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Nutrient bioavailability and microbial responses in fresh and coastal aquatic ecosystems

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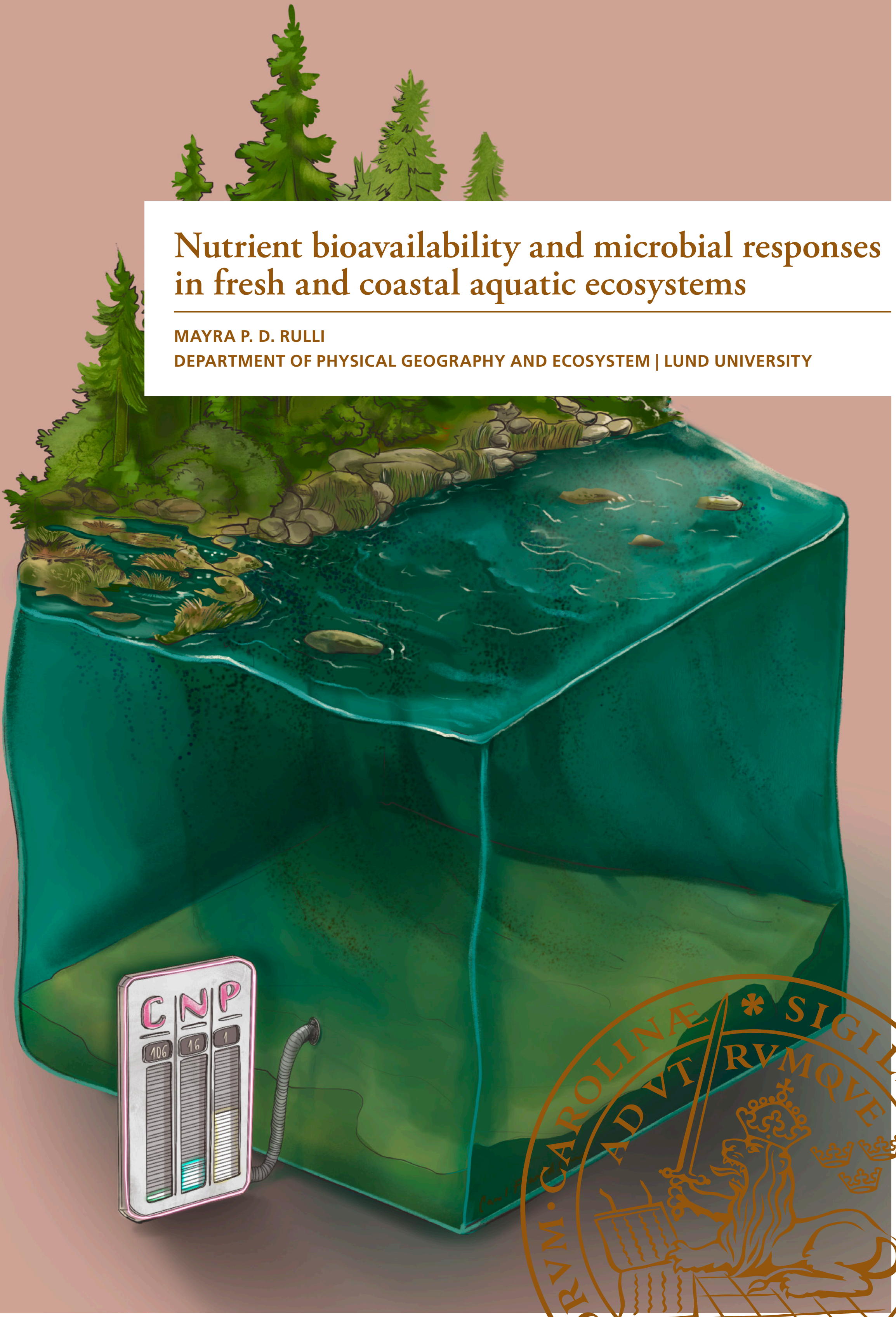

MAYRA RULLI studies how microbes interact with nutrients and organic matter across aquatic ecosystems — from forest streams to coastal waters. Originally from Brazil, they have a background in microbial ecology and a passion for understanding the invisible microbial processes that shape ecosystem dynamics. Her research explores how dissolved organic matter composition, quality and nutrient stoichiometry influence nutrient bioavailability, microbial activity, and broader ecological responses. In this thesis, she combines fieldwork, laboratory bioassays, and molecular tools to investigate carbon, nitrogen, and phosphorus dynamics along the land-to-sea continuum — shedding a brighter light on the nutrients microbes can truly use to sustain aquatic ecosystems.

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Nutrient bioavailability and microbial responses in fresh and coastal aquatic ecosystems

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Mayra P.D. Rulli

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DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the
Faculty of Science at Lund University to be publicly defended on
Friday, the 13th of June at 09.00 in Pangea auditorium, Geocentrum II,
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Abstract: Nutrients are the lifeblood of aquatic ecosystems, but not all nutrients are equally accessible to the organisms that depend on them. While traditional monitoring often tracks total or inorganic concentrations of carbon, nitrogen, and phosphorus, these measures do not reflect what microbial communities can actually use. This thesis explores that disconnect by investigating the bioavailability of key nutrients and their relationship to microbial community structure and function across a land-sea continuum. To bridge this gap, I developed a standardized, high-throughput multi-element bioassay to estimate bioavailable carbon, nitrogen, and phosphorus in aquatic ecosystems. Combined with dissolved organic matter (DOM) quality analysis and microbial community sequencing, this approach offered a consistent framework to compare nutrient accessibility and microbial responses across diverse ecosystems. The results reveal that bioavailable nutrient fractions often diverge substantially from total concentrations — and that these differences matter. Microbial communities, particularly bacteria and eukaryotes, responded not only to nutrient levels, but also to nutrient form and stoichiometry. DOM composition further influenced nutrient availability, with fresh, microbial-produced and protein-like DOM closely linked to greater bioavailability, while aromatic, humic-rich DOM often constrained microbial access. Meanwhile, landscape features such as lake size, catchment vegetation, and water colour influenced both DOM characteristics and microbial community structure. Furthermore, functional analyses indicated that microbes adapt to nitrogen and phosphorus limitation by modulating nutrient acquisition pathways. Together, these findings challenge the assumption that more nutrients always mean more microbial activity. They underscore the importance of measuring not just what is present in the water, but what is actually accessible. By combining chemical and biological perspectives, this work highlights the need for bioavailability-based approaches to better understand nutrient cycling and manage aquatic ecosystems in a rapidly changing world.

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To myself — for continuing, growing, and finding light even when the path was hard.

"It is good to have an end to journey toward; but it is the journey that matters, in the end."
Ursula K. Le Guin, *The Left Hand of Darkness*

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Paper II

Berggren, M., **Rulli, M.P.D.**, Bergström, A-K., Sponseller, R. A., & Hensgens, G. Does dissolved organic matter composition help explain the concentrations of bioavailable macronutrients in organic matter-rich freshwaters? *Under review at Freshwater Biology.*

Paper III

Rulli, M.P.D., Salis, R.K., Bergström, A-K., Sponseller, R. A., & Berggren, M. Microbial community structure in lakes of varying size and organic matter quality: The role of stoichiometric constraints and bioavailable nutrients. *Manuscript.*

Paper IV

Rulli, M.P.D., Garnier, A., Huss, M., Sponseller, R. A., Bergström, A-K., Younes, H., Bell, O., & Berggren, M. Nutrient, carbon and darkening impacts on coastal dissolved phosphorus bioavailability – a mesocosm study. *Under review at Limnology and Oceanography.*

Contributions

Paper I: I co-led the study design together with MB, including decisions on the number and identity of the sampling sites. I also coordinated field samplings for all four streams and carried out the laboratory work. I adapted the bioassay approach to suit our experimental goals. I conducted all statistical analyses, created all figures and tables, and wrote the first draft of the manuscript. I also led the manuscript revision process.

Paper II: I contributed to the design of my specific study within a broader oxygenation experiment, using the same four headwater streams as in Paper I and two additional lakes. I was responsible for coordinating and conducting field sampling and laboratory work for my part of the study. I participated in manuscript revisions and contributed to data interpretation.

Paper III: I led the study design, including lake selection, fieldwork planning, and the decision to include microbial community composition analysis. I developed and implemented the modified bioassay protocol in deep 96-well plates to accommodate a much larger number of samples. I selected the DNA extraction method and sequencing strategy and carried out all field and laboratory work. I performed part of the statistical analysis and interpreted all results, created all tables and part of the figures, and wrote the first draft. I also coordinated manuscript revisions.

Paper IV: I contributed to the experimental design, supervised part of the fieldwork and carried out the rest of the sampling and laboratory work myself. I implemented the microplate-based bioassay approach and performed all statistical analyses. I created all figures and tables and wrote the first draft of the manuscript. I also led the manuscript revision process.

Abstract

Nutrients are the lifeblood of aquatic ecosystems, but not all nutrients are equally accessible to the organisms that depend on them. While traditional monitoring often tracks total or inorganic concentrations of carbon, nitrogen, and phosphorus, these measures do not reflect what microbial communities can actually use. This thesis explores that disconnect by investigating the bioavailability of key nutrients and their relationship to microbial community structure and function across a land-sea continuum. To bridge this gap, I developed a standardized, high-throughput multi-element bioassay to estimate bioavailable carbon, nitrogen, and phosphorus in aquatic ecosystems. Combined with dissolved organic matter (DOM) quality analysis and microbial community sequencing, this approach offered a consistent framework to compare nutrient accessibility and microbial responses across diverse ecosystems. The results reveal that bioavailable nutrient fractions often diverge substantially from total concentrations — and that these differences matter. Microbial communities, particularly bacteria and eukaryotes, responded not only to nutrient levels, but also to nutrient form and stoichiometry. DOM composition further influenced nutrient availability, with fresh, microbial-produced and protein-like DOM closely linked to greater bioavailability, while aromatic, humic-rich DOM often constrained microbial access. Meanwhile, landscape features such as lake size, catchment vegetation, and water colour influenced both DOM characteristics and microbial community structure. Furthermore, functional analyses indicated that microbes adapt to nitrogen and phosphorus limitation by modulating nutrient acquisition pathways. Together, these findings challenge the assumption that more nutrients always mean more microbial activity. They underscore the importance of measuring not just what is present in the water, but what is actually accessible. By combining chemical and biological perspectives, this work highlights the need for bioavailability-based approaches to better understand nutrient cycling and manage aquatic ecosystems in a rapidly changing world.

