample. More growth leads to more hyphae, more nuclei, more total genomic DNA, and more target gene copies. This positive association between DNA abundance and decomposition indicates that molecular measurements have potential to be used as proxies for fungal activity.

It is important to note, however, that the use of total extracted DNA as a proxy for fungal biomass is only meaningful in the context of pure culture experiments such as this one. In a field setting, environmental DNA samples would contain genetic material from a wide range of organisms, making it impossible to attribute total

DNA quantity to a single fungal species or even a whole fungal community separate from other DNA. This represents a fundamental limitation of total DNA quantification as a meaningful metric and underlines the need for gene-specific primers targeting functional genes in any future application of qPCR to real-world eDNA monitoring.

The requirement for gene-specific primers also raises the question of primer quality and design. The development of the β-glucosidase primers in this study illustrates some of the challenges involved.

The β-glucosidase primers designed in this study did not produce successful amplification under the tested conditions. There can be several reasons for primers not to perform in PCR. Reasons may include:

- Mismatches, especially near the 3' end (Green et al. 2015)
- Unequal melting temperatures (Tm) across sequences (Green et al. 2015)
- Wrong annealing temperature: too high increases mismatch bias or too low increasing nonspecific products and mismatched binding (Green et al. 2015)
- Formation of primer dimers or hairpin structures (Bustin & Huggett 2017)
- Reduced DNA quality and/or quantity
- High GC content, especially at the 3' end (Bustin & Huggett 2017)
- No GC clamp

The lack of aquatic fungi DNA sequences was recognised as a gap and identified as a priority target even a decade ago (Duarte et al. 2016; Khomich et al. 2018), yet the representation has barely improved. Difficulties in finding well conserved regions during primer design of β-glucosidase led to accepted gaps in consensus of chosen regions. Furthermore, only a small number of sequences (30) was used for primer design in this work and a higher number may change the quality of the consensus.
As the same samples were used to validate the new cellobiohydrolase (CBHI) primers and produced a positive result, quality and quantity of DNA should be sufficient in those samples, ruling out the plausibility of amplification failure cause by samples. Due to time limitations of this study, no further testing on possible primer shortcomings was performed.

In this study relative abundance of the functional gene CBHI did not significantly differ between nutrient treatments and did not correlate with decomposition. This suggests that gene copy alone may not adequately reflect decomposition activity. Even though decomposition rates varied greatly across nutrient treatments, CBHI showed little to no change in abundance. Abundance of a gene does not indicate

activation of that gene, and no prediction can be made as to the degree of gene expression. Fungal functional response may therefore be more regulated at the level of gene expression or enzyme activity than gene abundance alone.

Interpretation of gene copy abundance is further complicated by the occurrence of gene copy number polymorphisms in fungi. This means that different individuals, strains or populations within one species can possess different numbers of copies for a particular gene in their genome. Previous studies on arbuscular mycorrhizal fungi have shown that copy number variation can occur in rDNA and protein-coding regions and may influence gene expression, fitness, and adaptation (Corradi et al. 2007). While such variation may contribute to ecological flexibility and contribute to better adaptations to selective pressures (Tang & Amon 2013), it also introduces uncertainty when gene copy abundance is used as a direct proxy for biomass. Nevertheless, the present study primarily interprets increased gene copy abundance as a consequence of increased fungal growth and biomass production rather than changes in copy number per genome.

Furthermore, normalising the Cq value with a standard curve and a reference gene would give a more precise and absolute copy number abundance compared to the attempt to normalise with the DNA concentration. Using DNA concentration to normalise qPCR signal (Cq/DNA amount) may provide additional information beyond the two metrics separately, partially accounting for differences in extraction yield and sample-specific variation. As previously mentioned, this is only possible if total DNA amount refers to pure culture DNA (as is the case here).
As we did not have a standard curve available, it is not possible to calculate the exact copy numbers of genes and the Cq was directly used as an approximation and compared between groups and ratios. This was possible as only one qPCR run was performed with all samples on one single 96-well plate, making Cq comparable within this run. To fit all samples on one plate and still have a meaningful selection of samples, the samples from the two “extreme” ratios (P-rich and P-poor) were chosen. With a validated standard curve absolute quantification of CBHI copy numbers would become possible and enable comparison across independent experiments and studies with a meaningful unit. This would also allow for including the balanced N:P treatment, possibly providing a more complete picture of gene abundance across the nutrient gradient. Including a reference gene (e.g. a house keeping gene) could normalise CBHI gene copy number against total fungal DNA, helping to interpret if changes in gene copy number come from changes in fungal biomass or DNA input amount. Including a standard curve and a reference gene would improve the results of qPCR and boost the applicability of it as a tool for linking molecular

measurements to ecosystem functioning and a prerequisite for intersite comparison in any real monitoring programme.

4.3 Assess the potential for fungal monitoring using eDNA approaches

eDNA metabarcoding and monitoring is increasingly used and validated for species detection (Taberlet et al. 2012; Ji et al. 2013; Pochon et al. 2025), but lacks application to underlying functional questions. Targeting genes associated with a specific function is not entirely a novel idea, it is, however, still an emerging field and mostly explored in soil microbiology and terrestrial systems (Barbi et al. 2020; Wemheuer et al. 2020). This thesis attempts to apply a similar logic to aquatic fungi and decomposition as a function.

Leaf litter decomposition has been found to be a powerful, integrative indicator of stream ecosystem functioning, linking terrestrial and aquatic ecosystems across multiple organism groups (Frainer et al. 2021). The Water Framework Directive (WFD) that aims to restore water bodies to reach good chemical and ecological status and prevent deterioration, bases this ecological status on structure and functioning of aquatic ecosystems, but indicators only address structural metrics and neglect function (Frainer et al. 2021). Therefore, functional impairment often goes unnoticed or is underestimated, as tolerant taxa can often compensate for functional deficit or functioning can be reduced through stress without changing community composition. Most importantly, structural metrics often miss microbial processes or inadequately represent their dynamics (Frainer et al. 2021). The WFD specifically includes fish, macroinvertebrates, macrophytes and phytoplankton/phytobenthos, entirely lacking any mention of aquatic fungi, despite their key role in stream ecosystem health. Decomposition responds sensitively to many stressors like nutrient enrichment, pesticides, hydro morphological alteration, sedimentation, acidification, mining and urbanisation and can therefore be used as an indicator for the magnitude of these stressors (Gessner & Chauvet 2002; Young et al. 2008). Understanding which stressors most predictably alter functional gene signals and in which way are necessary requirements for applying monitoring using eDNA to agricultural streams.

The results of this study provide partial support for the hypothesis that increased phosphorus availability stimulates fungal growth, drives decomposition, and is reflected in greater DNA abundance. The positive association between DNA abundance and decomposition within species groups indicates that molecular measurements have potential to be used as proxies for fungal activity. However, a lack of predictable correlation between relative gene copy numbers and

decomposition makes it difficult at this stage to link qPCR results directly to ecosystem functioning. This study therefore represents a proof of concept, conducted under controlled conditions, that requires further testing and validation under real-world conditions including from field-collected eDNA samples.

