



Doctoral (PhD) Dissertation

Revealing diatom diversity, rarity patterns, and environmental drivers through complementary microscopic and eDNA metabarcoding approaches

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Introduction

Diatoms are among the most diverse and ecologically informative groups of microscopic algae in aquatic ecosystems. They occur in marine and freshwater habitats and contribute substantially to primary production, carbon fixation, nutrient cycling, and aquatic food webs. Their rapid responses to environmental change and species-specific ecological preferences have also made them central to freshwater biomonitoring and ecological-status assessment (B-Béres et al. 2023; Rimet 2012; Almeida et al. 2014).

Traditional diatom assessment relies on light microscopy and the morphology of siliceous valves. This approach provides direct taxonomic evidence and continuity with established monitoring systems, but it is labour-intensive, requires specialist expertise, and examines only a limited number of valves per sample. Rare, weakly silicified, cryptic, or morphologically similar taxa can therefore be missed or incompletely resolved (Pawlowski et al. 2016; Kermarrec et al. 2013).

At the same time, freshwater biodiversity is under increasing pressure from pollution, land-use change, eutrophication, salinization, hydromorphological alteration, habitat fragmentation, and climate-related disturbances (Reid et al. 2019). Rare species are particularly vulnerable, yet their ecological roles remain poorly understood in microbial and algal communities (Violle et al. 2017; Jousset et al. 2017). In benthic diatoms, rarity may reflect low population size, habitat specialization, restricted geographic distribution, or combinations of these characteristics.

DNA metabarcoding offers a complementary perspective. By sequencing selected genetic markers from environmental samples, it can process complex communities at high throughput, improve the detection of low-abundance taxa, and retain fine sequence-level variation through amplicon sequence variants (ASVs) (Keck et al. 2022; Callahan et al. 2016). However, molecular results remain influenced by marker choice, reference-library completeness, laboratory protocols, bioinformatic processing, and the imperfect relationship between sequence reads and organism abundance (Rimet et al. 2019).

Introduction

This dissertation addresses these linked challenges through three complementary components. The first synthesizes the development, applications, and methodological limitations of diatom DNA metabarcoding. The second examines the ecological significance of rare benthic diatom taxa using a multidimensional rarity framework in a river network. The third directly compares phytobenthos microscopy, diatom microscopy, and diatom DNA metabarcoding to determine how methodological choice affects biodiversity estimates and the detection of environmental responses.

The central perspective of the dissertation is that microscopy and metabarcoding should not be treated as competing approaches. Microscopy remains essential for morphological interpretation, taxonomic continuity, and compatibility with established assessment systems. Metabarcoding increases detection sensitivity and can reveal cryptic or low-abundance diversity that is difficult to observe microscopically. Their combined use provides a broader basis for understanding diatom diversity, rarity, and community-environment relationships (Keck et al. 2022; Bailet et al. 2020).

A second unifying theme is that biodiversity cannot be represented adequately by dominant taxa or species richness alone. Rare species can contribute strongly to spatial turnover, beta diversity, and community structure (Jousset et al. 2017; Violle et al. 2017). By integrating methodological evaluation with rarity ecology and environmental-gradient analysis, the dissertation provides a coherent assessment of how diatom biodiversity can be measured and interpreted more effectively.

Aims

The overall aim of this dissertation was to improve understanding of diatom diversity, rarity, and environmental responses through complementary microscopic and molecular approaches.

- Evaluate the development, methodological variability, applications, and remaining limitations of diatom DNA metabarcoding, with particular attention to taxonomic detection, reference libraries, bioassessment, and methodological standardization.
- Investigate the ecological significance of rare benthic diatom species in river networks by classifying different forms of rarity and assessing their contribution to community variability, beta diversity, and ecological network structure.
- Compare phytobenthos microscopy, diatom microscopy, and diatom DNA metabarcoding in their ability to characterize biodiversity and reveal community responses to environmental gradients.

These three objectives correspond directly to the three main chapters of the dissertation and provide the basis for the thesis points presented in the results section of this booklet.

Dissertation Methodology

The dissertation combines systematic literature synthesis with river-network community ecology and a direct comparison of morphological and molecular identification approaches. The three components are linked by a common question: how can diatom biodiversity be described more completely and interpreted more effectively in relation to ecological conditions?

