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Responses of diatom communities to browning and eutrophication – a field experiment

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Tiivistelmä - Referat – Abstract

Freshwater browning (increased dissolved organic matter) and eutrophication (nutrient enrichment) are pervasive trends in boreal lakes and streams. Browning, driven by higher inputs of terrestrial organic matter and iron, tends to shift algal assemblages toward mixotrophic or planktonic, whereas eutrophication typically boosts algal biomass and favors tolerant generalists at the expense of overall diversity. Diatoms are important primary producers and sensitive bioindicators of such environmental change. In this study, diatom species richness and community composition responses to controlled browning and nutrient treatment were tested. Also, likely dispersal sources for colonizing diatoms (nearby lakes vs. streams) were assessed.

Experimental pools at Lammi biological station (southern Finland) were set up using water from five differently colored lakes and added nutrients (nitrogen and phosphorus). Diatoms were sampled from each pool and species richness, Shannon-Wiener diversity and Pielou’s evenness were calculated. Community composition was analyzed using nonmetric multidimensional scaling (NMDS) and analysis of similarity (ANOSIM). Indicator species analysis identified taxa associated with each treatment. The results suggested that increased water color significantly altered diatom assemblages: NMDS/ANOSIM showed distinct communities by color ($R \approx 0.25$, $p \approx 0.03$) and diversity metrics varied across treatments (medium brown pools had the highest richness, clearest pools the lowest). *Nitzschia brevissima* emerged as an indicator of darker water. Nutrient addition had little effect on community composition, and the diatom communities bacame more similar to each other. *Pinnularia sinistra* was associated with nutrient-enriched pools, and *Asterionella formosa* emerged as an indicator for medium colored enriched pools.

Over the experiment, pool water color converged and diatom communities became more similar. Comparisons with nearby lake and stream samples showed that pool assemblages were very distinct from nearby lakes and streams. These results indicate that both browning and eutrophication can restructure diatom diversity in new habitats. This insight helps predict how algal communities in boreal freshwaters may shift under changing environmental conditions.

Keywords
diatoms, dispersal, eutrophication, browning, experimental mesocosms

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Tiivistelmä - Referat - Abstract

Förbruning (tillskott av organiskt material) och eutrofiering (tillskott av näringsämnen) är utbredda trender i boreala sjöar och vattendrag. Förbruning orsakas av ett tillskott av terrestriskt organiskt material och har en tendens att förändra algsamhällen mot mixotrofa eller planktoniska arter, medan eutrofiering vanligtvis ökar algbiomassan och gynnar toleranta generalister på bekostnad av artmångfalden. Kiselalger är viktiga primärproducenter och känsliga bioindikatorer för denna typ av miljöförändringar. I denna studie testades det hur kiselalgernas artrikedom och artsammansättning reagerade på färg- och näringsämnesbehandling. De sannolika spridningskällorna för koloniserande kiselalger (det vill säga närliggande sjöar eller vattendrag) utforskades även.

Experimentet utfördes med experimentella dammar vid Lammi biologiska station (södra Finland), fyllda med vatten från fem sjöar med olika färggrad samt näringsämnen (kväve och fosfor). Kiselalgsprover togs från varje damm och artrikedom, Shannon-Wieners diversitet och Pielous jämnhet beräknades. Artsammansättningen analyserades med icke-metrisk multidimensionell skalning (NMDS) och ANOSIM test. Indikatorartsanalys identifierade arter kopplade till respektive behandling. Resultaten visade att ökad vattenfärg signifikant påverkade kiselalgsamhällena: NMDS/ANOSIM visade tydliga skillnader mellan färggrupperna ($R \approx 0{,}25$, $p \approx 0{,}03$), och diversitetsmåtten varierade mellan behandlingar (dammar med medelhög färg hade högst artrikedom, de klaraste dammarna lägst). *Nitzschia brevissima* identifierades som indikatorart för mörkare vatten. Näringsämnestillsats hade liten effekt på samhällssammansättningen och kiselalgsamhällena blev mer lika varandra. *Pinnularia sinistra* var associerad med näringsberikade dammar, och *Asterionella formosa* framträdde som indikatorart för medelfärgade, berikade dammar.

Under experimentets gång blev vattenfärgen i dammarna mer jämn, och kiselalgsamhällena blev mer likartade. Jämförelser med närliggande sjöar och vattendrag visade att dammsamhällena var distinkta från dessa naturliga miljöer. Resultaten visar att både färgmörkning och eutrofiering kan omstrukturera kiselalgers mångfald i nya habitat. Denna kunskap kan bidra till att förutsäga hur algsamhällen i boreala sötvatten förändras i takt med förändrade miljöförhållanden.