As noted in section 4.2, total DNA quantity cannot serve as a meaningful proxy in field settings where environmental samples contain genetic material from many organisms simultaneously. Gene-specific qPCR targeting aquatic fungal functional genes such as CBHI therefore represents the more promising avenue for eDNA-based monitoring of decomposition function, provided that the relationship between gene copy abundance and ecosystem functioning can be established more robustly. In a mixed environmental sample, the relative abundance of a decomposition-related gene across the full fungal community may be more ecologically informative than the total DNA signal from any single species.

A further consideration is the substrate used in this study. Cellulose filter discs represent a simplified analogue of leaf litter, used here as a basic and controllable carbon source that eliminates the additional nutrient input that would occur with natural substrate. While this was appropriate for a proof-of-concept study, in situ eDNA samples would involve far more variable substrates, as nutrient and lignin content vary greatly among and even within tree species (Gessner & Chauvet 2002). The relationship between gene copy abundance and decomposition would need to be validated under those more complex conditions.

To be able to adopt this method from controlled laboratory conditions to field conditions several considerations need to be made and understood. It starts by identifying sampling locations within a stream cross section. An eDNA sample taken directly from leaf litter (sampling of hyphae) will most likely yield different results for functional genes compared to sampling done on benthic substrate or the water column (mainly spores).
By nature, an eDNA sample includes DNA from numerous species and not limited to fungal DNA making it impossible to use total DNA as an indicator. Even when using functional gene primers, they would need to be fungi specific and not amplify other organisms with homologue genes.
To be able to draw meaningful conclusions from functional gene copy numbers, time series data — periodic measurements over a longer time period — would be ideal to establish baseline levels and allow results to be compared and interpreted. Given the distinct variation in DNA amount but only minor variation in CBHI gene copies between N:P ratios, this particular gene may show comparable

abundance across species, which could be an advantage when comparing results across sites or conditions.
Additionally, more information on individual species of aquatic fungi and better availability of their DNA sequences could better inform primer design, help explain differences in growth characteristics and molecular signals across species and improve the understanding of single species contribution to community-level decomposition processes when subjected to stressors.

Considering the different responses of TEMA, FISP and NELU to phosphorus enrichment, the relative proportions in an eDNA sample could possibly signal stoichiometric stress and might be more informative than a single functional gene count. Fernandez Nuñez et al. (2021) proposed ratios of different fungal and bacterial groups as indicators to monitor terrestrial soil ecosystem recovery after mining. Potentially this could be an idea worth exploring in the context of stressors affecting aquatic fungi and their ecosystem functions. Appropriate indicator taxa would need to be determined and a consistent relationship between chosen groups established.

5. Conclusions and Future Outlook

Decomposition is one of the most fundamental ecosystem functions in freshwater streams, driving nutrient cycling and energy flow within aquatic food webs, and can be strongly influenced by environmental impacts like nutrient enrichment from agricultural land use. Aquatic hyphomycetes are key drivers of these decomposition processes in streams, but their functional response on a molecular level still remains understudied. This thesis investigated the dynamics of decomposition rate, total DNA quantity and abundance of a fungal decomposition-related functional gene (CBHI) of three aquatic hyphomycete species and a community mixture of the three species, across three different N:P ratios. The goal was to take the first step towards developing an eDNA-based tool for monitoring freshwater ecosystem functioning.

Decomposition increased with increasing P-level for all species, though species differed in the strength of their response. Together with an increase in DNA abundance with higher P-levels for most groups, this shows a link between P availability, growth and decomposition rate. However, no clear correlation was observed between CBHI gene copies and decomposition for the conditions that were tested and no significant difference in CBHI gene abundance between P-rich and P-poor nutrient treatments. This leads to the conclusion that CBHI does not seem to be a good indicator for monitoring aquatic fungal decomposition. This does not, however, undermine the potential of molecular methods for monitoring entirely, but does demonstrate that CBHI abundance alone cannot yet predict ecosystem functioning. Further research is needed to evaluate the applicability of fungal functional gene-based approaches for monitoring decomposition processes in freshwater ecosystems.

The multitude of open and unanswered questions illustrate the complexity of developing a functioning tool and the journey it takes to gain usable and comparable end results. The approach tested in this thesis is still at the very beginning of being developed and this study is a first step in testing the viability of this proposed method. Further testing and adaptations will be necessary to fully assess the applicability of the method.

References

Barbi, F., Kohler, A., Barry, K., Baskaran, P., Daum, C., Fauchery, L., Ihrmark, K., Kuo, A., LaButti, K., Lipzen, A., Morin, E., Grigoriev, I.V., Henrissat, B., Lindahl, B.D. & Martin, F. (2020). Fungal ecological strategies reflected in gene transcription - a case study of two litter decomposers. *Environmental Microbiology*, 22 (3), 1089–1103. https://doi.org/10.1111/1462-2920.14873

Bärlocher, F. (1985). The role of fungi in the nutrition of stream invertebrates. https://doi.org/10.1111/j.1095-8339.1985.tb01137.xDigital Object Identifier (DOI)

Bärlocher, F. (2009). Reproduction and dispersal in aquatic hyphomycetes. *Mycoscience*, 50 (1), 3–8. https://doi.org/10.1007/S10267-008-0449-X

Bärlocher, F. & Kendrick, B. (1974). Dynamics of the Fungal Population on Leaves in a Stream. *The Journal of Ecology*, 62 (3), 761. https://doi.org/10.2307/2258954

Bustin, S. & Huggett, J. (2017). qPCR primer design revisited. *Biomolecular Detection and Quantification*, 14, 19–28. https://doi.org/10.1016/j.bdq.2017.11.001

Camelo, F.R.B., Tonin, A.M., Salgueiro, L., Sena, G., Braga, I., Medeiros, A.O. & Gonçalves Júnior, J.F. (2022). Tropical stream microcosms of isolated fungal species suggest nutrient enrichment does not accelerate decomposition but might inhibit fungal biomass production. *FEMS Microbiology Letters*, 369 (1), fnac113. https://doi.org/10.1093/femsle/fnac113

Chambers, P.A., Meissner, R., Wrona, F.J., Rupp, H., Guhr, H., Seeger, J., Culp, J.M. & Brua, R.B. (2006). Changes in Nutrient Loading in an Agricultural Watershed and Its Effects on Water Quality and Stream Biota. *Hydrobiologia*, 556 (1), 399–415. https://doi.org/10.1007/s10750-005-1202-5

Corradi, N., Croll, D., Colard, A., Kuhn, G., Ehinger, M. & Sanders, I.R. (2007). Gene Copy Number Polymorphisms in an Arbuscular Mycorrhizal Fungal Population. *Applied and Environmental Microbiology*, 73 (1), 366–369. https://doi.org/10.1128/AEM.01574-06