Popular science summary

Every drop of water carries a story. From forest streams to coastal seas, these stories are shaped by what the water picks up along the way — especially nutrients and organic matter, like the natural 'tea' that seeps into the from leaves and soil. Among the key characters in this story are carbon, nitrogen, and phosphorus — the nutrients that fuel microbial life and shape how ecosystems work. But not all nutrients are created equal, and not everything in the water is actually usable to the microbes trying to make a living there.

This thesis explores this idea by looking at not just how much of each nutrient is present in aquatic systems, but how much is bioavailable — that is, in a form microbes can actually use. Microbes — the tiny bacteria and single-celled organisms that drive much of the recycling in these waters — can only take up certain forms of carbon, nitrogen, and phosphorus. Like us, they need their food in the right form to grow, reproduce, and keep the ecosystem running. It turns out that in many lakes and streams, a big portion of these nutrients are locked away in forms that are not immediately accessible. That's a big deal, because we usually base environmental monitoring and management on total nutrient concentrations. But microbes, the engines of nutrient cycling, experience a very different reality — one that cannot always be captured with the usual methods used in water monitoring.

To understand what nutrients are actually usable by microbes, I developed a method to measure their bioavailability in a simple, reproducible way. The idea was to create a tool that could work across different types of aquatic ecosystems — not just where I happened to be working, but anywhere one wants to ask: "What's available, how much, and to whom?". I applied this method to boreal streams, a diverse set of lakes, and controlled coastal experiments designed to mimic real-world conditions. Together, they offered insights into how nutrient forms and microbial access can vary across environments — but the method itself is flexible enough to be used well beyond these cases.

At the same time, I looked at the microbes themselves: who was there, what they were doing, and how they responded to the chemistry around them. Some thrived on fresh, easily digestible organic matter — like takeaway meals or a ripe fruit, ready to fuel them fast with little to no effort. Others were better at scraping by with tougher stuff — think dried beans or raw potatoes, and the effort it takes to turn them into something edible. And while some environments were stocked with nutrients, only a portion — ranging from meagre scraps to generous servings — was actually on the microbial menu. They can tell the difference, even if our usual tests cannot.

As climate change and human activity continue to reshape the landscape, many waters are getting darker from increased runoff of organic matter — a process known as browning — and receiving more nutrients from agriculture and other sources, driving eutrophication. These changes alter the foodscape for microbes, affecting what they can use and how ecosystems function.

The results showed that bioavailable nutrients shift not only across space, but also with the seasons and environmental conditions. And microbes are not just along for the ride — they actively shape the nutrient landscape. Both the balance between carbon, nitrogen, and phosphorus, and the type of organic matter available matter for how ecosystems function.

So, what now? As aquatic ecosystems endure increasing pressures from climate and landscape changes, we must stop assuming that microbes can simply use whatever nutrients are present. Instead, we need to pay closer attention to what they can actually access and use. If we want to understand and manage aquatic ecosystems in a changing world, it is not just about measuring more — it is about measuring smarter, through the eyes (or maybe mouths) of the microbes themselves.

Resumo de ciência popular

Cada gota d'água carrega uma história. De riachos florestais a mares costeiros, essas histórias são moldadas pelo o que a água vai coletando pelo caminho — especialmente nutrientes e matéria orgânica, como o "chá natural" que infiltra na água a partir de folhas e do solo. Entre os personagens principais dessa história estão o carbono, o nitrogênio e o fósforo — nutrientes que alimentam a vida microbiana e moldam o funcionamento dos ecossistemas. Mas nem todos os nutrientes são criados iguais, e nem tudo o que está na água pode ser usado pelos microrganismos que tentam sobreviver ali.

Esta tese explora essa ideia olhando não apenas para a quantidade total de nutrientes presents em ecossistemas aquáticos, mas para o quanto que é biodisponível — ou seja, em uma forma que os microrganismos consigam usar. Os micróbios — pequenas bactérias e organismos unicelulares que são o motor da reciclagem na água — só conseguem absorver certos tipos de carbono, nitrogênio e fósforo. Assim como nós, eles precisam que seu "alimento" esteja na forma certa para crescer, se reproduzir e manter o ecossistema funcionando. Descobri que, em muitos lagos e riachos, grande parte desses nutrientes está presa em formas que não podem ser utilizadas de imediato. Isso é importante pois o monitoramento e a gestão ambiental normalmente se baseiam nas concentrações totais de nutrientes. Mas para os microrganismos — que são quem realmente faz o trabalho — a realidade é bem diferente, e nem sempre pode ser captada pelos métodos de análise tradicionais.

Para entender melhor o que realmente está disponível para os micróbios, desenvolvi um método simples e reprodutível para medir a biodisponibilidade dos nutrientes. A ideia foi criar uma ferramenta que pudesse ser aplicada em qualquer tipo de ambiente aquático — não apenas nos locais que estudei, mas onde quer que surjam perguntas como: "O que está disponível, em que quantidade e para quem?". Apliquei esse método em riachos boreais, em um conjunto diversificado de lagos e em experimentos costeiros controlados que simulavam condições reais. Eles forneceram uma janela para observar como a forma dos nutrientes e o acesso microbiano variam entre ambientes — mas o método é flexível o bastante para ir muito além desses exemplos.

Ao mesmo tempo, investiguei os próprios microrganismos: quem estava lá, o que estavam fazendo e como respondiam à química ao redor deles. Alguns prosperavam em matéria orgânica fresca e fácil de digerir — como uma refeição entregue em casa ou uma fruta madura, prontas para serem usadas rapidamente sem muito esforço. Outros precisavam se virar com materiais mais duros — como feijões secos ou batatas cruas, que exigem preparo antes de serem consumidos. E, embora alguns ambientes estivessem cheios de nutrientes, apenas uma parte — variando de migalhas a verdadeiros banquetes — estava realmente disponível no cardápio dos microrganismos. Eles sabem muito bem a diferença, mesmo quando nossos testes tradicionais não conseguem mostrar isso.

À medida que as mudanças climáticas e a atividade humana continuam transformando a paisagem, muitos corpos d’água estão ficando mais escuros devido ao aumento do escoamento de matéria orgânica — um processo conhecido como “browning” — e recebendo mais nutrientes da agricultura e de outras fontes, impulsionando a eutrofização. Essas mudanças alteram o “buffet” disponível para os micróbios, afetando o que eles conseguem utilizar e como os ecossistemas funcionam.

Os resultados mostraram que a biodisponibilidade dos nutrientes muda não só de um lugar para outro, mas também com as estações do ano e com as condições ambientais. E os microorganismos não são apenas passageiros — eles também moldam ativamente a paisagem de nutrientes. Tanto o equilíbrio entre carbono, nitrogênio e fósforo quanto o tipo de matéria orgânica disponível são fatores-chave no funcionamento dos ecossistemas.

E agora? À medida que os ecossistemas aquáticos enfrentam pressões cada vez maiores devido às mudanças climáticas e às transformações no uso do solo, precisamos parar de assumir que os microrganismos conseguem simplesmente usar tudo o que está presente. Precisamos prestar mais atenção ao que eles realmente conseguem acessar e utilizar. Se quisermos entender e gerenciar melhor os ecossistemas aquáticos em um mundo em mudança, não basta medir mais — precisamos medir de forma mais inteligente, vendo o ambiente pelos olhos (ou melhor, pelas “bocas”) dos próprios micróbios.

Acknowledgments

It has been almost seven long years. Not all of them were good — three of them, in fact, were a rollercoaster of ups and downs and very little work happening. When I think about that now, I like to remind myself that it practically means I did do this in four years… kind of. The important thing is that when things were not great, I had a lot of support from so many directions, and that made all the difference.

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To everyone who shared space with me during these years — even if remotely — or helped with field work or the various analyses, thank you. Thanks also to Magnus for having the doors of your lab open whenever I came knocking to run plates and plates on the flow cytometer, or when I needed to use any other spaces or instruments we didn't have at our department. Very special thanks to Romana for taking on the challenge I presented to you, the collaboration, teachings, and encouragement these last few years, your support was essential to get me here.

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To Bruna, Mariel, and Carol (yes, you again), for still being part of my life — even if at a long distance — after all these years. Special thanks to the friends I wasn't expecting to find during this journey, and who are so important to me now, Ju and Ditte, thank you for the friendship, the distractions, the many books discussions, the constant encouragement, and all the love. Getting here would have been so much harder without you.

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Abreviations

BDOC: Bioavailable dissolved organic carbon

BDOP: Bioavailable dissolved organic nitrogen

BDOP: Bioavailable dissolved organic phosphorus

BTDN: Bioavailable total dissolved nitrogen

BTDP: Bioavailable total dissolved phosphorus

BP: Bacterial production

C: Carbon

DIN: Dissolved inorganic nitrogen

DNA: Deoxyribonucleic acid

DOM: Dissolved organic matter

DOC: Dissolved organic carbon

DON: Dissolved organic nitrogen

DOP: Dissolved organic phosphorus

EEM: Excitation-emission matrix

SRP: Soluble reactive phosphorus

SUVA: Specific UV absorbance

N: Nitrogen

NMDS: Non-metric multidimensional scaling

PERMANOVA: Permutational multivariate analysis of variance

P: Phosphorus

RDA: Redundancy analysis

16S: 16S ribosomal RNA gene

18S: 18S ribosomal RNA gene

Why this, why now – rationale and structure

Aquatic ecosystems are dynamic interfaces where carbon (C) and nutrients meet biology, with microbes serving as key biological agents mediating these interactions. In this thesis, I approach microbial nutrient cycling from a perspective that integrates three main layers: resource quality (dissolved organic matter [DOM] composition and nutrient forms), microbial responses (community structure and function), and landscape context (hydrology, land use, and ecosystems). These elements form the conceptual foundation for understanding how bioavailable nutrient fractions influence, and are influenced by, microbial processes along a land-to-sea continuum. *This may sound like a complex mix — and truthfully, it often is — but in ecosystems, these layers are always intertwined.*

A central assumption of the thesis is that bioavailable fractions of C, nitrogen (N), and phosphorus (P) offer a more ecologically meaningful view of nutrient supply than total or inorganic concentrations alone (Stepanauskas et al., 2002; Berggren et al., 2015). Especially in DOM-rich waters, where nutrients are often bound to organic molecules, relying on standard chemical metrics may obscure important controls on microbial metabolism (Fellman et al., 2009b; Jansson et al., 2012). Instead, short-term bioassays can reveal what is actually accessible to microbial communities, allowing us to interpret nutrient cycling and limitation through a biological lens (Stepanauskas et al., 2000b; Stepanauskas et al., 2002; Soares et al., 2017).