Keywords
kiselalger, spridning, eutrofiering, förbruning, experimentella dammar

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1. Introduction

Freshwater ecosystems are shaped by a combination of physical, chemical, and biological factors, influencing species distribution, ecosystem function, and biodiversity (Battarbee et al., 2010). Freshwaters are increasingly threatened by anthropogenic activities. These include climate and land use change, causing, among a plethora of consequences, lake or stream browning and eutrophication (Woodward et al., 2010). Browning and eutrophication represent two major environmental stressors affecting aquatic systems, particularly in boreal and subarctic regions (Bergström & Karlsson, 2019; Finstad et al., 2016).

Through changes in land use including intense agriculture, deforestation and urbanization, nutrients are leached from the catchments and transported to lakes and streams via runoff (Lyche Solheim et al., 2023). The most important nutrients for aquatic ecosystems, nitrogen and phosphorus, are usually limiting factors in lakes and streams, making their increase impactful for the ecosystem dynamics. Eutrophication causes biomass growth depending on prevailing light conditions (Isles et al., 2021), and in the worst case, oxygen is depleted causing anoxic zones at the bottom of lakes.

Browning of freshwaters is caused by accumulation of dissolved organic matter and iron from the catchment (Bergström & Karlsson, 2019; de Wit et al., 2016; Kritzberg et al., 2020; Lyche Solheim et al., 2023). Browning is becoming more common, particularly in Nordic lakes, but may be occurring worldwide where wetlands or forests cause organic matter to leach to waters (Blanchet et al., 2022). This is because of land use changes and climate change increasing soil leaching and runoff from the catchments (Finstad et al., 2016). Although browning is principally a natural phenomenon, it impacts ecosystems by decreasing light penetration into the water. This causes community shifts in photosynthetic aquatic organisms, but it also influences the whole food web via biotic interactions.

Diatoms are unicellular microalgae inhabiting aquatic ecosystems and live both as plankton and in benthos. They are primary producers and efficient in nutrient cycling and therefore the base of food webs, which makes them crucial for ecosystem function. Diatoms are sensitive to environmental change, making them useful bioindicators. (Rühland et al., 2015). One of the many factors controlling diatom communities is light availability, impacted by the accumulation of organic and inorganic matter. In addition to light, other environmental factors such as pH, conductivity and nutrients control diatom communities (Smol & Stoermer, 2010).

By investigating diatom communities, one can get an understanding about the ecological status of the aquatic ecosystem, and thus, diatom sampling and investigation has been an established method in freshwater monitoring (Heino et al., 2009).

To predict how browning and eutrophication will affect freshwater diatom communities, it is important to understand the dispersal and ecology of diatoms by investigating the key environmental and spatial factors controlling diatoms. Dispersal plays a key role in structuring diatom communities, yet the extent to which diatoms originate from surrounding water bodies, airborne sources, or sedimentary seed banks is still debated (Kristiansen, 1996). While diatoms are known for their high dispersal capability, environmental filtering rather than dispersal limitation often dictates which species successfully establish in a habitat (Soininen, 2007).

This study aims to investigate the effects of browning and eutrophication on diatom communities, while also considering the role of dispersal in shaping these observed patterns. Possible dispersal routes of diatoms are investigated by comparing diatoms of natural lentic and lotic freshwater systems with freshly established diatom colonies in experimental pools. The study area is in southern Finland, where lakes and streams are subjected to both land use changes and climate change effects, which impacts the diatom communities through changes in both chemical and physical conditions. In Finland, increasing browning and eutrophication are changing aquatic ecosystems, making it crucial to understand how diatom communities respond to these stressors. While previous studies have focused on large-scale trends (Rühland et al., 2015), fewer studies have examined localized experimental systems like the one used in this study. The experimental setting provides a unique environment for distinguishing the effects of specific environmental factors.

Study questions:

1. How do diatom species richness and community composition respond to browning and eutrophication?

2. From where did the diatoms most likely disperse into the experimental pools?

By addressing these questions, this study provides insight into the mechanisms driving diatom community structure. This also contributes to a broader understanding of freshwater ecosystem response to multiple stressors. The findings are relevant for assessing the ecological status of aquatic ecosystems, while also providing information useful for conservation and management strategies.

2. Browning freshwater

The color of water is naturally varying in freshwater ecosystems. Some lakes and streams are naturally clear and oligotrophic, for example arctic waters, while lakes and streams near agricultural land are more turbid and often eutrophic. Lakes in forests and near wetlands are usually browner and more acidic because of dissolved organic material comprising humic acids. Although dark colored water occurs naturally, the browning of freshwater ecosystems is increasing (de Wit et al., 2016; Finstad et al., 2016; Kritzberg et al., 2020), altering aquatic ecosystems by reducing light levels and possibly increasing water acidity.