Costello, D.M., Tiegs, S.D., Boyero, L., Canhoto, C., Capps, K.A., Danger, M., Frost, P.C., Gessner, M.O., Griffiths, N.A., Halvorson, H.M., Kuehn, K.A., Marcarelli, A.M., Royer, T.V., Mathie, D.M., Albariño, R.J., Arango, C.P., Aroviita, J., Baxter, C.V., Bellinger, B.J., Bruder, A., Burdon, F.J., Callisto, M., Camacho, A., Colas, F., Cornut, J., Crespo-Pérez, V., Cross, W.F., Derry, A.M., Douglas, M.M., Elosegi, A., De Eyto, E., Ferreira, V., Ferriol, C., Fleituch, T., Follstad Shah, J.J., Frainer, A., Garcia, E.A., García, L., García, P.E., Giling, D.P., Gonzales-Pomar, R.K., Graça, M.A.S., Grossart, H., Guérold, F., Hepp, L.U., Higgins, S.N., Hishi, T., Iñiguez-Armijos, C., Iwata, T., Kirkwood, A.E., Koning, A.A., Kosten, S., Laudon, H., Leavitt, P.R., Lemes Da Silva, A.L., Leroux, S.J., LeRoy, C.J., Lisi, P.J., Masese, F.O., McIntyre, P.B., McKie, B.G., Medeiros, A.O., Miliša, M., Miyake, Y., Mooney, R.J., Muotka, T., Nimptsch, J., Paavola, R., Pardo, I., Parnikoza, I.Y., Patrick, C.J., Peeters, E.T.H.M., Pozo, J., Reid, B., Richardson, J.S., Rincón, J., Risnoveanu, G., Robinson, C.T., Santamans, A.C., Simiyu, G.M., Skuja, A., Smykla, J., Sponseller, R.A., Teixeira-de Mello, F., Vilbaste, S., Villanueva, V.D., Webster, J.R., Woelfl, S., Xenopoulos, M.A., Yates, A.G., Yule, C.M., Zhang, Y. & Zwart, J.A. (2022). Global Patterns and Controls of Nutrient Immobilization on Decomposing Cellulose in Riverine Ecosystems.

Global Biogeochemical Cycles, 36 (3), e2021GB007163. https://doi.org/10.1029/2021GB007163

Cummins, K.W. (1974). Structure and Function of Stream Ecosystems. *BioScience*, 24 (11), 631–641. https://doi.org/10.2307/1296676

Danger, M., Gessner, M.O. & Bärlocher, F. (2016). Ecological stoichiometry of aquatic fungi: current knowledge and perspectives. *Fungal Ecology*, 19, 100–111. https://doi.org/10.1016/j.funeco.2015.09.004

Duarte, S., Bärlocher, F., Pascoal, C. & Cássio, F. (2016). Biogeography of aquatic hyphomycetes: Current knowledge and future perspectives. *Fungal Ecology*, 19, 169–181. https://doi.org/10.1016/j.funeco.2015.06.002

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A., Soto, D., Stiassny, M.L.J. & Sullivan, C.A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, 81 (2), 163–182. https://doi.org/10.1017/S1464793105006950

Elser, J.J., Fagan, W.F., Denno, R.F., Dobberfuhl, D.R., Folarin, A., Huberty, A., Interlandi, S., Kilham, S.S., McCauley, E., Schulz, K.L., Siemann, E.H. & Sterner, R.W. (2000). Nutritional constraints in terrestrial and freshwater food webs. *Nature*, 408 (6812), 578–580. https://doi.org/10.1038/35046058

Falkowski, P., Hogberg, P., Seitzinger, S., Smetacek, V. & Steffen', W. (2000). The Global Carbon Cycle: A Test of Our Knowledge of Earth as a System.

Feckler, A., Schnurr, J., Kalčíková, G., Truchy, A., McKie, B.G., Schäfer, R.B., Schulz, R. & Bundschuh, M. (2023). Agricultural land use weakens the relationship between biodiversity and ecosystem functioning. *Oikos*, 2023 (10), e09936. https://doi.org/10.1111/oik.09936

Fernandez Nuñez, N., Maggia, L., Stenger, P.-L., Lelievre, M., Letellier, K., Gigante, S., Manez, A., Mournet, P., Ripoll, J. & Carriconde, F. (2021). Potential of high-throughput eDNA sequencing of soil fungi and bacteria for monitoring ecological restoration in ultramafic substrates: The case study of the New Caledonian biodiversity hotspot. *Ecological Engineering*, 173, 106416. https://doi.org/10.1016/j.ecoleng.2021.106416

Frainer, A., Bruder, A., Colas, F., Ferreira, V. & McKie, B.G. (2021). Plant Litter Decomposition as a Tool for Stream Ecosystem Assessment. In: Swan, C.M., Boyero, L., & Canhoto, C. (eds) *The Ecology of Plant Litter Decomposition in Stream Ecosystems*. Springer International Publishing. 483–509. https://doi.org/10.1007/978-3-030-72854-0_21

Gessner, M.O. & Chauvet, E. (1993). Ergosterol-to-Biomass Conversion Factors for Aquatic Hyphomycetes. *Applied and Environmental Microbiology*, 59 (2), 502–507. https://doi.org/10.1128/aem.59.2.502-507.1993

Gessner, M.O. & Chauvet, E. (2002). A CASE FOR USING LITTER BREAKDOWN TO ASSESS FUNCTIONAL STREAM INTEGRITY. *Ecological Applications*, 12 (2), 498–510. https://doi.org/10.1890/1051-0761(2002)012%5B0498:ACFULB%5D2.0.CO;2

Gessner, M.O., Gulis, V., Kuehn, K.A., Chauvet, E. & Suberkropp, K. (2007). 17 Fungal Decomposers of Plant Litter in Aquatic Ecosystems. In: *The Mycota*. vol 4. Springer, Berlin, Heidelberg. https://doi.org/https://doi.org/10.1007/978-3-540-71840-6_17

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H. & Hättenschwiler, S. (2010). Diversity meets decomposition. *Trends in Ecology & Evolution*, 25 (6), 372–380. https://doi.org/10.1016/j.tree.2010.01.010

Göthe, E., Wiberg-Larsen, P., Kristensen, E.A., Baattrup-Pedersen, A., Sandin, L. & Friberg, N. (2015). Impacts of habitat degradation and stream spatial

location on biodiversity in a disturbed riverine landscape. *Biodiversity and Conservation*, 24 (6), 1423–1441. https://doi.org/10.1007/s10531-015-0865-0

Graça, D., Arias-Real, R., Fernandes, I., Cássio, F. & Pascoal, C. (2024). Fungal identity mediates the impacts of multiple stressors on freshwater ecosystems. *Science of The Total Environment*, 937, 173466. https://doi.org/10.1016/j.scitotenv.2024.173466

Green, S.J., Venkatramanan, R. & Naqib, A. (2015). Deconstructing the Polymerase Chain Reaction: Understanding and Correcting Bias Associated with Primer Degeneracies and Primer-Template Mismatches. Ugaz, V.M. (ed.) (Ugaz, V. M., ed.) *PLOS ONE*, 10 (5), e0128122. https://doi.org/10.1371/journal.pone.0128122

Grossart, H.-P., Van Den Wyngaert, S., Kagami, M., Wurzbacher, C., Cunliffe, M. & Rojas-Jimenez, K. (2019). Fungi in aquatic ecosystems. *Nature Reviews Microbiology*, 17 (6), 339–354. https://doi.org/10.1038/s41579-019-0175-8