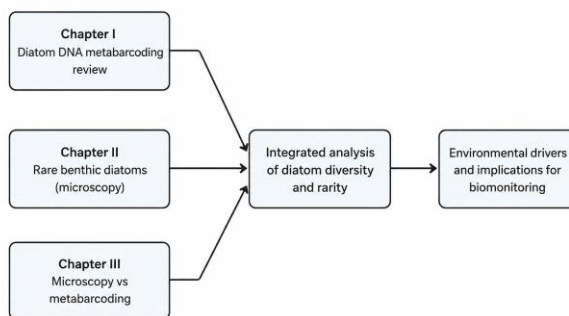


Figure 1. Overall conceptual structure of the dissertation.

Section 3 used a systematic literature search to characterize the global development of diatom DNA metabarcoding and to compare methodological choices across studies. The synthesis considered sampling and preservation, DNA extraction, barcode markers, sequencing technologies, bioinformatic pipelines, reference libraries, taxonomic correspondence with microscopy, ecological assessment, and other applications (Pawlowski et al. 2018; Bailet et al. 2020).

Dissertation Methodology

Section 4 examined rare benthic diatom species across a Central European river network using microscopy-derived community data. Rarity was classified along three dimensions: population size, habitat specificity, and geographic range. Rare and common subcommunities were then compared in terms of community variability, beta diversity, species replacement, and ecological network position.

Section 5 compared three biological datasets generated from the same river-network system: phytobenthos microscopy, diatom microscopy, and diatom DNA metabarcoding. The analyses evaluated taxonomic inventories, within-sample richness, Shannon diversity, agreement in relative abundance for shared taxa, environmental gradients, diversity-environment relationships, and community-environment co-structure.

For the molecular dataset, sequence reads were processed to infer ASVs using the DADA2 framework (Callahan et al. 2016) and assign taxonomy using the curated diat.barcode reference library (Rimet et al. 2019). Environmental data were summarized using principal component analysis, while co-inertia analysis was used to compare the strength of community-environment relationships among the three biological datasets.

Dissertation studies

Environmental DNA Metabarcoding of Diatoms: A Next-Generation Tool for Aquatic Biodiversity and Bioassessment

This review synthesizes the development of diatom DNA metabarcoding from field sampling to ecological interpretation. It evaluates geographical patterns in published research, common sampling matrices and habitats, DNA extraction procedures, marker selection, sequencing technologies, bioinformatic pipelines, reference libraries, and the correspondence between molecular and morphological inventories. It also examines applications in ecological-status assessment and identifies the main barriers to broader routine implementation.

Role of rare species in benthic diatom communities: patterns, processes and networks

This study investigates rarity as a multidimensional ecological property rather than a simple abundance threshold. Benthic diatom species were classified according to population size, habitat specificity, and geographic range, and the resulting rare subcommunities were compared with common taxa. The study evaluates how rare species contribute to community variability, beta diversity, species replacement, and the structural organization of ecological networks.

Using complementary approaches to study diatom diversity and its environmental drivers

This study compares phytobenthos microscopy, diatom microscopy, and diatom DNA metabarcoding within the same environmental framework. It evaluates how the three approaches differ in recorded taxonomic inventories, within-sample diversity, abundance relationships, and sensitivity to environmental gradients. The study also tests which dataset shows the strongest overall co-structure with measured environmental variables.

Results

Thesis I

I showed that diatom DNA metabarcoding has developed into a powerful but methodologically heterogeneous approach for biodiversity assessment and biomonitoring.

A systematic analysis of 128 publications demonstrated that research activity is geographically uneven and concentrated mainly in Europe, North America, and East Asia. River biofilms, the *rbcL* barcode, silica-column extraction methods, Illumina sequencing, and increasingly ASV-based bioinformatic processing form the dominant methodological framework, but substantial variation remains among studies.

The synthesis showed that disagreements between microscopy and metabarcoding are systematic rather than random. Metabarcoding generally detects higher richness within individual samples, whereas microscopy may recover a larger cumulative regional species pool. Agreement is strongest for abundant and frequently occurring taxa, while discrepancies are concentrated among rare, cryptic, weakly silicified, morphologically similar, or genetically unrepresented taxa (Kulaš et al. 2022; Pérez-Burillo et al. 2020).

These findings support the interpretation of microscopy and metabarcoding as complementary rather than interchangeable approaches. Wider routine application of metabarcoding will depend on improved reference libraries, harmonized laboratory and bioinformatic protocols, and more robust treatment of sequence-read abundance (Rimet et al. 2019; Bailet et al. 2020; Pawlowski et al. 2018).

Results

Thesis II

I demonstrated that rare benthic diatoms are major contributors to spatial community organization rather than incidental components of biodiversity.

Of 409 recorded species, 204 were rare according to at least one dimension. Small population size was the most frequent form of rarity, while many rare taxa were also habitat specialists or geographically restricted. Eighty-two species fulfilled all three rarity criteria and were classified as absolutely rare.