Browning of freshwater is caused by increasing dissolved organic matter (Vyverman et al., 2007) including dissolved organic carbon (DOC), and iron (Fe) (Kritzberg & Ekström, 2012; Lyche Solheim et al., 2023). Organic matter accumulates in the water through runoff from the catchment area and is escalated by rain events and prolonged growth seasons (Jansson et al., 2008). This material originates from decomposing plants including vascular plants and mosses, containing mainly dissolved organic carbon, nutrients and humic acid (Evans et al., 2005). Dissolved organic matter contains light absorbing particles, and by absorbing short-wavelength radiation it makes the water appear yellow- and brownish (Vyverman et al., 2007). The light-absorbing particles in DOM are often referred to as colored dissolved organic matter, cDOM.

2.1 Causes of browning

The browning of lakes and streams has been increasing in the last decade in the northern hemisphere because of climate change, land use changes and melting of permafrost (de Wit et al., 2016; Larsen et al., 2011; Sun et al., 2018). According to predictions, climate change causes a shift in climate conditions towards a wetter climate. Wetter climate increases the mobilization of carbon in soil, meaning increased concentrations of DOM (Bergström &

Karlsson, 2019; de Wit et al., 2016). Dissolved Fe levels also increase together with DOM contributing to browning (Kritzberg & Ekström, 2012). Moreover, browning of freshwater is partly induced by the greening of catchments and emission recovery (Finstad et al., 2016). Due to prolonged growing seasons vegetation is increased in catchments. This adds organic material into the soil, which in turn ends up in lakes and streams through runoff. Plants also release carbon into the soil when decomposing (de Wit et al., 2016).

In addition, depositions from anthropogenic sources, particularly sulphates, historically acidified the soil but through restrictions emissions decreased resulting in soil recovery (Monteith et al., 2007). This long-term trend of recovery causes soil to release previously stored carbon, which accumulates in aquatic ecosystems as cDOM causing browning. This also causes increasing humic acids in boreal catchments (Bergström & Karlsson, 2019). Agricultural land adds nutrients and carbon into the soil and aquatic ecosystems, increasing browning (Kritzberg et al., 2020). Fertilizers contain nutrients, which combine with cDOM in aquatic ecosystems, exacerbating browning. Different agricultural practices, such as plowing, may also increase erosion, further adding to cDOM. Other land use changes that impact lake browning include deforestation and wetland drainage (Bergström & Karlsson, 2019; Kritzberg et al., 2020; Larsen et al., 2011). Wetland drainage is usually done for agricultural purposes and has several strong impacts on the catchment. Both wetlands and forests store carbon and decrease soil erosion, while also naturally contributing to the DOM, DOC and Fe levels. When these are removed, the carbon from the soil is mobilized and transported to nearby lakes and streams with runoff.

While DOM is the main driver of browning, recent research highlights the important role of iron (Fe) in enhancing water color and affecting aquatic biogeochemistry (Kritzberg & Ekström, 2012). Fe forms complexes with DOM, increasing light absorption and contributing to the overall darkening of freshwater systems. Weyhenmeyer et al. (2014) found Fe to increase cDOM by binding with DOC until saturated in aquatic ecosystems with less than 1 mg L^{-1} Fe, which is the case for most boreal lakes. Additionally, Fe acts as a cofactor in many microbial and chemical processes, influencing nutrient cycling and oxygen availability (Knorr, 2013).

In boreal waters, Fe concentrations have increased parallel with DOM due to reduced acid deposition and changes in land use (Kritzberg & Ekström, 2012). This means that Fe may indirectly contribute to the effects of browning on diatom communities, not only by intensifying light attenuation but also by modifying nutrient availability, particularly phosphorus (P) (Knorr, 2013). Under some conditions, Fe can bind P in sediments, making it less bioavailable to primary producers (Kleeberg et al., 2013). However, in anoxic conditions, Fe-bound P can be released back into the water column, potentially intensifying eutrophication in some environments (Kleeberg et al., 2013).

2.2. Ecological impacts of browning

Fennoscandian lakes and streams are often sensitive to browning because of their exposure to multiple stressors (Knoll et al., 2018; Solomon et al., 2015). Some of the most critical impacts of browning are light limitation and changes in nutrient dynamics. While DOM can work as a nutrient source and light damper, its effects depend on the balance between these factors. It is also important to note that DOM protects the ecosystem from harmful UV-radiation. Furthermore, browning can impact aquatic ecosystems by lowering pH because of increases in humic acid (Futter et al., 2014). In addition to these impacts, ecological impacts also include changes in microbial activity and whole food webs by altering biotic interactions such as primary production, grazing and predation. This is caused by DOM restricting available habitats and resources (Blanchet et al., 2022; Brüsecke et al., 2023). Since diatoms are well-established bioindicators of water quality, their response to browning can be used to assess its ecological impacts.