Gulis, V., Kuehn, K.A., Schoettle, L.N., Leach, D., Benstead, J.P. & Rosemond, A.D. (2017). Changes in nutrient stoichiometry, elemental homeostasis and growth rate of aquatic litter-associated fungi in response to inorganic nutrient supply. *The ISME Journal*, 11 (12), 2729–2739. https://doi.org/10.1038/ismej.2017.123

Güsewell, S. & Gessner, M.O. (2009). N : P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. *Functional Ecology*, 23 (1), 211–219. https://doi.org/10.1111/j.1365-2435.2008.01478.x

Harper, L.R., Buxton, A.S., Rees, H.C., Bruce, K., Brys, R., Halfmaerten, D., Read, D.S., Watson, H.V., Sayer, C.D., Jones, E.P., Priestley, V., Mächler, E., Múrria, C., Garcés-Pastor, S., Medupin, C., Burgess, K., Benson, G., Boonham, N., Griffiths, R.A., Lawson Handley, L. & Hänfling, B. (2019). Prospects and challenges of environmental DNA (eDNA) monitoring in freshwater ponds. *Hydrobiologia*, 826 (1), 25–41. https://doi.org/10.1007/s10750-018-3750-5

Ingold, C.T. (1942). Aquatic hyphomycetes of decaying alder leaves. *Transactions of the British Mycological Society*, 25 (4), 339-IN6. https://doi.org/10.1016/S0007-1536(42)80001-7

Ji, Y., Ashton, L., Pedley, S.M., Edwards, D.P., Tang, Y., Nakamura, A., Kitching, R., Dolman, P.M., Woodcock, P., Edwards, F.A., Larsen, T.H., Hsu, W.W., Benedick, S., Hamer, K.C., Wilcove, D.S., Bruce, C., Wang, X., Levi, T., Lott, M., Emerson, B.C. & Yu, D.W. (2013). Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. Holyoak, M. (ed.) (Holyoak, M., ed.) *Ecology Letters*, 16 (10), 1245–1257. https://doi.org/10.1111/ele.12162

Khomich, M., Cox, F., Andrew, C.J., Andersen, T., Kauserud, H. & Davey, M.L. (2018). Coming up short: Identifying substrate and geographic biases in fungal sequence databases. *Fungal Ecology*, 36, 75–80. https://doi.org/10.1016/j.funeco.2018.08.002

Pérez, J., Martínez, A., Descals, E. & Pozo, J. (2018). Responses of Aquatic Hyphomycetes to Temperature and Nutrient Availability: a Cross-transplantation Experiment. *Microbial Ecology*, 76 (2), 328–339. https://doi.org/10.1007/s00248-018-1148-6

Pinckney, J.L., Paerl, H.W., Tester, P. & Richardson, T.L. (2001). The Role of Nutrient Loading and Eutrophication in Estuarine Ecology. *Enviornmental Health Perspectives*, 109, 699–706

Pochon, X., Bowers, H.A., Zaiko, A. & Wood, S.A. (2025). Advancing the environmental DNA and RNA toolkit for aquatic ecosystem monitoring and management. *PeerJ*, 13, e19119. https://doi.org/10.7717/peerj.19119

Sayer, C.A., Fernando, E., Jimenez, R.R., Macfarlane, N.B.W., Rapacciuolo, G., Böhm, M., Brooks, T.M., Contreras-MacBeath, T., Cox, N.A., Harrison, I., Hoffmann, M., Jenkins, R., Smith, K.G., Vié, J.-C., Abbott, J.C., Allen, D.J., Allen, G.R., Barrios, V., Boudot, J.-P., Carrizo, S.F., Charvet, P., Clausnitzer, V., Congiu, L., Crandall, K.A., Cumberlidge, N., Cuttelod, A., Dalton, J., Daniels, A.G., De Grave, S., De Knijf, G., Dijkstra, K.-D.B., Dow, R.A., Freyhof, J., García, N., Gessner, J., Getahun, A., Gibson, C., Gollock, M.J., Grant, M.I., Groom, A.E.R., Hammer, M.P., Hammerson, G.A., Hilton-Taylor, C., Hodgkinson, L., Holland, R.A., Jabado, R.W., Juffe Bignoli, D., Kalkman, V.J., Karimov, B.K., Kipping, J., Kottelat, M., Lalèyè, P.A., Larson, H.K., Lintermans, M., Lozano, F., Ludwig, A., Lyons, T.J., Máiz-Tomé, L., Molur, S., Ng, H.H., Numa, C., Palmer-Newton, A.F., Pike, C., Pippard, H.E., Polaz, C.N.M., Pollock, C.M., Raghavan, R., Rand, P.S., Ravelomanana, T., Reis, R.E., Rigby, C.L., Scott, J.A., Skelton, P.H., Sloat, M.R., Snoeks, J., Stiassny, M.L.J., Tan, H.H., Taniguchi, Y., Thorstad, E.B., Tognelli, M.F., Torres, A.G., Torres, Y., Tweddle, D., Watanabe, K., Westrip, J.R.S., Wright, E.G.E., Zhang, E. & Darwall, W.R.T. (2025). One-quarter of freshwater fauna threatened with extinction. *Nature*, 638 (8049), 138–145. https://doi.org/10.1038/s41586-024-08375-z

Schröter, M., Bonn, A., Klotz, S., Seppelt, R. & Baessler, C. (eds) (2019). *Atlas of Ecosystem Services: Drivers, Risks, and Societal Responses*. Springer International Publishing. https://doi.org/10.1007/978-3-319-96229-0

Shearer, C.A., Descals, E., Kohlmeyer, B., Kohlmeyer, J., Marvanová, L., Padgett, D., Porter, D., Raja, H.A., Schmit, J.P., Thorton, H.A. & Voglymayr, H. (2007). Fungal biodiversity in aquatic habitats. *Biodiversity and Conservation*, 16 (1), 49–67. https://doi.org/10.1007/s10531-006-9120-z

Taberlet, P., Coissac, E., Hajibabaei, M. & Rieseberg, L.H. (2012). Environmental DNA. *Molecular Ecology*, 21 (8), 1789–1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x

Tang, Y.-C. & Amon, A. (2013). Gene Copy-Number Alterations: A Cost-Benefit Analysis. *Cell*, 152 (3), 394–405. https://doi.org/10.1016/j.cell.2012.11.043

Tant, C.J., Rosemond, A.D., Mehring, A.S., Kuehn, K.A. & Davis, J.M. (2015). The role of aquatic fungi in transformations of organic matter mediated by nutrients. *Freshwater Biology*, 60 (7), 1354–1363. https://doi.org/10.1111/fwb.12573

Wemheuer, F., Taylor, J.A., Daniel, R., Johnston, E., Meinicke, P., Thomas, T. & Wemheuer, B. (2020). Tax4Fun2: prediction of habitat-specific functional profiles and functional redundancy based on 16S rRNA gene sequences. *Environmental Microbiome*, 15 (1), 11. https://doi.org/10.1186/s40793-020-00358-7

Young, R.G., Matthaei, C.D. & Townsend, C.R. (2008). Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *Journal of the North American Benthological Society*, 27 (3), 605–625. https://doi.org/10.1899/07-121.1