This microbial lens is closely tied to DOM quality. Aromaticity and fluorescence signatures of DOM provide proxies for its lability, origin, and nutrient-carrying potential (Berggren, 2009; Fellman et al., 2009b). In this thesis, I explore whether these characteristics can help predict the bioavailability of organic nutrients and, in turn, how these nutrients shape microbial community composition and functional traits (Logue et al., 2016). A further layer involves stoichiometry, which determines whether microbes experience a balanced resource supply or face nutrient limitation (Sterner & Elser, 2002). By comparing C:N:P ratios in bioavailable and bulk fractions, the thesis aims to better understand how stoichiometric imbalances influence microbial strategies for nutrient uptake.

Lastly, this framework is applied across multiple ecosystem types, from headwater streams to lakes and a coastal mesocosm experiment. These systems vary in hydrological connectivity, DOM sources, nutrients concentrations, and residence times (Cole et al., 2007), offering a natural gradient to examine how nutrient availability and microbial communities shift along the inland-coastal axis. In this way, the thesis adopts a land-to-sea perspective (Aufdenkampe et al., 2011; Regnier et al., 2013), seeking patterns that transcend individual environments and speak to broader questions of nutrient limitation, ecosystem response to water browning/darkening, and microbial mediation of aquatic biogeochemistry.

Each paper addresses a different part of this conceptual structure: Paper I quantifies seasonal and spatial variation in nutrient bioavailability in boreal streams; Paper II links bioavailable nutrient concentrations to DOM composition across streams and lakes; Paper III explores how bioavailable nutrients and DOM quality influence microbial communities in lakes; and Paper IV tests how nutrient and carbon enrichment under darkened conditions affects bioavailability and microbial responses in coastal waters. Together, these studies aim to connect nutrient chemistry, microbial responses, and ecosystem dynamics through a shared framework, grounded in bioavailability, stoichiometry, and environmental gradients.

Setting the stage

Aquatic ecosystems, especially in boreal regions, are deeply shaped by what flows in from the land — organic carbon, nutrients, and everything in between. These terrestrial inputs are more than just background noise; they set the stage for microbial activity, plankton growth, and food web dynamics. Among them, nitrogen and phosphorus are key players. These macronutrients regulate phytoplankton primary production and bacterioplankton secondary production, driving processes like organic matter cycling, energy transfer, and biodiversity maintenance (Jones, 1998). Dissolved organic carbon (DOC), alongside N and P, fuels heterotrophic microbial activity and sustains microbial loops, which connect bacteria to higher trophic levels and form an essential part of aquatic ecosystems (Tranvik, 1998; Karlsson et al., 2012).

Microorganisms are at the heart of these processes, orchestrating the transformation and mineralization of organic matter, and ultimately controlling the flow and fate of carbon and nutrients in aquatic systems (Wetzel, 2001). While internal microbial recycling keeps ecosystems running, the availability of nutrients is often constrained by what the land delivers — organic and inorganic inputs from soils, forests, and wetlands. In brown-water systems, DOM is not only a dominant source of C but also often carries substantial fractions of N and P, further emphasising its importance as a resource for microbial communities (Jansson et al., 2000). The increasing concentrations of DOM observed across many boreal waters in recent decades (Monteith et al., 2007), suggest that such systems may become increasingly widespread under future climate scenarios.

Despite the ecological significance of terrestrial DOM inputs, it remains challenging to determine the fraction of organic C, N, and P that is bioavailable, i.e., the fraction accessible and usable by microbial communities. This is particularly true in DOM-rich systems, where nutrients are often bound to or associated with organic molecules of varying composition and lability (Battin et al., 2008; Berggren & Del Giorgio, 2015; Soares et al., 2017). Standard chemical analyses typically quantify total or inorganic nutrient concentrations but do not distinguish between biologically accessible and refractory forms. As a result, they may substantially misrepresent the nutrient pool available to microorganisms, especially when a large portion is tied up in organic matter (Jani & Toor, 2018).

These limitations have prompted increasing interest in using bioavailability assays as a more biologically meaningful way to estimate the true fraction of resources accessible to microbes. Such assays typically involve short-term incubations with natural microbial inocula and nutrient amendments to determine how much of the DOM-associated nutrient pool supports microbial growth (Berggren et al., 2015). However, methodological differences across studies, including incubation time, inoculum source, and nutrient treatments, can complicate comparisons and reduce reproducibility (Soares et al., 2017). Moreover, few studies have assessed the bioavailability of C, N, and P simultaneously using standardized approaches, leaving major gaps in our understanding of relative nutrient availability and its ecological consequences.

Understanding how organic nutrient fractions contribute to overall nutrient supply to microbes is important not only from a theoretical or biogeochemical perspective, but it also has direct implications for predicting how aquatic ecosystems respond to land use, climate change, and restoration efforts. As DOM concentrations rise and become more complex, particularly in boreal and temperate waters experiencing browning or under other climate-driven shifts, resource availability may change in ways that affect microbial processing, productivity, and even ecosystem stability (Solomon et al., 2015; Kritzberg et al., 2020).

Despite its ecological importance, the bioavailability of organic nutrients remains poorly quantified in most aquatic systems — partially due to the lack of standardised methods and the logistical challenges of large-scale assessments. This thesis takes a rare and ambitious step toward addressing that gap by applying a unified, multi-elemental bioassay approach to simultaneously estimate the bioavailability of C, N, and P across a range of aquatic environments. Such comprehensive assessments are still uncommon, particularly ones assessing multiple aquatic ecosystems. By combining this methodological consistency with ecological breadth, this work provides novel insights into how resource availability varies along the aquatic continuum and how these patterns influence microbial communities and the functions they perform.

From DOM chemistry to stoichiometric constraints

The biological availability of nutrients associated with DOM depends not only on concentration but also on chemical composition and quality (Jørgensen et al., 2014; Jørgensen et al., 2015). DOM is a highly heterogeneous mixture, composed of molecules of varying size, structure, and origin — ranging from freshly produced, labile compounds to complex, refractory humic substances (Berggren et al., 2010a; Mann et al., 2014). The quality of DOM — often assessed through indicators such as aromaticity, humification, or fluorescence signatures — influences microbial access to both carbon and nutrients by affecting degradation rates, uptake efficiency, and enzyme requirements (Fellman et al., 2009b). In this context, freshly produced

DOM (e.g., phytoplankton exudates) is generally considered more bioavailable than terrestrially derived, humified DOM (Fellman et al., 2009a; Berggren et al., 2010d).

The bioavailability of dissolved organic N (DON) and P (DOP) is also strongly shaped by DOM quality (Stepanauskas et al., 2002; Bronk et al., 2007). For instance, protein-like or microbially-derived compounds are more likely to carry bioavailable forms of N and P (Berggren et al., 2010a; Fellman et al., 2010), whereas humic-bound nutrients may be less accessible due to their structural complexity or chemical binding with metals such as iron (Amon & Benner, 1996; Kritzberg et al., 2020). However, DOM-nutrient relationships remain difficult to generalize, as different nutrient elements interact with DOM in distinct ways and under different environmental conditions (Bianchi, 2011).

Another key factor influencing nutrient accessibility is stoichiometry — the relative balance of C, N, and P available in the environment. Microbial communities require nutrients in specific ratios, and imbalances in ambient C:N:P ratios can lead to nutrient limitation or excess. For example, high C:N or N:P ratios may indicate potential N or P limitation, respectively, especially in environments dominated by organic nutrient sources. In such systems, microbes may activate specific metabolic pathways to acquire limiting nutrients, such as phosphatase production under P limitation or nitrogen assimilation pathways under low N availability (Hall et al., 2009; Godwin & Cotner, 2015). These metabolic adjustments reflect the flexibility of microbial communities to respond to resource constraints, particularly in nutrient-poor or DOM-rich environments (Sterner & Elser, 2002). Understanding these stoichiometric constraints is therefore essential to predict how microbial metabolism responds to changes in DOM quality and nutrient supply.

Despite this recognition, studies that directly link DOM composition, nutrient stoichiometry, and bioavailability across multiple elements remain rare. The interplay between DOM chemistry and microbial nutrient acquisition strategies is likely to be an important regulator of nutrient cycling in inland waters, particularly in ecosystems subject to increasing DOM concentrations and terrestrial influence.

What shapes microbial communities?

Microbial communities are central to aquatic biogeochemical cycles, mediating the uptake, transformation, and mineralization of nutrients and organic matter (Azam et al., 1983; Falkowski et al., 2008). Through their metabolic activity, both bacterial and eukaryotic microbes influence ecosystem productivity, nutrient retention, and energy transfer through aquatic food webs (Fenchel, 2008; Worden et al., 2015). However, microbial communities are not passive actors — they also respond dynamically to environmental gradients, including changes in DOM composition, nutrient concentrations, and elemental ratios (Logue et al., 2016).

In DOM-rich systems, microbial community composition is shaped by the availability and quality of organic substrates, as well as by the relative supply of C, N, and P (Berggren et al., 2010c). Bacteria, as osmotrophic organisms, are particularly sensitive to DOM properties such as molecular size, aromaticity, and lability, which influence their access to carbon and associated nutrients (Kellerman et al., 2014). Eukaryotic microbes, including heterotrophs, autotrophs, and mixotrophs, are often structured by both nutrient availability and food web interactions, such as grazing and competition (Fouilland & Mostajir, 2010). The balance of these interactions is likely to shift under changing DOM and nutrient regimes (Jansson et al., 2007) — *which, in microbe terms, can mean a total reshuffling of who gets to thrive and who's left scrambling for scraps.*

Despite this complexity, most studies have focused on how inorganic nutrients shape microbial community structure, with less attention to how bioavailable organic nutrients and DOM quality influence microbial diversity and function (Stepanauskas et al., 2000b; Cotner & Biddanda, 2002). Moreover, functional traits — such as metabolic pathways involved in nutrient acquisition — are rarely considered alongside taxonomic composition (Comte & Del Giorgio, 2011). This limits our understanding of how microbial communities respond to and help regulate nutrient cycling under conditions of stoichiometric imbalance or organic matter enrichment (Godwin & Cotner, 2015; Maranger et al., 2018).

To advance microbial ecology in aquatic systems, it is crucial to integrate measures of community composition, functional potential, and bioavailable nutrient pools (Prosser et al., 2007; Graham et al., 2016). Such an approach allows for a more complete view of how microbial communities mediate ecosystem functioning — and how they may shift in response to climate- and land use-driven changes in DOM and nutrient inputs (Jansson et al., 2007).

Why this matters: gaps and opportunities

Despite growing recognition of the role DOM quality and stoichiometry play in regulating microbial activity, there are still some surprisingly persistent blind spots in our understanding of aquatic nutrient cycling (Karl, 2000; Cotner & Biddanda, 2002; Jansson et al., 2012). Chief among them is a limited grasp of how much C, N, and P — especially in organic forms — is truly available to microorganisms. Standard measurements often stop at inorganic nutrients or bulk concentrations, which tell us little about the true nutritional landscape facing microbial communities (Stepanauskas et al., 2000b; Berggren et al., 2010b). In DOM-rich systems, this means that entire pools of organic N and P — arguably the dominant fractions — are often ignored.