2.2.1 Effects on light availability

Light availability is the most important factor at explaining variations in primary production in northern lakes (Karlsson et al., 2009; Seekell et al., 2015). Browning reduces light availability below the surface by absorbing light, particularly in the ultraviolet (100-400 nm) and blue wavelength (400-500 nm), leading to darker water and decreased photic depth (de Wit et al., 2016; Evans et al., 2005). This makes the water appear brownish or red and as a result, light-dependent primary producers including diatoms, experience reduced photosynthetically active radiation (Karlsson et al., 2009; Senar et al., 2021)

Many diatom species thrive in oligotrophic, clear-water conditions, making them particularly sensitive to light availability. Reduced light conditions favor mixotrophic species adapted to shade and using alternative sources of energy (Isles et al., 2021). Especially small, high-metabolism planktonic species resistant to sedimentation are favored (Finkel et al., 2010; Lyche Solheim et al., 2023; Rühland et al., 2015). Additionally, low-light tolerant diatom species may become more dominant in brown-water conditions (Bergström & Karlsson, 2019).

The impact of light limitation on algal biomass is complex. Some studies show a general decline in primary production because of reduced light (Bergström & Karlsson, 2019), others suggest that some species are still able to thrive when light is limited (Senar et al., 2021). These findings suggest that the extent of which light limitation affects primary production depends on other factors, such as nutrient availability. Moreover, the depth in which algae grow has importance on how strongly browning influences primary production. For algae growing near the surface, browning effects are much smaller than on algae growing in deeper water layers.

2.2.2 Effects on nutrient dynamics

Browning does not only limit light availability, but it also influences nutrient transportation and cycling. DOM can transport nutrients especially in catchments with high terrestrial input (Isles et al., 2021). The effect of browning on nutrient availability, however, depends on catchment characteristics, external inputs and lake mixing patterns. Phosphorus (P) and nitrogen (N) are the primary nutrients regulating algal growth, including diatoms. P is most commonly the main limiting factor, even though some boreal lakes are N-limited (Bergström & Karlsson, 2019), In low-DOC lakes, increased nutrient input promote biomass growth. However, as DOM levels increase, the efficiency of nutrient uptake for photosynthesis is reduced due to lower light intensities (Bergström & Karlsson, 2019).

Some larger diatom species prefer higher-nutrient environments, such as *Nitzschia spp.,* while *Asterionella formosa* for example prefers moderate-nutrient environments. In contrast, smaller species like *Achnanthes minutissima* and *Tabellaria flocculosa* typically thrive in low-nutrient environments. (Hall & Smol, 2010). Because of the limited number of studies using diatoms when investigating browning, phytoplankton such as cyanobacteria are often included. Cyanobacteria impact nutrient dynamics by compensating for N-limitation by fixing N from the atmosphere, making them dependent on P. When total phosphorus (TP) exceeds 100 µg/L, cyanobacteria dominance increases, particularly in humic lakes (Lyche Solheim et al., 2023). However, some studies suggest that P leaching from forested catchments into freshwater has decreased because of climate change mitigation, reducing the likelihood of eutrophication locally (Eimers et al., 2009).

2.2.3 Interactions between light limitation and nutrients

As stated before, both light availability and nutrients independently regulate primary production. However, it is their combined effect that determines the structure of algal communities. To summarize, in clear, low-DOM freshwater ecosystems algal growth is limited by nutrients, especially P. In moderate-DOM systems light and nutrients interact favoring mixotrophs and other shade-adapted diatom species. In high-DOM systems (>8 mg/L DOC) light limitation dominated, causing declines in primary production despite nutrient availability (Bergström & Karlsson, 2019). According to another study, primary production stops increasing at the threshold of about 4.8 mg/L DOC, because of light limitation in northern lakes (Seekell et al., 2015). Both Solomon et al. (2015) and Horppila et al. (2023) found the threshold to be around 10-14 mg/L. These changing dynamics drive shifts in species composition. When nutrients and DOM is low, diatoms dominate over other algae. When DOM increases to moderate levels and P is available, cyanobacteria start outcompeting diatoms (Bopp et al., 2005). In high-DOM waters where both nutrients and light are limited, mixotrophic species dominate. Based on light and nutrient availability, primary production is impacted positively or negatively (Seekell et al., 2015). These shifts affect the food webs and ecosystem functions, as primary producers shape the availability of energy and nutrients for higher trophic levels.

2.3 Thermal stratification

One of many phenomena intensified by browning is thermal stratification (de Wit et al., 2016; Kellerman et al., 2015). Because of climate change, thermal stratification is already becoming stronger especially in northern aquatic ecosystems experiencing seasonality (Rühland et al., 2015). Stratification and browning create a positive feedback loop, making conditions more extreme. Thermal stratification is caused by differences in water density between the layers of water, making water mixing more difficult. Because of decreased light attenuation due to browning, much of the light is absorbed in the surface layer of the water (epilimnion), while the deeper layer (hypolimnion) stays darker and cooler. Colored water also absorbs more radiation, making the surface water warmer and causes warming earlier in the spring than in clear waters (Sarkkola et al., 2013).