Popular science summary

Freshwater ecosystems are home to 10% of all known species even though they only make up 1% of the Earth's surface. Yet approximately 24% of assessed freshwater fauna species are threatened by extinction. Agricultural intensification is one stressor that can cause pollution, nutrient enrichment and habitat degradation, affecting valuable living space for countless animals and plants and their interactions. This can affect ecosystem functions, the natural biological, chemical and physical processes within ecosystems and ultimately affect the benefits humans get from these natural dynamics (for example, clean drinking water, fishing, recreational activities, etc.). Decomposition, the biological process that breaks down organic matter such as leaf litter, is one of the most fundamental processes in stream ecosystems, needed to redistribute nutrients within the system and among organisms. Aquatic fungi and particularly aquatic hyphomycetes play an important role in decomposition in freshwater streams as they fragment leaf litter, release nutrients, and improve the digestibility of organic material for other organisms (insects, fish, etc.). Despite their ecological importance there is a lack of knowledge and adequate ways of monitoring these ecosystem functions and fungal biodiversity, to be able to protect them from damaging impacts like increased nutrient inputs. Traditional methods of monitoring are time consuming, labour intensive and expensive, so this thesis looks into the possibility of including aquatic fungi in freshwater ecosystem monitoring using environmental DNA (eDNA). eDNA refers to genetic material (DNA) released by organisms into their surrounding environment through processes such as excretion, reproduction, and decomposition and can be collected directly from environmental samples (water, soil, sediment, etc.).

The study aims to answer the question if fungal decomposition-related genes can help understand how environmental conditions affect decomposition processes and act as an indicator of freshwater ecosystem health. To assess changes in decomposition rate in different nutrient ratios of nitrogen and phosphorus (N:P) a laboratory experiment was set up with three species, a mix of the three species and a control (5 groups) in mineral solutions with three different N:P ratios and a carbon source of cellulose filter paper. Nitrogen was kept at the same level while three different amounts of phosphorus were added to get three N:P ratios. The decomposition rate was defined as the mass loss from the cellulose filter paper after fungi decomposed the carbon source. The DNA was extracted from the filter paper and the genes of an enzyme (cellobiohydrolase) in the fungal genome involved in decomposition was amplified using qPCR to determine the number of copies of this gene in the different samples and across the different N:P ratios. Mass loss was always highest with the P-rich treatment across all groups, agreeing

with previous studies that found nutrient enrichment to increase decomposition processes. The groups differed in their degree of mass loss showing the individuality of the different species. Decomposition also increased with the amount of DNA extracted. More phosphorus, more fungal DNA (for most groups) resulting in more decomposition. The qPCR result, however, did not show a significant difference of target gene copy numbers between the N:P ratios and no consistent relationship between copy number and decomposition.

The positive association between DNA abundance and decomposition within species groups indicates that molecular measurements have potential to be used as approximations for fungal activity. However, without a reliable relationship between gene copy numbers and decomposition it is difficult at this stage to link qPCR results directly to ecosystem functioning. Further work is needed to understand variations in species behaviour, collect time series data to establish a baseline for reference and to improve the availability of DNA sequences for aquatic fungi. This thesis was a first attempt at developing a tool to assess freshwater ecosystem function with fungal functional genes that could be added to monitoring programs with the use of eDNA, a monitoring scheme and network on the rise in Europe.

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Artificial intelligence (AI) was minimally used to support writing, coding in R and RStudio, and for background research and literature exploration. All scientific content, analyses, interpretations, and conclusions were developed and verified by the author.

Appendix 1

ANOVA script and results – Mass loss

```
> #Choose ONE of The following
> #load ggpubr for "%>%" to work
> DATA <- MassAll
> #   --
> #  |3|   Comparing means of more than two Groups  =====>>>>Test parametric (for normally distributed data)
> #  --
> #
> #Two-way ANOVA
> #Two-way ANOVA with internation effet
> # aov(variable ~ Factor1 * Factor2, data = My Data)
> anov_interact <- aov(ML_pc ~ Group * Ratio, data = DATA)
> anov_interact
Call:
   aov(formula = ML_pc ~ Group * Ratio, data = DATA)

Terms:
                    Group    Ratio Group:Ratio Residuals
Sum of Squares  4977.692 2699.570    1574.609  2047.024
Deg. of Freedom        4        2           8        60

Residual standard error: 5.840982
Estimated effects may be unbalanced
> summary(anov_interact)
            Df Sum Sq Mean Sq F value   Pr(>F)
Group        4   4978  1244.4  36.475 1.92e-15 ***
Ratio        2   2700  1349.8  39.563 1.10e-11 ***
Group:Ratio  8   1575   196.8   5.769 1.88e-05 ***
Residuals   60   2047    34.1
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> #If the interaction between the 2 conditions is significant, meaning the relationships between Variable and Factor1
depend on Factor2
> #If the interaction between the 2 conditions is not significant, then must use the >>>>additive model<<<<
> #Normality verification
> #If p-value > 0.05, Normal distribution ==> parametric test
> #If p-value < 0.05, Not normal distribution ==> no-parametric
> shapiro.test(anov_interact$residuals)
```

```
                    Shapiro-Wilk normality test

data:  anov_interact$residuals
W = 0.98025, p-value = 0.2916

> plot(anov_interact,1)
> plot(anov_interact,2)
> #TukeyHSD
> TukeyHSD(anov_interact, which = "Group")
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = ML_pc ~ Group * Ratio, data = DATA)

$Group
               diff         lwr         upr     p adj
TEMA-CTRL  23.111111  17.1126269  29.1095953 0.0000000
FISP-CTRL  14.179894   8.1814100  20.1783784 0.0000001
NELU-CTRL   6.433862   0.4353782  12.4323466 0.0296171
MIX-CTRL   17.375661  11.3771772  23.3741456 0.0000000
FISP-TEMA  -8.931217 -14.9297011  -2.9327327 0.0008641
NELU-TEMA -16.677249 -22.6757329 -10.6787645 0.0000000
MIX-TEMA   -5.735450 -11.7339339   0.2630345 0.0674948
NELU-FISP  -7.746032 -13.7445159  -1.7475476 0.0051080
MIX-FISP    3.195767  -2.8027170   9.1942514 0.5675686
MIX-NELU   10.941799   4.9433148  16.9402831 0.0000316

> TukeyHSD(anov_interact, which = "Ratio")
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = ML_pc ~ Group * Ratio, data = DATA)

$Ratio
              diff       lwr      upr    p adj
mid-high  7.631746  3.661442 11.60205 6.13e-05
low-high 14.692063 10.721759 18.66237 0.00e+00
low-mid   7.060317  3.090013 11.03062 2.04e-04

> TukeyHSD(anov_interact, which = "Group:Ratio")
```

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = ML_pc ~ Group * Ratio, data = DATA)