While bioavailability assays provide a more realistic view of these accessible pools, they remain underused and rarely applied across all three nutrient elements at once.

Among these, dissolved organic P (DOP) stands out as one of the least studied, despite its ecological importance (Cotner & Wetzel, 1992; Karl, 2000). Methods that estimate bioavailable DOP are still in development, and few studies attempt to link these estimates to DOM properties or microbial processes (Bergström, 2010; Jansson et al., 2012).

Adding to this, is the challenge of DOM heterogeneity. We know that microbial access to nutrients is influenced by DOM composition — but what exactly makes a molecule "available"? Optical tools such as fluorescence spectroscopy and specific ultraviolet absorbance (SUVA) offer valuable clues, helping to characterize DOM origin and reactivity (Weishaar et al., 2003; Fellman et al., 2010). Yet these tools are rarely integrated into multi-element bioavailability studies, limiting our ability to predict how microbial nutrient dynamics shift under different DOM regimes (Berggren et al., 2010a).

Finally, microbial communities themselves — key agents in the transformation of DOM and nutrients — are often studied in isolation from nutrient availability (Jansson et al., 2000; Logue et al., 2016). We know that microbes mediate biogeochemical cycling, but we still lack a clear picture of how bioavailable nutrient fractions, DOM quality, and stoichiometric constraints shape microbial composition and functional traits. These gaps are especially pronounced when we look across the land–sea continuum, where systems face diverse and shifting nutrient regimes (Regnier et al., 2013).

This thesis aims to fill in some of these blanks. By combining field surveys, laboratory assays, molecular sequencing, and experimental approaches across a gradient from streams to coastal waters, it provides a more integrated look at how nutrient bioavailability and DOM quality interact to influence microbial communities — and how these relationships affect aquatic ecosystem functioning in a changing world.

Aims and objectives

This PhD project investigates the bioavailability of carbon (C), nitrogen (N), and phosphorus (P) in inland and coastal freshwater systems, focusing on how dissolved organic matter (DOM) quality and nutrient stoichiometry shape microbial accessibility and ecological functioning. While total nutrient concentrations are often measured in aquatic ecosystems, the bioavailable fraction — the portion accessible to microorganisms — varies widely and plays a critical role in ecosystem dynamics under changing environmental conditions.

The overarching aim of the thesis was to identify the environmental and chemical factors regulating nutrient bioavailability along the land-to-sea continuum, and to examine how DOM characteristics and stoichiometric constraints influence microbial responses.

The following research questions (RQ) guided the work:

1. Seasonal dynamics: How do the relative abundances of bioavailable carbon, nitrogen, and phosphorus vary seasonally in boreal headwater streams, and what environmental factors shape these patterns? (Paper I)

2. Chemical drivers: Can DOM composition, as assessed by fluorescence-based indicators, help explain the variation in bioavailable macronutrient concentrations across organic-rich streams and lakes? (Paper II)

3. Microbial community responses: How do microbial community structures in lakes respond to gradients in lake size, DOM quality, and bioavailable nutrient ratios? (Paper III)

4. Experimental manipulations: How do labile carbon additions, nutrient enrichment, and increased water colour interact to influence the bioavailability of dissolved organic phosphorus in coastal waters? (Paper IV)

Papers overview

Paper I – Seasonal patterns in nutrient bioavailability in boreal headwater streams

This study examined seasonal variation in the bioavailability of dissolved organic carbon (DOC), nitrogen (DON), and phosphorus (DOP) in four boreal headwater streams in northern Sweden. Using short-term nutrient addition bioassays designed to isolate individual nutrient effects, the study quantified the bioavailable fractions of total dissolved organic nutrients across seasons. The findings revealed distinct temporal patterns: bioavailable fractions were consistently lowest for carbon and highest for phosphorus, with particularly elevated concentrations of all three nutrients in autumn. Bioavailable N:P ratios also peaked during autumn, likely due to combined effects of leaf litter input and the rewetting of soils following summer drought conditions. While streams draining forest and mire catchments showed similar seasonal patterns, mire streams tended to exhibit higher absolute concentrations of bioavailable nutrients in autumn. The study highlights the importance of autumn as a critical period for the delivery of bioavailable resources to stream ecosystems and suggests that climate-related changes in hydrology may strongly influence nutrient stoichiometry and microbial productivity.

(Addresses research question 1 and tests hypothesis 1).

Paper II – Does dissolved organic matter composition help explain the concentrations of bioavailable macronutrients in organic matter-rich freshwaters?

This study tested whether variation in DOM composition can explain the bioavailability of carbon (C), nitrogen (N), and phosphorus (P) across organic-rich, unproductive freshwaters. The study combined bacterial nutrient bioassays with fluorescence excitation-emission matrix (EEM) analysis across eight stream and lake sites in northern Sweden, including systems with known differences in DOM sources and redox conditions. The main hypothesis was that specific DOM components — such as freshly produced humic-like and protein-like substances — would correspond to higher bioavailability of C and N, while bioavailable P would be linked to microbially-derived DOM components or DOM-Fe complexes.

The results supported the hypothesis for carbon, with strong positive correlations between BDOC and recently produced, low-aromatic DOM. Phosphorus bioavailability was also linked to fluorescence components indicative of microbial origin, suggesting that microbially-processed DOM may serve as a key source of organic P. In contrast, nitrogen bioavailability was poorly related to DOM composition and instead driven primarily by dissolved inorganic nitrogen (DIN) concentrations. DOM composition variables improved statistical models of bioavailability for all three elements, explaining additional variation beyond standard nutrient metrics.

This study deepens the mechanistic understanding of DOM–nutrient interactions introduced in Paper I, demonstrating that DOM quality — not just quantity — plays a critical role in determining nutrient accessibility. It also demonstrates the potential of fluorescence-based DOM characterization as a predictive tool for bioavailable nutrient pools in inland waters.

(Addresses research question 2 and tests hypothesis 2)

Paper III – Microbial community structure in lakes of varying size and organic matter quality: The role of stoichiometric constraints and bioavailable nutrients

This study investigates how microbial community composition and metabolic functional diversity in boreal lakes respond to variation in nutrient stoichiometry, bioavailability, and DOM quality. Using data from 34 lakes in southern Sweden spanning a broad range of lake sizes and DOM characteristics, the study combined 16S and 18S rRNA sequencing with nutrient bioavailability assays and functional pathway analysis. The primary hypothesis was that microbial metabolic pathways related to nitrogen and phosphorus cycling would be increasingly represented in lakes with relative deficiencies of these nutrients.

The results revealed that DOC and phosphorus were major drivers of microbial community composition, with bacterial communities most strongly associated with total DOC and C:N:P stoichiometry, while eukaryotic communities were influenced by both BDOC and bioavailable total dissolved phosphorus (BTDP). Functional analysis supported the hypothesis, showing that N- and P-related pathways correlated with stoichiometric imbalances, suggesting that microbial communities modulate their metabolic strategies under nutrient-limited conditions. Additionally, lake size and DOM aromaticity (as measured by SUVA) emerged as important environmental factors shaping both community structure and functional potential.

This study adds an ecological perspective to the patterns and mechanisms explored in Papers I and II, highlighting how nutrient bioavailability and stoichiometry interact with lake characteristics to influence microbial dynamics. It underscores the importance of integrating microbial functional traits into assessments of freshwater biogeochemistry.

(Addresses research question 3 and tests hypothesis 3)

Paper IV – Nutrient, carbon and darkening impacts on coastal dissolved phosphorus bioavailability – a mesocosm study

This study tested how total dissolved phosphorus bioavailability is influenced by water colour, labile carbon inputs, and inorganic nutrient enrichment in a coastal brackish water system. Using a fully factorial mesocosm experiment, the study explored the individual and interactive effects of these drivers on bioavailable total dissolved phosphorus (BTDP). The central hypothesis was that nutrient and carbon additions would enhance BTDP through microbial activity and phytoplankton exudation in clear waters, but that darkened waters would constrain these processes, limiting transformations of phosphorus into bioavailable organic forms.

The results supported this hypothesis: in clear water mesocosms, nutrient enrichment led to increased BTDP concentrations, suggesting enhanced microbial and algal processing. In contrast, in darkened conditions, added inorganic phosphorus remained largely untransformed, persisting as PO_4 rather than contributing to the organic bioavailable pool. Labile carbon additions (glucose) had limited effects, highlighting the context-dependence of microbial priming.

This study complements the observational findings in Papers 1–3 by experimentally testing how DOM quality and nutrient enrichment interact to influence phosphorus bioavailability. It reinforces the idea that DOM-nutrient interactions are light- and context-dependent, and that both eutrophication and water darkening must be addressed to mitigate shifts in phosphorus cycling in coastal ecosystems.

(Addresses research question 4 and tests hypothesis 4)

How the science happened

My project combined field experiments and surveys, laboratory bioassays, and microbial community analysis to explore how organic matter and nutrient availability influence microbial dynamics across the land-sea continuum. Rather than aiming to provide a complete picture of biogeochemical cycling in each system, the goal was to apply a consistent analytical lens to diverse environments — ranging from forested headwater streams to lakes and coastal mesocosms — in order to uncover common patterns and ecosystem-specific processes. The study design was guided by a desire to link microbial activity with nutrient supply in a way that was mechanistically informative and comparable across systems. To do so, we focused on short-term nutrient bioavailability assays, DOM quality indicators, and microbial sequencing, using variations of the same core protocols throughout the project. This approach aimed to uncover not just where nutrients are found, but which ones are accessible — and to whom. To keep the thesis accessible to a wide audience, key methods and concepts are briefly introduced along the way. Each study contributed to one or several pieces of the puzzle, helping illuminate the microbial and biogeochemical responses to nutrient inputs in systems shaped by land-water interactions.

From forest floor to coastal shore

The four studies in this thesis span a broad environmental gradient — from headwater streams and lakes draining fully to partially forested catchments in northern Sweden, to many more lakes in southern Sweden, and finally to nutrient-rich coastal waters on the Baltic Sea coast (Figure 1). While each system was chosen for its own ecological and methodological interest, their combination was also strategic. The headwater streams in northern Sweden provided natural variation in catchment characteristics and a rich range of long-term monitoring data; the lakes in southern Sweden offered a wide range in size and DOM quantity and quality; and the coastal mesocosm experiment allowed us to manipulate light and nutrient regimes under controlled conditions. Together, they form a simplified land-sea continuum, i.e., a progression of environments influenced by different combinations of nutrient supply, DOM inputs, and hydrological dynamics. The importance of tracing nutrient and organic matter transformations along the land–sea continuum is increasingly recognized in biogeochemical research (e.g., (Raymond et al., 2013; Regnier et al., 2013)), and this study was designed with that broader framework in mind.