Lakes in Nordic countries experiencing four seasons usually go through a mixing process in autumn when the weather cools, and in spring after snowmelt. Kellerman et al. (2015) suggested DOM as a reason for prolonged stratification periods in northern European lakes. This prolonging means a later onset autumn overturn (de Wit et al., 2016). Prolonged stratification limits nutrient cycling and oxygen distribution in the lake, causing hypoxia. Hypoxic and anoxic conditions limit the survival and growth of aquatic organisms. Anoxia also causes internal loading by DOC, P and Fe being released from the sediment (Knoll et al., 2018).

3. Eutrophication

The accumulation and enrichment of aquatic ecosystems by inorganic nutrients has become a global threat to biodiversity (Heikonen et al., 2023; Lyche Solheim et al., 2023). Eutrophication occurs when typical nutrient concentrations are exceeded, leading to excessive primary production. While this process does occur naturally, it is often driven by human activities such land use changes, especially agriculture and wastewater discharge. Most cases of eutrophication in aquatic ecosystems are directly linked to anthropogenic nutrient enrichment (Smith et al., 1999).

The primary nutrients responsible for eutrophication are N and P, which enter aquatic ecosystems through agricultural fertilizer runoff, soil erosion and sewage water (Devlin & Brodie, 2023). Once in the water, they stimulate excessive algal growth leading to oxygen depletion and biodiversity loss. In Europe, eutrophication has significantly impacted freshwater ecosystems, with 22% of lakes in the European Union and Norway experiencing degradation due to nutrient pollution. In Central Europe, 60% of streams have been affected, while in Northern Europe, over 20% of streams show signs of eutrophication (Lyche Solheim et al., 2023).

In Finland, land use changes, particularly agricultural expansion and peatland drainage, have played a key role in freshwater eutrophication. Heikonen et al. (2023) identified land use change as the main driver of eutrophication in Finnish lakes. Increased precipitation and storm events, predicted under future climate change scenarios, are expected to exacerbate nutrient runoff, further increasing eutrophication risk. Runoff from the catchment area makes nutrients accumulate in the water. Warmer water temperatures can intensify microbial activity and algal growth, amplifying the impacts of eutrophication.

Eutrophication stimulates the excessive growth of diatoms, cyanobacteria, and other photosynthetically active primary producers. This alters microbial communities and disrupts food web dynamics (Hall & Smol, 2010). One major consequence is the formation of cyanobacterial blooms, as N-fixing cyanobacteria thrive in P-rich environments. These blooms block light penetration, reducing photosynthesis in deeper water while their decomposition depletes oxygen, leading to hypoxic conditions. Additionally, cyanobacteria can produce harmful toxins, making eutrophication an ecological economic, and public health concern (Lyche Solheim et al., 2023).

Although anthropogenic nutrient inputs have declined in Finland, many aquatic ecosystems still suffer from internal nutrient loading. P stored in lake sediments can be released under low-oxygen conditions, prolonging eutrophication even after external nutrient inputs are reduced (Sondergaard et al., 2001). This internal cycling of nutrients means that management efforts must go beyond reducing surface runoff; oxygen depletion in sediments must also be addressed to prevent long-term eutrophication effects.

4. Environmental factors affecting diatoms

Diatoms are controlled by environmental and spatial factors (Heino et al., 2009; Soininen, 2007). These factors impact each other, influencing the biogeographical distribution patterns of diatoms. Both water chemistry and physical characteristics control diatoms growth and distribution. The most important environmental factors for diatoms are pH, conductivity, nutrients, light and temperature (Soininen, 2007). The relative importance of environmental factors, of course, depends on the aquatic ecosystem in question.

4.1 Physical factors

Physical factors, including depth, flow, sediment, and temperature, play a crucial role in structuring diatom communities. Flowing vs. stagnant waters create distinct habitats, influencing which diatom species can thrive. Streams and rivers often favor attached species adapted to flow, like *Gomphonema spp*, while lakes and ponds tend to support more planktonic and benthic communities (Soininen, 2007).

Water temperature is a key factor affecting diatom growth and metabolism. Many diatom species exhibit temperature optima, meaning shifts in water temperatures, whether seasonal or climate-induced, can alter species composition. Since temperature directly affects metabolic rates, species distributions change seasonally but also annually. (Heino et al., 2009). Warmer water temperatures tend to favor cyanobacteria over diatoms, as many cyanobacteria species have higher thermal optima and can outcompete diatoms under warming conditions (Rühland et al., 2015).

Sediment resuspension and turbulence can further impact diatoms by altering light conditions, thus possibly creating competitive shifts (Hall & Smol, 2010). Water depth and shape determine light penetration, influencing diatom growth and competition. Additionally, sediment interactions influence diatom distribution, particularly in benthic environments. Some diatom species prefer sandy or silty substrates, while others attach to rocks, macrophytes, or organic debris (Battarbee et al., 2010). Sediments can also trap nutrients, creating localized zones of high productivity or anoxia, which can further shape community dynamics.