$`Group:Ratio`
                          diff         lwr       upr     p adj
TEMA:high-CTRL:high   6.5396825  -6.5239988  19.603364 0.9004424
FISP:high-CTRL:high  11.1111111  -1.9525703  24.174792 0.1806971
NELU:high-CTRL:high   1.3968254 -11.6668560  14.460507 1.0000000
MIX:high-CTRL:high   10.3492063  -2.7144750  23.412888 0.2741952
CTRL:mid-CTRL:high    2.2222222 -10.8414591  15.285904 0.9999989
TEMA:mid-CTRL:high   27.0476190  13.9839377  40.111300 0.0000001
FISP:mid-CTRL:high   12.5714286  -0.4922528  25.635110 0.0709617
NELU:mid-CTRL:high    9.5873016  -3.4763798  22.650983 0.3936691
MIX:mid-CTRL:high    16.1269841   3.0633028  29.190665 0.0041637
CTRL:low-CTRL:high    1.0793651 -11.9843163  14.143046 1.0000000
TEMA:low-CTRL:high   39.0476190  25.9839377  52.111300 0.0000000
FISP:low-CTRL:high   22.1587302   9.0950488  35.222412 0.0000121
NELU:low-CTRL:high   11.6190476  -1.4446338  24.682729 0.1330436
MIX:low-CTRL:high    28.9523810  15.8886996  42.016062 0.0000000
FISP:high-TEMA:high   4.5714286  -8.4922528  17.635110 0.9950340
NELU:high-TEMA:high  -5.1428571 -18.2065385   7.920824 0.9849988
MIX:high-TEMA:high    3.8095238  -9.2541576  16.873205 0.9992549
CTRL:mid-TEMA:high   -4.3174603 -17.3811417   8.746221 0.9971980
TEMA:mid-TEMA:high   20.5079365   7.4442551  33.571618 0.0000648
FISP:mid-TEMA:high    6.0317460  -7.0319353  19.095427 0.9441038
NELU:mid-TEMA:high    3.0476190 -10.0160623  16.111300 0.9999425
MIX:mid-TEMA:high     9.5873016  -3.4763798  22.650983 0.3936691
CTRL:low-TEMA:high   -5.4603175 -18.5239988   7.603364 0.9747619
TEMA:low-TEMA:high   32.5079365  19.4442551  45.571618 0.0000000
FISP:low-TEMA:high   15.6190476   2.5553662  28.682729 0.0064853
NELU:low-TEMA:high    5.0793651  -7.9843163  18.143046 0.9865827
MIX:low-TEMA:high    22.4126984   9.3490170  35.476380 0.0000093
NELU:high-FISP:high  -9.7142857 -22.7779671   3.349396 0.3721480
MIX:high-FISP:high   -0.7619048 -13.8255861  12.301777 1.0000000
CTRL:mid-FISP:high   -8.8888889 -21.9525703   4.174792 0.5202399
TEMA:mid-FISP:high   15.9365079   2.8728266  29.000189 0.0049225
FISP:mid-FISP:high    1.4603175 -11.6033639  14.523999 1.0000000
NELU:mid-FISP:high   -1.5238095 -14.5874909  11.539872 1.0000000
MIX:mid-FISP:high     5.0158730  -8.0478084  18.079554 0.9880311
```

CTRL:low-FISP:high -10.0317460 -23.0954274 3.031935 0.3210375

TEMA:low-FISP:high 27.9365079 14.8728266 41.000189 0.0000000

FISP:low-FISP:high 11.0476190 -2.0160623 24.111300 0.1874588

NELU:low-FISP:high 0.5079365 -12.5557449 13.571618 1.0000000

MIX:low-FISP:high 17.8412698 4.7775885 30.904951 0.0008703

MIX:high-NELU:high 8.9523810 -4.1113004 22.016062 0.5083232

CTRL:mid-NELU:high 0.8253968 -12.2382845 13.889078 1.0000000

TEMA:mid-NELU:high 25.6507937 12.5871123 38.714475 0.0000003

FISP:mid-NELU:high 11.1746032 -1.8890782 24.238285 0.1741188

NELU:mid-NELU:high 8.1904762 -4.8732052 21.254158 0.6518491

MIX:mid-NELU:high 14.7301587 1.6664774 27.793840 0.0137092

CTRL:low-NELU:high -0.3174603 -13.3811417 12.746221 1.0000000

TEMA:low-NELU:high 37.6507937 24.5871123 50.714475 0.0000000

FISP:low-NELU:high 20.7619048 7.6982234 33.825586 0.0000502

NELU:low-NELU:high 10.2222222 -2.8414591 23.285904 0.2923940

MIX:low-NELU:high 27.5555556 14.4918742 40.619237 0.0000000

CTRL:mid-MIX:high -8.1269841 -21.1906655 4.936697 0.6635653

TEMA:mid-MIX:high 16.6984127 3.6347313 29.762094 0.0024990

FISP:mid-MIX:high 2.2222222 -10.8414591 15.285904 0.9999989

NELU:mid-MIX:high -0.7619048 -13.8255861 12.301777 1.0000000

MIX:mid-MIX:high 5.7777778 -7.2859036 18.841459 0.9599205

CTRL:low-MIX:high -9.2698413 -22.3335226 3.793840 0.4497497

TEMA:low-MIX:high 28.6984127 15.6347313 41.762094 0.0000000

FISP:low-MIX:high 11.8095238 -1.2541576 24.873205 0.1179736

NELU:low-MIX:high 1.2698413 -11.7938401 14.333523 1.0000000

MIX:low-MIX:high 18.6031746 5.5394932 31.666856 0.0004216

TEMA:mid-CTRL:mid 24.8253968 11.7617155 37.889078 0.0000008

FISP:mid-CTRL:mid 10.3492063 -2.7144750 23.412888 0.2741952

NELU:mid-CTRL:mid 7.3650794 -5.6986020 20.428761 0.7937784

MIX:mid-CTRL:mid 13.9047619 0.8410805 26.968443 0.0265350

CTRL:low-CTRL:mid -1.1428571 -14.2065385 11.920824 1.0000000

TEMA:low-CTRL:mid 36.8253968 23.7617155 49.889078 0.0000000

FISP:low-CTRL:mid 19.9365079 6.8728266 33.000189 0.0001145

NELU:low-CTRL:mid 9.3968254 -3.6668560 22.460507 0.4269616

MIX:low-CTRL:mid 26.7301587 13.6664774 39.793840 0.0000001

FISP:mid-TEMA:mid -14.4761905 -27.5398718 -1.412509 0.0168617

NELU:mid-TEMA:mid -17.4603175 -30.5239988 -4.396636 0.0012428

MIX:mid-TEMA:mid -10.9206349 -23.9843163 2.143046 0.2015380

CTRL:low-TEMA:mid -25.9682540 -39.0319353 -12.904573 0.0000002

TEMA:low-TEMA:mid 12.0000000 -1.0636814 25.063681 0.1043155

FISP:low-TEMA:mid -4.8888889 -17.9525703 8.174792 0.9905538

NELU:low-TEMA:mid -15.4285714 -28.4922528 -2.364890 0.0076364

MIX:low-TEMA:mid 1.9047619 -11.1589195 14.968443 0.9999998

NELU:mid-FISP:mid -2.9841270 -16.0478084 10.079554 0.9999553

MIX:mid-FISP:mid 3.5555556 -9.5081258 16.619237 0.9996541

CTRL:low-FISP:mid -11.4920635 -24.5557449 1.571618 0.1439134

TEMA:low-FISP:mid 26.4761905 13.4125091 39.539872 0.0000001

FISP:low-FISP:mid 9.5873016 -3.4763798 22.650983 0.3936691

NELU:low-FISP:mid -0.9523810 -14.0160623 12.111300 1.0000000

MIX:low-FISP:mid 16.3809524 3.3172710 29.444634 0.0033235

MIX:mid-NELU:mid 6.5396825 -6.5239988 19.603364 0.9004424

CTRL:low-NELU:mid -8.5079365 -21.5716179 4.555745 0.5922989

TEMA:low-NELU:mid 29.4603175 16.3966361 42.523999 0.0000000

FISP:low-NELU:mid 12.5714286 -0.4922528 25.635110 0.0709617

NELU:low-NELU:mid 2.0317460 -11.0319353 15.095427 0.9999996

MIX:low-NELU:mid 19.3650794 6.3013980 32.428761 0.0002012

CTRL:low-MIX:mid -15.0476190 -28.1113004 -1.983938 0.0105371

TEMA:low-MIX:mid 22.9206349 9.8569535 35.984316 0.0000055

FISP:low-MIX:mid 6.0317460 -7.0319353 19.095427 0.9441038

NELU:low-MIX:mid -4.5079365 -17.5716179 8.555745 0.9956741

MIX:low-MIX:mid 12.8253968 -0.2382845 25.889078 0.0593541

TEMA:low-CTRL:low 37.9682540 24.9045726 51.031935 0.0000000

FISP:low-CTRL:low 21.0793651 8.0156837 34.143046 0.0000364

NELU:low-CTRL:low 10.5396825 -2.5239988 23.603364 0.2482833

MIX:low-CTRL:low 27.8730159 14.8093345 40.936697 0.0000000

FISP:low-TEMA:low -16.8888889 -29.9525703 -3.825208 0.0021024

NELU:low-TEMA:low -27.4285714 -40.4922528 -14.364890 0.0000000

MIX:low-TEMA:low -10.0952381 -23.1589195 2.968443 0.3113139

NELU:low-FISP:low -10.5396825 -23.6033639 2.523999 0.2482833

MIX:low-FISP:low 6.7936508 -6.2700306 19.857332 0.8722417

MIX:low-NELU:low 17.3333333 4.2696520 30.397015 0.0013981

Appendix 2

ANOVA script and results – DNA amount