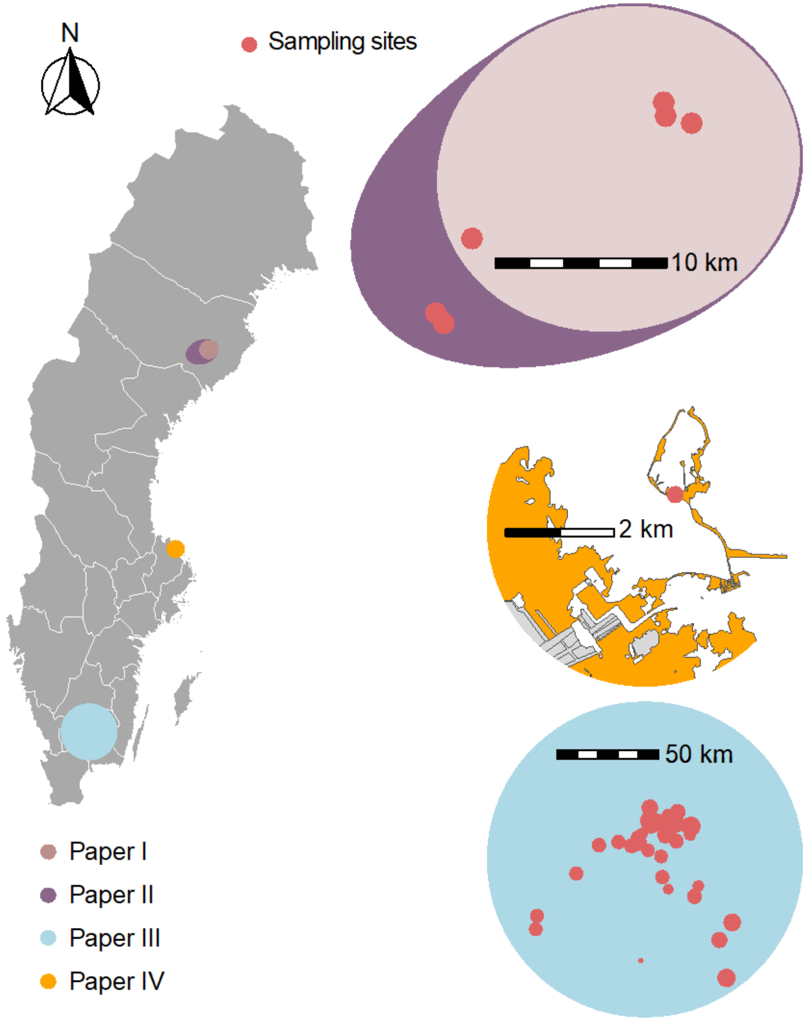

Figure 1. Study sites associated with each paper included in this thesis.
Paper I focuses on boreal headwater streams in northern Sweden (top, light pink), while Paper II expands on this by including the same streams plus two nearby lakes (top, purple). Paper III examines lakes in southern Sweden across a gradient in DOM quality and lake size (bottom, light blue), and Paper IV uses a coastal mesocosm experiment based on seawater collected from the Baltic Sea (middle, yellow). The sites represent a progression along the land-to-sea continuum, spanning different hydrological and biogeochemical settings.

To make sense of this complexity, I used a shared methodological core, including bioavailability assays, DOM quality analyses, and microbial community characterization, I aimed to trace patterns and contrasts in nutrient availability and microbial structure across these systems. Some comparisons were direct and quantitative, such as between stream and lake bioavailability estimates; others were more conceptual, as in the experimental contrasts in Paper IV. Each study contributed different pieces to the broader narrative, and while the environmental context varied, the questions remained the same: which resources are available, to whom, and under what conditions?

This comparative approach also helped clarify which patterns were system-specific and which might hold more broadly. For example, while BDOC concentrations were consistently low across stream and lake environments, coastal dynamics were more strongly affected by interactions between light and nutrient enrichment. Likewise, microbial responses in different systems revealed both shared traits and ecosystem-specific sensitivities. Rather than forcing a uniform interpretation, the land-sea lens allowed for differences to be informative, offering insights into how microbial communities and nutrient cycling interact under contrasting environmental pressures.

Working across such a broad range of environments also meant adapting to wildly different conditions — ecologically, methodologically, and sometimes physically. Some field days felt idyllic, with clear skies and warm weather. Others were less forgiving. Imagine this: you're originally from a tropical country, it is December, and you're heading out for your first sampling trip without a supervisor fresh into your PhD education…in northern Sweden. I can tell you with confidence that checking the forecast, seeing that it will be sunny (despite the –20 °C), and thinking "*Cold, but I can handle that*" is not enough preparation. Not snowing does not mean there will not be snow. I learned that the hard way, spending nearly 90 minutes — on a route that should have taken 40 minutes round trip — trying to locate a stream past a forest and mire clearing, knee-deep in snow the entire time (*have you ever tried to walk through that much snow?*). All the while, daylight was slipping away, and this was only the first of several sites. Thankfully, we managed to finish just as the sun dipped below the horizon. I did learn from the experience, though — *and have since done my best to avoid winter sampling altogether* — taking precautions and being prepared when sampling during winter is unavoidable. That day was a stark reminder that fieldwork is not just about data collection, but it is also about adapting, learning, and navigating real-world unpredictability. I am glad for that experience and am completely able to laugh about it… *now*.

Measuring what really matters

How much of the C, N, or P dissolved in water is actually available to microorganisms? This is a deceptively simple question, and yet one that aquatic ecologists/biogeochemists have long struggled to answer. For decades, nutrient availability has often been inferred indirectly — from total or inorganic nutrient concentrations, stoichiometric ratios, or changes in biomass (Berggren et al., 2015). But these approaches offer limited insight into the actual usability of resources by microbial communities, particularly in systems where organic matter dominates.

Bioassays offer a more direct way in: they are tests that measure the biological response to a chemical substance — in this case, the availability of dissolved nutrients to microorganisms. By incubating natural microbial communities with ambient water — sometimes enriched with nutrients — and tracking microbial growth over time, bioassays allow for estimates of how much of the nutrient pool can be accessed and metabolized. Early bioassay studies, such as those by Stepanauskas et al. (2000a); Stepanauskas et al. (2002), introduced the use of microbial regrowth combined with flow cytometry — a technique that uses lasers to rapidly count and characterize individual cells — as a way to estimate bioavailable DOC and DON. Later, Soares et al. (2017) adapted this approach using bacterial production (BP) — an estimate of how much biomass bacteria produce, often measured by tracking the incorporation of radioactive substrates like leucine — instead of cell counts as a proxy for microbial growth. Still, most studies have focused on individual nutrients — typically DOC — and methods have varied widely in terms of incubation time, detection techniques, and inoculum source (Berggren et al., 2015). As a result, comparisons across systems or studies remain rare and methodologically inconsistent. Attempts to quantify all three nutrients (C, N, and P) simultaneously are even scarcer.

To address this, I developed a standardized, high-throughput version of a multi-element bioavailability assay, combining microbial regrowth and flow cytometry, building on and modifying the approaches used (Stepanauskas et al., 2000a) and (Soares et al., 2017). The principle was simple: incubate natural inocula with filtered water — both in control and nutrient-enriched conditions — and measure cell abundance over short timescales (48–72 h). Microbial growth, as detected by cell counts, serves as a proxy for the bioavailable portion of the nutrient pool. While this approach builds on previous work, its application to multiple nutrient elements simultaneously — and across such a diverse land-to-sea gradient — marks a significant step forward in the effort to make bioavailability estimates more ecologically relevant and broadly comparable.

We chose flow cytometry over other approaches, such as BP measurements, because it offered a more streamlined and cost-effective solution (Gasol & Moran, 1999). The protocol is relatively straightforward, with fewer steps and lower costs than radiolabel-based methods like leucine incorporation. It also allows for sample preservation and later analysis — an advantage when handling large sets of samples from different studies. While interpreting growth curves still presents challenges, the method provided the flexibility and scalability needed to apply the same core protocol across systems.

To handle the large number of samples required — particularly in Papers III and IV — I adapted the protocol to a 96-well deep-well plate format (Figure 2). This setup allowed for parallel processing of dozens of samples, with consistent nutrient and inoculum volumes across treatments. It also enabled replication and the inclusion of internal controls on each plate. The modified assay made it possible to estimate bioavailable fractions of C, N, and P across more than 70 individual and 30 sites samples, including lakes (34 samples) and coastal mesocosms (36 samples). While flow cytometry had previously been used in bioavailability assays, its use in this multi-element, microplate-based format was, to our knowledge, unprecedented, and scaling up required both technical and logistical adjustments.