4.2 Water pH

Climate change causes warming and increased precipitation, which further exacerbates acidification. Anthropogenic emissions also contribute to acid rainfall, causing leaching of heavy metals from soil. Water pH not only influences diatom metabolism but also affects nutrient availability, shaping competition between species adapted to different acidity levels. Acidification is an increasing problem caused by heightened levels of carbon dioxide in the atmosphere (Rantala et al., 2017). Aquatic ecosystems in the boreal zone of northern Europe are more susceptible because of their naturally higher pH caused by the low pH soils and humic acid content.

Aquatic alkalinity or acidity is one of the most influential factors controlling diatoms (Battarbee et al., 2010; Heino et al., 2009). When pH levels decrease and water acidifies, diatoms presently adapted to neutral and alkaline waters disappear, causing a decline in biodiversity (Battarbee et al., 2010). The prevailing pH conditions determine both diatom growth and distribution and even small changes can cause shifts in the community composition. Diatoms exhibit distinct preferences for pH conditions, and this sensitivity is great enough for diatoms to be used as bioindicators for alkaline or acid waters. Also, pH levels impact the bioavailability of nutrients and solubility of harmful substances, such as metals. Acid and alkaline waters often exhibit dominance of one or a few tolerant species. Low pH tolerant species include *Eunotia incisa* and *Tabellaria quadriseptata*, for example (Battarbee et al., 2010).

4.3 Conductivity

Conductivity, meaning the concentration of dissolved ions in water, is an important environmental factor in aquatic ecosystems. Conductivity levels are influenced by the amount of nutrients, meaning that eutrophic waters tend to have higher dissolved ion concentrations, making conductivity a useful indicator of nutrient enrichment and land use changes (Hall & Smol, 2010). Naturally, saline waters have higher conductivity than brackish or freshwater, but human activities such as eutrophication and pollution also contribute to increased conductivity. Similarly to pH, conductivity affects both nutrient bioavailability and diatom metabolism, with some species being more tolerant of high conductivity than others (Kahlert et al., 2021). Certain aerial diatoms, which are often found in nutrient-poor environments, are frequently associated with higher conductivity habitats (Johansen, 2010). These patterns highlight the close relationship between conductivity, nutrient availability, and diatom community shifts, making diatoms valuable indicators of the ecological state of aquatic ecosystems.

4.4 Lentic and lotic systems

Lentic systems, meaning lakes and ponds, are often more stable, and relatively isolated ecosystems (Soininen & Weckström, 2009). Lotic systems, meaning rivers and streams, are more unstable and connected, additionally changing with time. Although similarities exist, there are fundamental differences especially in their hydrology and morphology and therefore their diatom communities. Some diatom species prefer lentic over lotic systems, vice versa, due to these differences. Because of the slow water movement in lentic systems, the substrate is usually finer, while lotic systems are often rich in coarse materials such as larger stones, therefore providing different kinds of habitats. Lentic systems change waters slower than lotic systems, and nutrient enrichment builds up gradually. Stratification is also often present and impacts nutrient cycling. Primary production is usually higher in lentic systems than in lotic systems, where allochthonous material is highly important to food webs because of the unidirectional flow. Lentic systems often have a larger algae community than lotic, albeit less diverse. In lakes, their often more isolated nature also makes airborne dispersal significant, while streams are constantly connected to a more complicated network. The diatom communities in both lakes and streams are controlled water chemistry, such as pH and conductivity, and physical factors, such as flow rates (Soininen, 2007).

Lakes and ponds with varying light conditions and still water support diatoms that can control their buoyancy (Kahlert & Gottschalk, 2014). Sun et al. (2018) found that diatoms in fast flowing streams are generally smaller than in stiller water. This is due to the benefits of buoyancy in flowing water. Kahlert & Gottschalk (2014) also pointed out their often streamlined appearance and attachment structures, making them able to withstand high flow. When comparing samples from lentic and lotic samples, the differences between these systems are evident. Lake samples represent longer periods of time and growing periods while lotic system samples only capture a short moment of time in the system. It is important to take physical disturbances into account when considering lotic samples and comparing them to lentic samples.

5. Diatom dispersal

The ability of diatoms to disperse across aquatic ecosystems is fundamental to their biogeography and ecological success. Dispersal mechanisms influence species distributions, genetic diversity, and community assembly processes in lakes and streams. Diatoms were long hypothesized to be cosmopolitan because of their small size and high reproduction rate (Vyverman et al., 2007). Some diatom species exhibit cosmopolitan traits but according to recent findings, diatoms do exhibit biogeographical patterns (Soininen & Teittinen, 2019;

Telford et al., 2006) influenced by historical, ecological and environmental factors limiting their dispersal.