```
> DATA <- df
> #Two-way ANOVA with internation effet
> # aov(variable ~ Factor1 * Factor2, data = My Data)
> anov_interact <- aov(DNA_amount ~ Group * Ratio, data = DATA)
> anov_interact
Call:
   aov(formula = DNA_amount ~ Group * Ratio, data = DATA)

Terms:
                    Group    Ratio Group:Ratio Residuals
Sum of Squares  4811.024 5620.520    5533.716  1735.504
Deg. of Freedom        3        2           6        24

Residual standard error: 8.503685
Estimated effects may be unbalanced
> summary(anov_interact)
            Df Sum Sq Mean Sq F value   Pr(>F)
Group        3   4811  1603.7   22.18 4.22e-07 ***
Ratio        2   5621  2810.3   38.86 2.97e-08 ***
Group:Ratio  6   5534   922.3   12.75 1.90e-06 ***
Residuals   24   1736    72.3
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> #If the interaction between the 2 conditions is significant, meaning the relationships between Variable and Factor1 depend on Factor2
> #If the interaction between the 2 conditions is not significant, then must use the >>>>additive model<<<<
> #Normality verification
> #If p-value > 0.05, Normal distribution ==> parametric test
> #If p-value < 0.05, Not normal distribution ==> no-parametric
> shapiro.test(anov_interact$residuals)