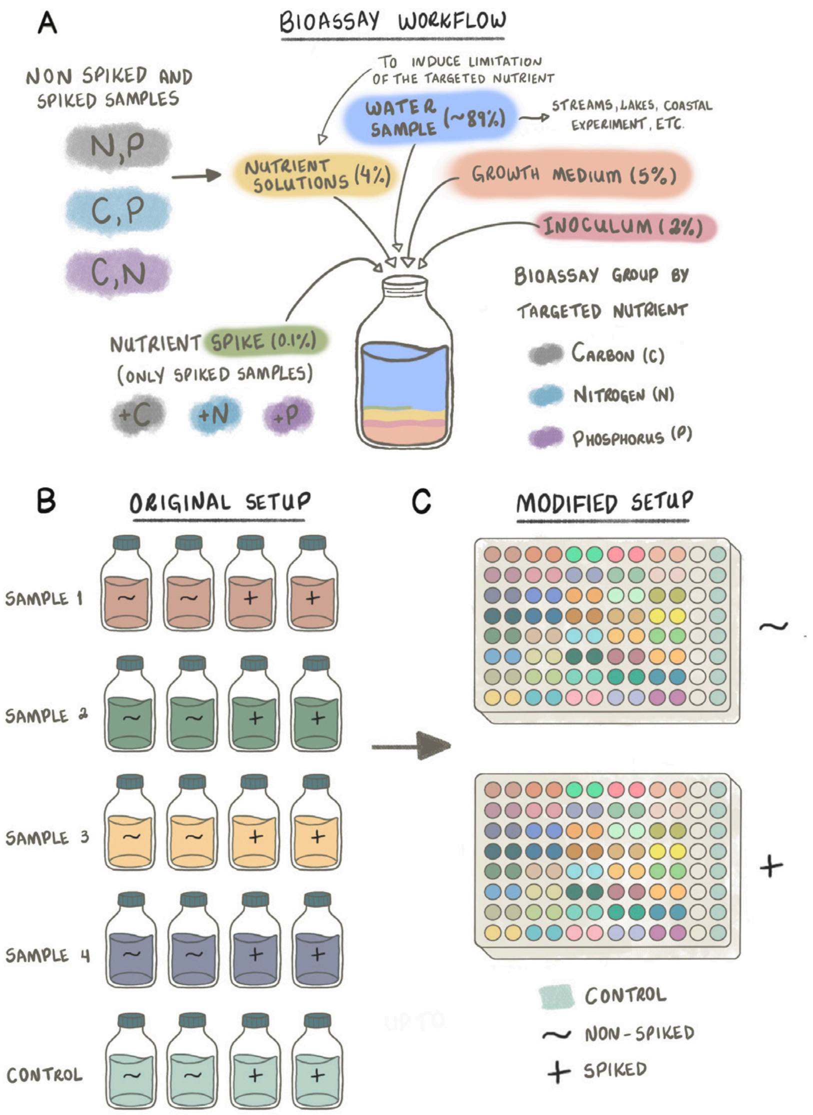

Figure 2. Bioassay workflow and experimental setup. Schematic overview of the multi-element bioassay approach developed and applied in this thesis to estimate bioavailable carbon (BDOC), nitrogen (BSON/BTDN), and phosphorus (BDOP/BTDP) across systems.
(A) Overview of the workflow used in the bioassays, including additions of a modified microbial growth medium, a microbial inoculum from each sample site or experimental treatment, the water sample, and inorganic nutrients to ensure that only the targeted nutrient (C, N, or P) was limiting. This workflow was applied across all studies, using either large bottles or 96-well plates. (B) Original setup used in Papers I and II, based on 250 mL Duran bottles, with four samples per run prepared in duplicate for spiked and non-spiked treatments, along with Milli-Q water controls (total of 60 bottles). (C) Modified high-throughput setup used in Papers III and IV, adapted to 96-deep-well plates to allow processing of up to 40 samples per plate, with internal duplicates (each colour representing a different sample) and a column reserved for plate-based Milli-Q water controls.

Adapting this bioassay method came with its fair share of challenges — from long days in the lab after sampling campaigns, filtering litres of water (twice: once for chlorophyll-*a*, and again to ensure a sterile inoculum), to preparing rows of bioassays and then spending even longer hours running multiple 96-well plates on the flow cytometer (Figure 3) to count cells. Since my department does not have its own instrument, this part of the work always took place in a different building, adding another layer of logistics to an already time-intensive process. And then, of course, came the data. Sometimes the results were confusing, or not quite what I had hoped for. Sometimes they made no sense at all — or perhaps, they do, and we just do not yet understand them. Accepting the limitations of the method was part of the process. But so was pushing through them.

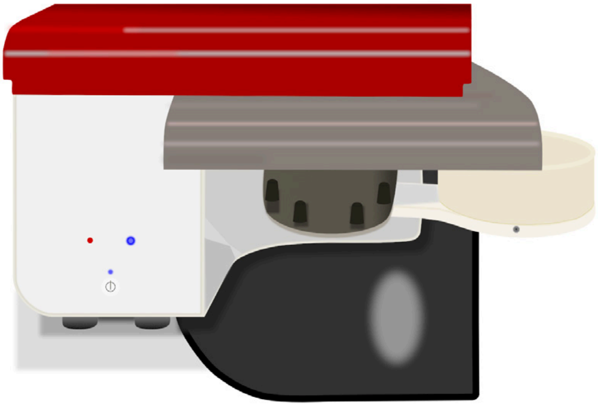

Figure 3. Flow cytometry in action.
Illustration of the instrument used to count bacterial cells in all bioassay samples throughout this thesis.

Adopting this method across such varied systems came with its share of risks. The sensitivity of the assay, the variability of microbial communities, and the complex behaviour of organic nutrients all posed challenges. Still, these risks were part of venturing into uncharted territory and, ultimately, worth taking for what we hoped to learn. By applying the same framework to multiple systems, the bioassays provided new insights into which nutrients were accessible, when, and where, and revealed patterns in nutrient availability that would have been missed by bulk measurements alone.

A landscape-infused brew

Understanding DOM composition was essential for interpreting patterns in microbial activity and nutrient bioavailability across the different environments studied in this thesis. DOM is a complex and heterogeneous mixture, and small differences in composition — such as aromaticity, molecular weight, or degree of humification — can influence how easily microorganisms can access the associated nutrients. These chemical differences also provide clues about the source and transformation history of DOM, from freshly produced phytoplankton exudates to terrestrial humic substances transported from soils.

To account for this variability, I included optical analyses of DOM quality in Papers II and III. The main tools were SUVA — a measure of ultraviolet absorbance per unit carbon concentration that serves as a proxy for DOM aromaticity and reactivity — and fluorescence excitation-emission matrix (EEM) spectroscopy — a technique that maps how DOM absorbs and emits light to reveal its chemical composition — which allows for a more detailed decomposition of DOM into protein-like and humic-like components (Catalan et al., 2017). These approaches have proven useful in characterizing DOM sources and reactivity (e.g., Berggren et al. (2010a); Fellman et al. (2010)) but are still rarely integrated with nutrient bioavailability data.

The rationale for including these methods was to bridge the gap between chemical composition and ecological function. Since many organic nutrients are carried by specific types of DOM, such as proteins or microbial exudates, linking DOM quality to nutrient uptake potential was key to understanding not just how much C, N, and P were present, but in what form and how accessible they were. This was particularly important in environments like dark humic streams and lakes, where standard bulk measurements are often poor predictors of bioavailability.

Casting the players

Microbial community composition was not part of the original project plan. But as the work unfolded, it became clear that understanding which microbes were present — and how they responded to DOM and nutrient conditions — would be essential to link bioavailability patterns with ecosystem function (*and if I am being honest, this was an aspect I had hoped to explore from the very beginning — even before it officially became part of the project)*. Amplicon sequencing of the 16S and 18S rRNA genes provided a practical and informative approach, allowing the characterisation of both bacterial and eukaryotic communities across a wide range of environments (Caporaso et al., 2012).

The decision to use this method was partly shaped by familiarity. Having previously worked with 16S rRNA sequencing during my master's, I knew the workflow well enough to confidently build it into the project. More advanced approaches like metagenomics would likely have offered deeper insights into microbial function, but such techniques require more infrastructure, technical support, computational resources, and expertise than were available to me at the time. In that sense, this choice reflected both the ambition of the project and a need to stay grounded in what was feasible.

To enable consistent future comparisons across systems, I selected protocols suitable for use with both Sterivex filters (Paper III) and other 0.2 μm filters (mesocosm samples), optimized DNA extraction steps for aquatic samples, and outsourced sequencing to a facility equipped for high-throughput processing. The result was a dataset that captured broad community patterns across lakes. While I later chose to focus primarily on compositional shifts rather than fine-scale taxonomy, these data added a valuable layer to the project. They helped frame bioavailability not only as a chemical property of the environment, but as something shaped and sensed by microbial communities themselves. In doing so, the community composition analysis provided a crucial link between resource availability and microbial ecology, complementing the bioavailability assays and DOM characterisation to create a more holistic view of ecosystem function.

The stats behind the story

Across the thesis, I approached the selection of statistical analyses with the aim of answering ecological questions in a clear, transparent, and purposeful way. For much of the work — particularly in Papers I, II, and IV — these questions were best addressed using classical approaches such as ANOVAs, t-tests, correlations, and linear and mixed models. These tools provided robust and interpretable results without overcomplicating the structure of the data. Where needed, they were paired with post hoc comparisons or correlation tests to explore specific patterns in nutrient availability and microbial responses.

While more advanced techniques are increasingly common in microbial ecology, the simpler analyses used here proved to be well suited to the nature of the data and the hypotheses being tested. In cases where community composition and multivariate relationships were of interest (Paper III), methods such as non-metric multidimensional scaling (NMDS), permutational multivariate analysis of variance (PERMANOVA), and redundancy analysis (RDA) were applied. Even then, interpretation remained grounded in ecological reasoning, with care taken to avoid overfitting or excessive dimensionality.

Throughout the project, the statistical choices reflected a balance between rigour and accessibility, with an emphasis on reproducibility and transparent communication of findings. More than testing for significance, the analyses were meant to reveal the shape and direction of ecological patterns across environments, supporting the broader goal of understanding microbial nutrient dynamics from land to sea.

Synthesis – Links across the land-to-sea continuum

Still with me? Good — because this is where it all comes together. It is time to step back and try to weave the threads of the past few years into a broader perspective that, hopefully, makes sense. Here we go…

What is actually on the microbial menu

Across all studies, a consistent pattern emerged: total or inorganic nutrient concentrations were often poor proxies for what microbial communities could actually access. This was particularly evident for C and P, where bioavailability varied substantially across environments and seasons. The use of short-term regrowth bioassays, applied across multiple systems with a standardized approach, allowed us to directly estimate which fractions of C, N, and P were biologically available (Figure 4). In doing so, these assays helped bridge a persistent gap between biogeochemical monitoring and ecological function, i.e., microbes rely on usable nutrients, not just those we can more easily quantify.

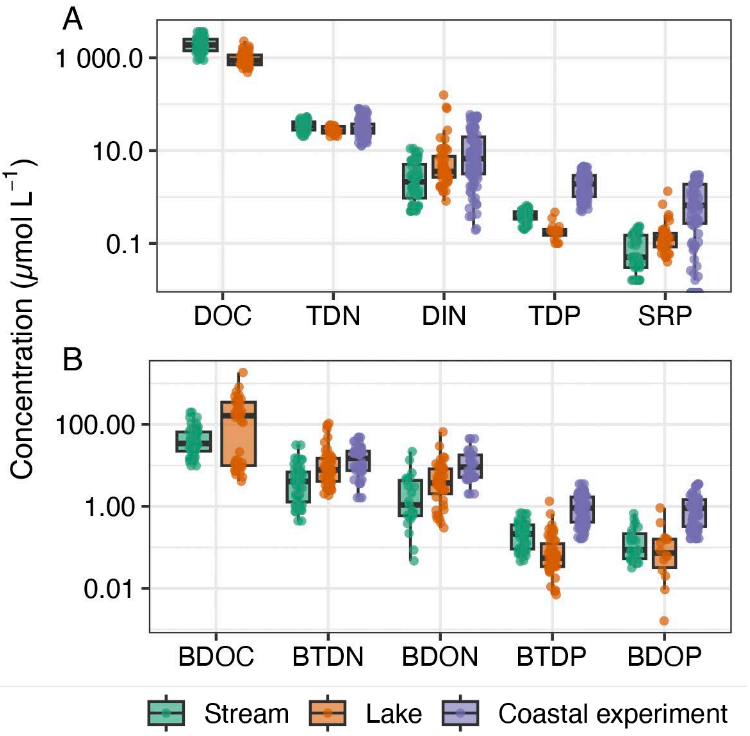

Figure 4. Boxplot summary of A) total or inorganic resouce concentration and B) bioavailable resource concentration for each aquatic environment.
This figure illustrates the differences between bulk and bioavailable fractions of carbon, nitrogen, and phosphorus. Data are drawn from Papers I (streams), II (streams and lakes), III (lakes), and IV (coastal mesocosms), reflecting environments along the land-to-sea continuum.