Rare species contributed disproportionately to community variability. Depending on the rarity category, rare subcommunities showed approximately 25-50% greater variability than their complementary sets of common species. Beta diversity was also strongly associated with rare taxa and was driven mainly by species replacement, particularly among habitat specialists and geographically restricted species.

Network analysis showed that numerical rarity did not imply structural insignificance. Rare species represented approximately 79-82% of core taxa in the diatom association network, indicating that low-abundance or restricted species can occupy central structural positions in community organization. This is consistent with the broader view that the rare biosphere can make important contributions to ecosystem organization and resilience (Jousset et al. 2017).

Results

Thesis III

I showed that estimates of benthic algal diversity and environmental response are strongly method-dependent, and that ASV-based metabarcoding provides particularly sensitive ecological information.

The three-way comparison demonstrated that diatom metabarcoding produced the highest within-sample richness, diatom microscopy produced intermediate estimates, and phytobenthos microscopy produced lower diatom-focused richness while representing a broader range of algal groups. The taxonomic inventories overlapped only partially, confirming that no single approach provided a complete representation of the community.

For taxa detected by both microscopy and metabarcoding, abundance relationships were taxon-specific. Strong rank correlations occurred for several abundant and frequently detected species, whereas weak or inconsistent relationships were more common among low-abundance taxa. Relative sequence-read abundance therefore cannot be treated as a universal substitute for microscopic valve counts (Kulaš et al. 2022; Pérez-Burillo et al. 2020).

Community-environment coupling was strongest for ASV-level metabarcoding. Co-inertia analysis produced RV coefficients of 0.36 for metabarcoding, 0.27 for diatom microscopy, and 0.19 for phytobenthos microscopy, with all relationships significant. Shannon diversity based on diatom ASVs also showed the best-supported response to the main environmental gradient.

Results

Overall scientific contribution

Taken together, the dissertation demonstrates that diatom biodiversity and ecological responses cannot be fully characterized by a single identification method or by focusing primarily on dominant species. Microscopy preserves morphological evidence and continuity with established monitoring frameworks, whereas DNA metabarcoding increases within-sample detection sensitivity and retains fine sequence-level variation. Rare taxa, meanwhile, contribute substantially to spatial turnover, beta diversity, and network structure.

The combined findings support an integrated biomonitoring perspective in which morphology-based and molecular datasets are interpreted according to their respective strengths. This approach can improve biodiversity characterization while also increasing sensitivity to environmentally structured patterns (Almeida et al. 2014; Pawlowski et al. 2018).

Correspondence with the aims

- Objective 1 - evaluated through Thesis I: development, applications, methodological variability, and limitations of diatom DNA metabarcoding.
- Objective 2 - evaluated through Thesis II: rarity types and the contribution of rare species to variability, beta diversity, and network structure.
- Objective 3 - evaluated through Thesis III: comparative biodiversity estimates and community-environment relationships across microscopy and metabarcoding datasets.

Publication list

- [1] Abubaker, B., Stenger-Kovács, C. & Tapolczai, K. (2026). Using complementary approaches to study diatom diversity and its environmental drivers. *Ecological Indicators*, 187, 114970. <https://doi.org/10.1016/j.ecolind.2026.114970>.
- [2] Stenger-Kovács, C., Korponai, J., Abubaker, B. et al. (2025). Role of rare species in benthic diatom communities: patterns, processes and networks. *Biodiversity and Conservation*, 34, 2095-2118. <https://doi.org/10.1007/s10531-025-03063-4>.
- [3] Lengyel, E., Abubaker, B., B-Béres, V., Udovič, M. G., Grmasha, R. A., Hubai, K., et al. (2025). Ecological drivers of rarity and commonness in freshwater benthic diatoms. *Global Ecology and Conservation*, 64, e03991. <https://doi.org/10.1016/j.gecco.2025.e03991>.
- [4] Abubaker, B., Stenger-Kovács, C., Rimet, F., & Tapolczai, K. Environmental DNA metabarcoding of diatoms: A next-generation tool for aquatic biodiversity and bioassessment. *Metabarcoding and Metagenomics*.

Selected conference contributions

- Using complementary approaches to study the environmental drivers of diatom diversity - 27th International Diatom Symposium, South Africa, 2025.
- Environmental drivers of diatom diversity: insights from DNA metabarcoding - AquaEcOmics Conference, France, 2025.
- Responses of benthic algae to environmental constraints using different identification approaches - 3rd International Conference: Lakes & Reservoirs: Hot Spots and Topics in Limnology, Poland, 2024.

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