Diatom dispersal occurs primarily through passive mechanisms, as they lack long distance motility. Abiotic factors, such as water currents and wind, play a major role, while biotic vectors like animals and humans can also facilitate long-distance dispersal (Kristiansen, 1996). While wind and water transport diatoms passively, biological vectors also contribute to their movement, sometimes enabling long-distance dispersal across isolated water bodies. Different animals, for example water birds and insects, can facilitate passive diatom dispersal. Birds and aquatic insects facilitate diatom dispersal by transporting cells on their bodies, feathers, or via ingestion and excretion. Birds are also effective in long-distance dispersal of diatoms, for example *Nitzschia pusilla* (Manning et al., 2021).

Diatoms also disperse passively by being airborne with no specific agent like birds. Their small size and resting spore stage make dispersal by wind possible. Aerophilic diatoms are often resistant to changes in their environment, and therefore also climate change (Tornés et al., 2022). These diatoms can be found in soil, trees, moss and on rocks. Species like *Mayamaea atomus*, *Mayamaea permitis*, *Pinnularia borealis* and *Luticola mutica* have been found living in aerial habitats (Johansen, 2010). Dispersal is affected by a multitude of factors, both environmental and anthropogenic (Kristiansen, 1996). Temperature, light and nutrients impact colonization success in new environments. Human activity, for example irrigation, water transfer and boat traffic facilitate diatom dispersal in great lengths. While diatoms are highly dispersive, research suggests that environmental conditions and competition also shape community structure, limiting successful colonization.

6. Methods

This study was conducted at and around the Lammi biological station and Evo Hiking area in Southern Finland (Figure 1). Lammi is a former municipality with about 5000 inhabitants and has been part of Hämeenlinna since 2009. The village is around 611 km^2 and the surrounding land has been used for agriculture for hundreds of years and is very arable. Therefore, human activity in the catchment area has affected the surrounding lakes and streams for a long time (*Päiväretki Lammille*, 2024). In comparison, Evo Hiking area has been a conservation area since 1994, meaning that lakes and streams are undisturbed by human activity. However, the forest is managed by regular controlled burning (Metsähallitus, 2025). The area consists mainly of boreal forests, with both coniferous and deciduous areas. Evo Hiking area is also slightly elevated with typically nutrient-poor soils and small lakes. In conclusion, samples from the forested streams and lakes in Evo and arable land in Lammi represent very different conditions for diatoms.