In headwater streams (Paper I), a key finding was that DOC concentrations were often high, yet only a small fraction, typically less than 10%, was accessible to bacteria. This low BDOC availability suggests that much of the terrestrial C entering these systems is structurally complex or otherwise resistant to immediate microbial use, i.e., recalcitrant (Koehler et al., 2012). These results complicate the widespread assumption that DOC-rich systems are automatically carbon-rich from a microbial perspective — a view often reinforced by studies that focus on DOC concentrations alone or assume microbial uptake without actually measuring it. *Well, apparently not*. A similar pattern emerged in the study by Soares et al. (2017), who used a comparable bioassay to ours — but based on bacterial production instead of abundance — and likewise reported low proportions of usable DOC. The findings here highlight the importance of testing the real microbial accessibility rather than inferring availability from bulk resource concentrations.

These findings underscore the value of direct bioavailability measurements for capturing ecosystem-specific processes (Bronk et al., 2007). For instance, in Paper II, we found that the best predictors of BDOC, BTDN, and BTDP were DOC, dissolved inorganic N (DIN), and total P (TP), respectively. However, this predictability broke down in the broader lake survey (Paper III), where neither DOC nor inorganic P — often measured as soluble reactive P (SRP) — significantly explained variation in BDOC or BTDP. Notably, TP was not measured in Paper III *(believe me, I am still trying to understand how this happened, or didn't as was the case. Human error is so real)*, which may help explain the weaker relationship to BTDP. Even so, the contrasting results suggest that bulk nutrient concentrations may provide useful insights in some contexts but cannot be assumed to scale with bioavailability across gradients or ecosystem types (Hood et al., 2009; Berggren et al., 2010a). Instead, the bioavailable fraction must be treated as an ecologically meaningful variable in its own right — one that responds to multiple controls and cannot always be inferred from total or inorganic pools.

While nutrient ratios are commonly used to infer potential limitations (Guildford & Hecky, 2000; Sterner & Elser, 2002), this thesis showed that bulk and bioavailable pools can tell very different stories. In both headwater streams (Paper I) and lakes (Paper III), bioavailable C:N:P ratios often diverged from their total or inorganic counterparts — sometimes substantially. For instance, while total DOC concentration was high in many systems, the bioavailable DOC pool was much smaller — as discussed above — shifting the balance toward N or P limitation when viewed through a microbial lens. Similarly, bioavailable N:P ratios revealed different patterns than those based on DIN and SRP alone (Figure 5). These discrepancies underscore the need to assess stoichiometry not just through bulk or inorganic concentrations, but through the lens of what microbes can, in fact, access. Bioavailable ratios provided a more ecologically meaningful perspective on microbial nutrient supply (Berggren & Del Giorgio, 2015), and helped clarify how microbial communities may respond to mismatches in elemental demand and availability — whether by altering community composition or adjusting their functional strategies (discussed later in this synthesis).

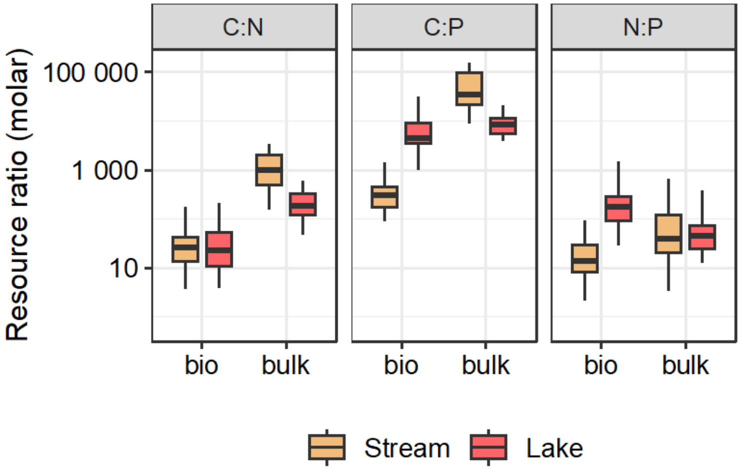

Figure 5. Comparison of bioavailable ("bio") and total/inorganic ("bulk") nutrient ratios (C:N, C:P, and N:P; molar units) across streams (Paper I) and lakes (Paper III).
Ratios are based on data from Papers I and III but are presented here together in a unified format. Points represent individual samples, with boxplots showing medians and interquartile ranges. Y-axes are shown on a $\log_{10}$ scale. Colours distinguish between water body types (streams vs. lakes).

The seasonal signal observed in headwater streams (Paper I) offered another important insight: autumn emerged as a key period of nutrient delivery, marked by high concentrations of bioavailable C, N, and P. These peaks coincided with falling water temperatures and catchment rewetting, likely reflecting a pulse of organic-rich material entering the aquatic ecosystem following summer drought (Raymond et al., 2016). This finding highlights how nutrient supply to aquatic systems is not only shaped by land cover or soil type, but also by hydrological timing. Such seasonal pulses are rarely captured in studies relying on single or summer-focused sampling campaigns, yet they may play a disproportionate role in shaping microbial metabolism and downstream nutrient export (Catalan et al., 2016). Recognising the importance of timing adds another layer of complexity to the already dynamic land-water interface and points to the need for seasonal bioavailability assessments in future work. This becomes especially relevant under climate change, as shifting patterns of drought and precipitation are likely to alter the timing and intensity of nutrient pulses entering aquatic systems (Creed et al., 2015; De Wit et al., 2016).

Together, these findings show that the concentrations of bioavailable nutrients — and not just the total pools — vary markedly across aquatic environments. Addressing research question 1, we found that the availability of C, N, and P is controlled by both system type and environmental conditions. In turn, this supports a more nuanced view of nutrient supply: one where microbial access to nutrients is shaped not only by how much is present, but also by how, when, and where it becomes available.

What is in the water matters

What is in the water — chemically and biologically — affects what microbes can do. Paper II set out to investigate whether DOM quality could help explain differences in nutrient bioavailability across streams and lakes, with a focus on optical indicators such as fluorescence components. The results were clear: DOM composition matters. Freshly produced humic-like and protein-like compounds helped explain patterns in BDOC and BTDP, highlighting that different types of DOM contribute differently to nutrient availability. These patterns, which emerged from consistent bioassays paired with DOM optical characterization, directly addressed research question 2 and helped illuminate how different DOM signatures support different elements of the nutrient pool. This has important implications. While DOM is often treated as a single pool — broadly divided into "labile" and "recalcitrant" — these results emphasize that its composition matters deeply for understanding nutrient cycling. The fluorescence-based indicators used here offer a tractable way to assess DOM quality in field studies and mesocosm experiments, providing insight into potential nutrient availability beyond what bulk DOC concentrations can reveal. By linking DOM quality to bioavailable nutrient fractions, the study adds nuance to the growing recognition that terrestrial inputs are not just carbon-rich, but chemically complex, and capable of influencing the elemental makeup of aquatic food webs.

The quality of DOM emerged as a common thread linking microbial processes across freshwater and coastal environments. In lakes (Paper III), shifts in DOM aromaticity and source characteristics were associated with changes in eukaryotic community structure, suggesting that DOM composition acts not just as a fuel, but as a selective force shaping microbial assemblages (Logue et al., 2016). In coastal mesocosms (Paper IV), water colour — reflecting both DOM concentration and quality — altered how P became bioavailable, particularly under enriched conditions. These findings highlight how the chemical character of organic matter influences both microbial composition and the processing of nutrients. From inland waters to the coast, DOM quality influenced not only what resources were available, but how they were used — and by whom.

Who is there and what are they doing

Microbial communities form the foundation of aquatic nutrient cycling, quietly orchestrating the uptake, transformation, and release of C, N, and P (Azam et al., 1983; Falkowski et al., 2008). In this thesis, they are both the protagonists and the storytellers — being shaped by the chemistry of their environment and, in turn, shaping the fate of nutrients across aquatic systems. Understanding who is there, i.e., the structure of the microbial communities, and what they are doing, their functional responses to resource gradients, is key to interpreting ecosystem-level processes (Louca et al., 2016). Yet, most studies have examined one or the other in isolation. By combining community composition and functional trait perspectives, this work contributes a more holistic view of microbial dynamics across streams, lakes, and coastal waters. In doing so, it sheds light on how microbes respond not just to the amount of nutrients, but to the form in which they are delivered, and how that response varies across environments with distinct DOM sources and stoichiometric imbalances. These dynamics were explored most directly in Paper III, which addressed RQ3 by investigating how microbial communities in lakes respond to gradients in DOM quality, nutrient ratios, and lake characteristics.

Microbial communities do not respond to nutrient gradients in uniform ways. Instead, their structure and function appear to be influenced by the type and accessibility of available resources, with bacteria and eukaryotes responding along different axes (Logue et al., 2016). We expected that bacterial communities would be tightly coupled to bioavailable C — after all, as primarily osmotrophs, bacteria rely directly on dissolved organic compounds for growth (Azam & Malfatti, 2007). But in Paper III, it was not BDOC that aligned most closely with bacterial community structure, it was DOC. This was outcome challenged our expectations. It suggests that bacterial community composition is shaped by more than just the immediately accessible C pool. Perhaps long-term exposure to persistent DOC types influences community assembly, selecting for certain taxa over time (Lennon & Jones, 2011). Or perhaps other, unmeasured factors such as viral interactions, past nutrient conditions, or grazing pressure, help explain the patterns we observed. In any case, this finding nudges us away from simple assumptions: even for bacteria, whose metabolism is tightly linked to C availability, the drivers of community structure may be broader and slower-moving than we tend to assume.

This also raises the question: is total DOC similarly important for structuring bacterial community in other aquatic systems, such as streams and coastal waters? While we did not analyse microbial communities in those environments, the possibility is compelling. Given the recurring patterns across studies, especially the mismatch between total and bioavailable nutrient pools and ratios, it seems plausible that similar dynamics may play out beyond lakes (Stepanauskas et al., 2000a; Kritzberg et al., 2020). Although we cannot confirm this, the parallels are striking enough to warrant further investigation. Notably, microbial community data from the coastal mesocosm experiment is already available and could offer valuable insight if analysed in future work. Due to time and scope constraints, this analysis was not included in the present thesis, but it remains a promising avenue for extending the land-sea continuum perspective explored here.