        Shapiro-Wilk normality test

data:  anov_interact$residuals
W = 0.97815, p-value = 0.6826

> TukeyHSD(anov_interact, which = "Group")
```

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = DNA_amount ~ Group * Ratio, data = DATA)

$Group
                diff        lwr       upr     p adj
FISP-TEMA  32.358889  21.300522 43.417256 0.0000002
NELU-TEMA  13.546667   2.488300 24.605034 0.0123733
MIX-TEMA   12.383333   1.324966 23.441700 0.0241634
NELU-FISP -18.812222 -29.870589 -7.753855 0.0004944
MIX-FISP  -19.975556 -31.033923 -8.917188 0.0002391
MIX-NELU   -1.163333 -12.221700  9.895034 0.9912648

> TukeyHSD(anov_interact, which = "Ratio")
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = DNA_amount ~ Group * Ratio, data = DATA)

$Ratio
              diff        lwr      upr     p adj
mid-high  7.908333 -0.7612854 16.57795 0.0784607
low-high 29.560000 20.8903813 38.22962 0.0000000
low-mid  21.651667 12.9820479 30.32129 0.0000056

> TukeyHSD(anov_interact, which = "Group:Ratio")
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = DNA_amount ~ Group * Ratio, data = DATA)

$`Group:Ratio`
                        diff          lwr        upr     p adj
FISP:high-TEMA:high  20.3633333  -4.67137786  45.3980445 0.1908939
NELU:high-TEMA:high  -5.3066667 -30.34137786  19.7280445 0.9996519
MIX:high-TEMA:high    3.3366667 -21.69804453  28.3713779 0.9999965
TEMA:mid-TEMA:high   -0.3966667 -25.43137786  24.6380445 1.0000000
FISP:mid-TEMA:high    7.0966667 -17.93804453  32.1313779 0.9954359
NELU:mid-TEMA:high   25.1966667   0.16195547  50.2313779 0.0475451
MIX:mid-TEMA:high    18.1300000  -6.90471119  43.1647112 0.3264459
```

```
TEMA:low-TEMA:high    7.9433333 -17.09137786  32.9780445 0.9887512
FISP:low-TEMA:high   77.1633333  52.12862214 102.1980445 0.0000000
NELU:low-TEMA:high   28.2966667   3.26195547  53.3313779 0.0175278
MIX:low-TEMA:high    23.2300000  -1.80471119  48.2647112 0.0862841
NELU:high-FISP:high -25.6700000 -50.70471119  -0.6352888 0.0409922
MIX:high-FISP:high  -17.0266667 -42.06137786   8.0080445 0.4116579
TEMA:mid-FISP:high  -20.7600000 -45.79471119   4.2747112 0.1721190
FISP:mid-FISP:high  -13.2666667 -38.30137786  11.7680445 0.7422709
NELU:mid-FISP:high    4.8333333 -20.20137786  29.8680445 0.9998566
MIX:mid-FISP:high    -2.2333333 -27.26804453  22.8013779 0.9999999
TEMA:low-FISP:high  -12.4200000 -37.45471119  12.6147112 0.8087485
FISP:low-FISP:high   56.8000000  31.76528881  81.8347112 0.0000012
NELU:low-FISP:high    7.9333333 -17.10137786  32.9680445 0.9888601
MIX:low-FISP:high     2.8666667 -22.16804453  27.9013779 0.9999993
MIX:high-NELU:high    8.6433333 -16.39137786  33.6780445 0.9788253
TEMA:mid-NELU:high    4.9100000 -20.12471119  29.9447112 0.9998332
FISP:mid-NELU:high   12.4033333 -12.63137786  37.4380445 0.8099727
NELU:mid-NELU:high   30.5033333   5.46862214  55.5380445 0.0083636
MIX:mid-NELU:high    23.4366667  -1.59804453  48.4713779 0.0811839
TEMA:low-NELU:high   13.2500000 -11.78471119  38.2847112 0.7436519
FISP:low-NELU:high   82.4700000  57.43528881 107.5047112 0.0000000
NELU:low-NELU:high   33.6033333   8.56862214  58.6380445 0.0028878
MIX:low-NELU:high    28.5366667   3.50195547  53.5713779 0.0161882
TEMA:mid-MIX:high    -3.7333333 -28.76804453  21.3013779 0.9999889
FISP:mid-MIX:high     3.7600000 -21.27471119  28.7947112 0.9999880
NELU:mid-MIX:high    21.8600000  -3.17471119  46.8947112 0.1277183
MIX:mid-MIX:high     14.7933333 -10.24137786  39.8280445 0.6081758
TEMA:low-MIX:high     4.6066667 -20.42804453  29.6413779 0.9999099
FISP:low-MIX:high    73.8266667  48.79195547  98.8613779 0.0000000
NELU:low-MIX:high    24.9600000  -0.07471119  49.9947112 0.0511712
MIX:low-MIX:high     19.8933333  -5.14137786  44.9280445 0.2151603
FISP:mid-TEMA:mid     7.4933333 -17.54137786  32.5280445 0.9928915
NELU:mid-TEMA:mid    25.5933333   0.55862214  50.6280445 0.0419935
MIX:mid-TEMA:mid     18.5266667  -6.50804453  43.5613779 0.2986276
TEMA:low-TEMA:mid     8.3400000 -16.69471119  33.3747112 0.9837179
FISP:low-TEMA:mid    77.5600000  52.52528881 102.5947112 0.0000000
NELU:low-TEMA:mid    28.6933333   3.65862214  53.7280445 0.0153672
MIX:low-TEMA:mid     23.6266667  -1.40804453  48.6613779 0.0767325
NELU:mid-FISP:mid    18.1000000  -6.93471119  43.1347112 0.3286134
MIX:mid-FISP:mid     11.0333333 -14.00137786  36.0680445 0.8969227
TEMA:low-FISP:mid     0.8466667 -24.18804453  25.8813779 1.0000000
```

```
FISP:low-FISP:mid     70.0666667  45.03195547  95.1013779 0.0000000
NELU:low-FISP:mid     21.2000000  -3.83471119  46.2347112 0.1530495
MIX:low-FISP:mid      16.1333333  -8.90137786  41.1680445 0.4876871
MIX:mid-NELU:mid      -7.0666667 -32.10137786  17.9680445 0.9955933
TEMA:low-NELU:mid    -17.2533333 -42.28804453   7.7813779 0.3932793
FISP:low-NELU:mid     51.9666667  26.93195547  77.0013779 0.0000056
NELU:low-NELU:mid      3.1000000 -21.93471119  28.1347112 0.9999984
MIX:low-NELU:mid      -1.9666667 -27.00137786  23.0680445 1.0000000
TEMA:low-MIX:mid     -10.1866667 -35.22137786  14.8480445 0.9358437
FISP:low-MIX:mid      59.0333333  33.99862214  84.0680445 0.0000006
NELU:low-MIX:mid      10.1666667 -14.86804453  35.2013779 0.9366235
MIX:low-MIX:mid        5.1000000 -19.93471119  30.1347112 0.9997605
FISP:low-TEMA:low     69.2200000  44.18528881  94.2547112 0.0000000
NELU:low-TEMA:low     20.3533333  -4.68137786  45.3880445 0.1913871
MIX:low-TEMA:low      15.2866667  -9.74804453  40.3213779 0.5634177
NELU:low-FISP:low    -48.8666667 -73.90137786 -23.8319555 0.0000154
MIX:low-FISP:low     -53.9333333 -78.96804453 -28.8986221 0.0000030
MIX:low-NELU:low      -5.0666667 -30.10137786  19.9680445 0.9997750
```

Appendix 3

Mass of cellulose filter discs after decomposition, mass loss and DNA amount

	130:1				13:1				1,3:1			
	Sample	Mass (g)	Massloss (%)	DNA ng/µL	Sample	Mass (g)	Massloss (%)	DNA ng/µL	Sample	Mass (g)	Massloss (%)	DNA ng/µL
	1	0,029	7,9%	9,01	26	0,0196	37,8%	9,27	51	0,0167	47,0%	16,4
	2	0,0308	2,2%	9,67	27	0,0246	21,9%	5,9	52	0,0204	35,2%	4,64
TEMA	3	0,0276	12,4%	4,23	28	0,0267	15,2%	6,55	53	0,0149	52,7%	25,7
	4	0,0271	14,0%	NA	29	0,0186	41,0%	NA	54	0,0166	47,3%	NA
	5	0,0275	12,7%	NA	30	0,0202	35,9%	NA	55	0,0222	29,5%	NA
	6	0,0268	14,9%	30	31	0,0248	21,3%	17,9	56	0,0259	17,8%	74,4
	7	0,0281	10,8%	23,5	32	0,026	17,5%	10	57	0,0255	19,0%	80,8
FI sp.	8	0,0281	10,8%	30,5	33	0,0258	18,1%	16,3	58	0,0197	37,5%	99,2
	9	0,0253	19,7%	NA	34	0,0275	12,7%	NA	59	0,0231	26,7%	NA
	10	0,0265	15,9%	NA	35	0,0284	9,8%	NA	60	0,0232	26,3%	NA
	11	0,0305	3,2%	2,37	36	0,0264	16,2%	37,3	61	0,029	7,9%	19,9
	12	0,0292	7,3%	2,54	37	0,0293	7,0%	23,9	62	0,0265	15,9%	41,1
NELU	13	0,0303	3,8%	2,08	38	0,0264	16,2%	37,3	63	0,0255	19,0%	46,8
	14	0,0299	5,1%	NA	39	0,0273	13,3%	NA	64	0,0266	15,6%	NA
	15	0,0302	4,1%	NA	40	0,0278	11,7%	NA	65	0,0264	16,2%	NA
	16	0,0283	10,2%	8,65	41	0,0239	24,1%	41,3	66	0,0235	25,4%	22,1
	17	0,0261	17,1%	16	42	0,0264	16,2%	13,6	67	0,023	27,0%	32,4
MIX	18	0,0269	14,6%	8,27	43	0,0273	13,3%	22,4	68	0,0214	32,1%	38,1
	19	0,0279	11,4%	NA	44	0,0231	26,7%	NA	69	0,0171	45,7%	NA
	20	0,0268	14,9%	NA	45	0,0262	16,8%	NA	70	0,0217	31,1%	NA
	21	0,0299	5,1%	NA	46	0,0296	6,0%	NA	71	0,0293	7,0%	NA
	22	0,0325	-3,2%	NA	47	0,0297	5,7%	NA	72	0,0295	6,3%	NA
CTRL	23	0,0304	3,5%	NA	48	0,0295	6,3%	NA	73	0,0306	2,9%	NA
	24	0,0304	3,5%	NA	49	0,0287	8,9%	NA	74	0,0318	-1,0%	NA
	25	0,0291	7,6%	NA	50	0,0313	0,6%	NA	75	0,0294	6,7%	NA

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