Eukaryotic communities, in contrast, showed stronger associations with BDOC and BTDP. i.e., nutrient fractions that more directly reflect short-term bioavailability. This points to a greater sensitivity among eukaryotes to immediately accessible resources, likely tied to their functional diversity and trophic strategies (Worden et al., 2015). These organisms include heterotrophs that rely on external C sources, autotrophs that require rapid access to inorganic nutrients, and mixotrophs that combine both strategies, making them particularly responsive to changes in resource form and availability.

The coastal mesocosm experiment (Paper IV) adds another layer to this picture. While we did not assess community composition directly, we observed that BDOP concentrations increased under nutrient addition in light-exposed mesocosms. This pattern suggests a potential link between primary production and the generation of bioavailable P — perhaps through phytoplankton exudation of labile organic compounds (Li et al., 2014). If so, eukaryotic microorganisms may not only respond to BTDP, as suggested in Paper III, but also contribute to its production under certain environmental conditions. While this points to phytoplankton as likely contributors to BDOP production in coastal systems, the structuring role of BDOP and BDOC in Paper III was more closely linked to heterotrophic and mixotrophic eukaryotes, suggesting that different eukaryotic groups influence and respond to these nutrient pools in distinct ways. This reciprocal relationship, where eukaryotes influence and are influenced by bioavailable nutrient pools, underscores the need to consider both biological drivers and feedback in understanding nutrient dynamics (Azam & Malfatti, 2007). These patterns suggest that differences in nutrient form and accessibility, rather than concentration alone, help explain the divergent structuring and roles of microbial communities across systems.

Reading the menu: microbial functions and nutrient demand

Aquatic microbes do not just respond passively to nutrient availability, they actively adjust their metabolic strategies to meet their needs (Falkowski et al., 2008). Just as community structure helps us understand who is there, functional traits help us understand what they might be doing. In Paper III, I explored how microbial functional pathways related to N and P acquisition varied across lake systems with distinct nutrient ratios and DOM quality. These pathways offered a window into how microbes adapt to stoichiometric imbalances, not just by changing their community composition, but by adjusting their functional potential. That is, we get clues to what microbes are “hungry” for and what they might be doing to satisfy those cravings.

Imagine the ecosystem as a restaurant. The microbes are the guests, each with different appetites — some need more carbon, others are craving nitrogen or phosphorus. The nutrients — C, N, and P — are the ingredients used to prepare the meals. The functional genes are the kitchen staff — chefs, prep cooks, and servers — each trained to work with certain ingredients. The menu represents the full set of biochemical processes that the restaurant (i.e., the ecosystem) is capable of preparing, depending on the available ingredients and demand. When the guests start asking for more P-rich meals, the restaurant responds by hiring more kitchen staff trained in phosphorus-based recipes. In other words, the microbial community upregulates P-acquisition genes to match the demand. Even if the delivery truck carrying phosphorus has not arrived yet, the staff are already in place — ready to slice, mix, and cook as soon as the ingredients show up. And just like you can tell a lot about a restaurant by looking at its staff and menu, we can tell a lot about an ecosystem’s nutrient needs by examining the genes present in its microbial community.

But not all ingredients arrive in ready-to-serve form. A tomato might come with the delivery, but you cannot just throw a whole tomato on a plate and call it dinner. Instead, it needs, e.g., to be sliced for a salad or crushed for a sauce, just as microbes need enzymes to break down complex organic compounds into usable forms. The functional genes encode these metabolic tools, allowing the community to transform raw nutrients into bioavailable forms. A similar logic applies to other nutrients, like N or P, when they arrive bound in organic molecules. In this way, the metabolic pathways encoded in microbial genomes do more than reflect the current menu, they hint at what the community is ready to serve if certain ingredients become available, and how they will process them. This is what makes functional gene profiles so valuable: they offer a glimpse not only into microbial capacities, but also into ecosystem-level nutrient demand.

In Paper III, bacterial communities in lakes with high C:N or low N:P ratios showed increased representation of N- and P-acquisition pathways. This supports the idea that stoichiometric imbalances select for microbial traits that help compensate for nutrient limitations, even when absolute nutrient concentrations are not extreme. These functional patterns build on the structural findings from RQ3, linking nutrient ratios and DOM quality to microbial metabolic readiness.

While this functional perspective was only applied to lake systems, it raises an important question: would similar microbial adaptations be observed in streams or coastal waters, where stoichiometric imbalances and bioavailability also vary? Though not originally part of the guiding research questions, this functional trait analysis adds a layer of insight into how microbes navigate nutrient constraints — offering a more complete picture of how microbes navigate nutrient constraints and shape ecosystem processes along the land–sea continuum.

From soil to sea: following the nutrient trail

Nutrients rarely move in straight lines. They hitch rides on water, cling to organic matter, and pass through the hands — so to speak — of microbes, plants, and sediments as they journey from upland soils to the sea. This thesis set out to trace part of that journey by exploring how nutrient bioavailability and microbial responses shift across different types of aquatic environments. Along the way, it became clear that while some patterns repeated across systems, others were highly specific to the physical and chemical context of each site.

In the boreal headwater streams studied in Paper I, autumn emerged as a key period of nutrient delivery, with peaks in the bioavailability of C, N, and P. These seasonal pulses, shaped by catchment characteristics such as forest cover and peat influence, reinforced the role of land-water connectivity in regulating microbial resource supply (RQ1). The long-term monitoring of these catchments allowed us to contextualise bioavailability within known seasonal and hydrological patterns — something that proved more difficult in systems without comparable background data.

As nutrients and coloured organic matter travel downstream, they shape not only the quantity of resources available to aquatic microbes, but also the form in which those resources are delivered. In lakes (Paper III), which addressed RQ3, landscape features like lake size and the associated DOM characteristics emerged as key drivers of both nutrient composition and microbial community structure. Smaller, more vegetation-rich catchments tended to host more aromatic DOM and distinct microbial communities, highlighting how upstream landscape gradients can set the stage for downstream biogeochemical processes.

Coastal systems are increasingly shaped by two overlapping pressures: terrestrial browning and nutrient enrichment. As DOM inputs from land darken the water column, they reduce light availability and potentially shift microbial processing pathways. At the same time, eutrophication supplies additional nutrients that may stimulate microbial activity and organic nutrient transformations. These forces do not act in isolation. In Paper IV, which addressed RQ4, we explored these effects through controlled mesocosm experiments simulating increased browning and nutrient enrichment. In clearer waters, nutrient enrichment appeared to promote the production of labile P, possibly through phytoplankton exudation. But as browning intensifies, the role of autotrophs could be diminished, shifting the burden of nutrient transformation to heterotrophs or slowing the regeneration of BDOP altogether. The outcome may not necessarily be a reduction in P availability, but rather a reconfiguration of its cycling routes — where light, nutrients supply, and DOM quality interact to shape microbial access to this critical resource.

This form-shift highlights a critical nuance: unless both browning and eutrophication are addressed together, management strategies may simply redirect bioavailable P from one form to another. Whether similar dynamics occur in inland waters remains an open question but given the widespread browning of lakes and streams (e.g., Monteith et al. (2007); De Wit et al. (2016)), and ongoing nutrient enrichment across many landscapes (Jeppesen et al., 2005), it is plausible that these interactions are already altering the nature of bioavailable P upstream. Recognizing this shift could help refine both scientific understanding and policy efforts aimed at nutrient management across the freshwater-marine continuum.

Across all these systems, the influence of terrestrial inputs, both natural and experimental, was clear. But it was equally clear that local factors also modulate how those inputs are processed. By applying a consistent methodological lens across streams, lakes, and coastal waters, this thesis highlights how both landscape position and system-specific characteristics shape the nutrient trail from soil to sea. As terrestrial influence intensifies with climate change and land use shifts, understanding these dynamics becomes ever more important — not only for predicting nutrient export, but also for anticipating how ecosystems will respond. Together, these studies — each tied to a distinct research question — offer clearer insights into how bioavailability and microbial responses shift along this land-to-sea continuum.

The nutrient journey, retold

Across the four studies in this thesis, a consistent picture begins to emerge: nutrient availability is not only about what is present in the water, but about what is accessible, to whom, and under which conditions. By pairing estimates of nutrient bioavailability with measures of DOM quality and microbial responses, this work shows how environmental context — from catchment characteristics to lake morphometry and coastal darkening — influences the resource landscape for aquatic microbes.

Some patterns aligned with expectations, such as low BDOC availability in forest-dominated systems. Others were more surprising, like BDOP concentrations being higher in light rather than dark conditions when nutrients were available, or the apparent decoupling of BDOC and bacterial community structure in lakes. Taken together, the findings point to the value of a cross-system, mechanistic perspective — one that treats microbial communities not only as responders to environmental change but as key drivers of nutrient cycling. While many details remain to be resolved, especially regarding the fate of organic nutrient forms, this work offers a step forward in understanding the invisible forces that link land to sea through water.

Lessons learned and the road ahead

This thesis set out to explore a deceptively simple question: how do nutrients move through aquatic ecosystems, and how much of what we measure is truly available to the organisms that need it. By following the journey of carbon, nitrogen, and phosphorus from headwater streams to coastal waters, it became clear that these connections — across landscapes, rivers, lakes, and seas — are influenced not only by how much is present, but also by how these nutrients are packaged, processed, and perceived by microbial communities.

These studies reveal that the most readily available portions of nutrients often behave differently from their bulk counterparts. Bioavailable C, N, and P do not always follow predictable patterns, and their relationships to DOM quality and environmental context shift across systems. Together, the findings make a case for using bioavailability as a distinct and informative lens in aquatic ecology — one that captures ecosystem-specific nuances that total concentrations alone may obscure.

By developing and applying a consistent approach to estimate nutrient bioavailability, this thesis offers both a methodological contribution and a conceptual framework for exploring how resource form and microbial function interact. It underscores the role of microorganisms not only as responders to environmental change, but also as drivers of nutrient transformations that shape broader ecosystem processes across different aquatic environments.

Looking forward, there is still much to uncover. Better linking of microbial activity and nutrient form with hydrological variability, seasonal dynamics, and landscape gradients will be critical. So will expanding bioavailability estimates to include particulate and sediment-associated nutrients or exploring how microbial traits evolve in response to long-term changes in resource supply. As aquatic systems continue to darken and nutrient inputs shift under climate and land-use change, understanding how nutrient bioavailability governs ecosystem responses will become increasingly essential — not only for predicting change, but for managing it.

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