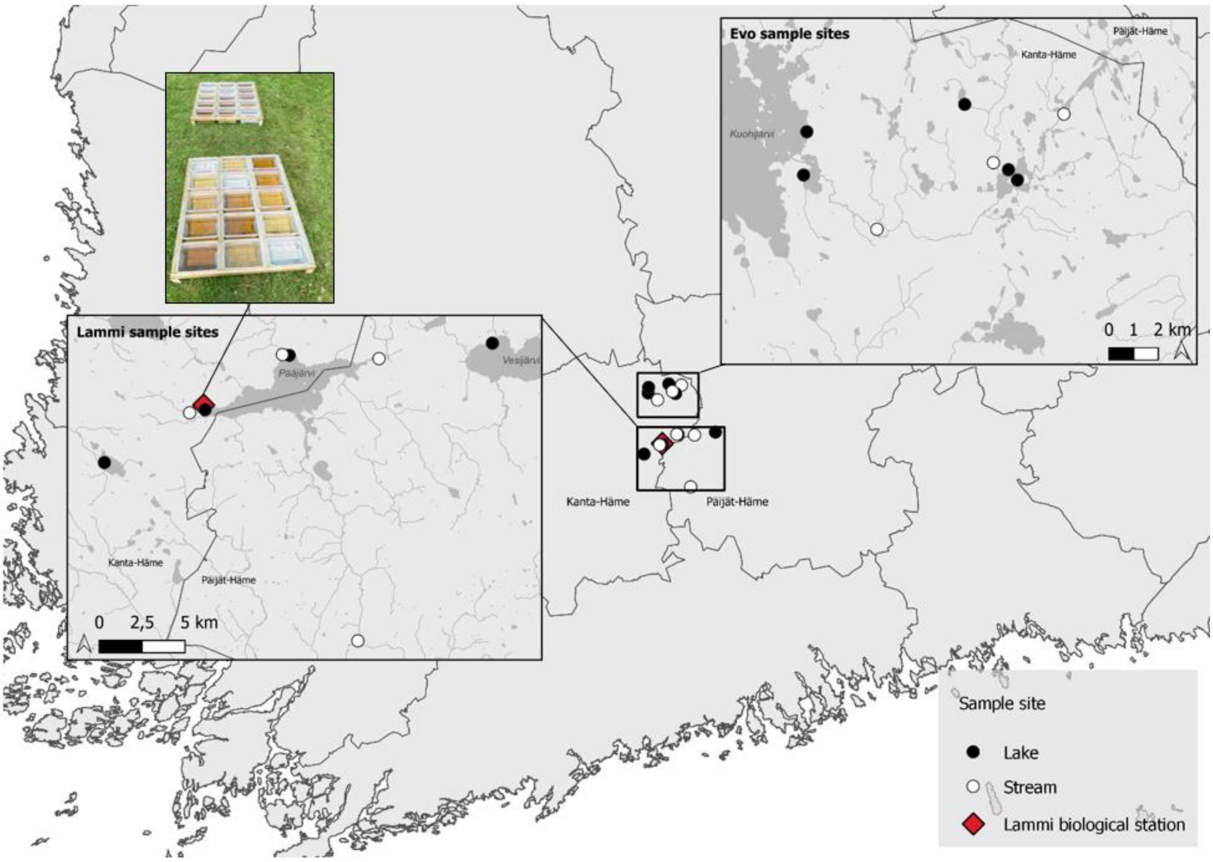


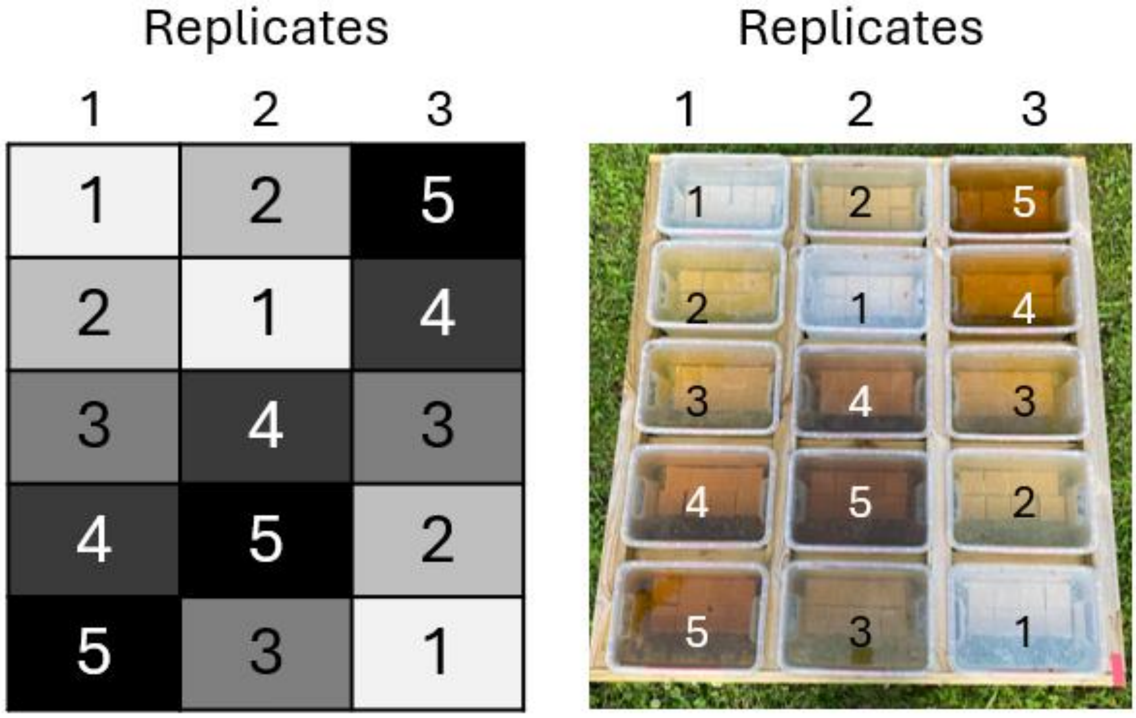
Figure 1: Map of natural sample sites, both lakes and streams, in Lammi and Evo (Kanta-Häme). The experimental setting site at Lammi biological station is pictured above Lammi sample sites. Picture: Emma Sinisalo

In this study the field data consists of diatom and water samples from lakes and streams around the biological station and from new experimental pools, similar to the experiment conducted in Teittinen et al. (2022). The diatom and water samples were collected from nine lakes and seven streams and 30 (15 x 2) experimental pools.

A flat plot was selected for the field experiment (Figure 1), which lasted from 26 June until 19 September 2023. The field experiment consisted of two parallel wooden grids, containing 15 plastic buckets each. The buckets were filled with 4 l of filtered water. The water was filtered through a 0.7-µm filter (Whatman Grade GF/F Filter 0,7 um, 1825-047) for the purpose of removing existing diatoms. Eight tiles (5 cm x 5 cm) were added to each bucket before filling them with water.

Five grades of colored water from nearby lakes were added into the buckets. The different colors of water were chosen to represent naturally occurring differences in water color in the study region. Each one had three replicates placed in the grid (Figure 2). The clearest water was collected from lake Syrjänalunen, the second clearest water was from lake Tavilammi, and the in-between color was from lake Rajajärvet. The darker watercolors were from lake Tiponen and lake Horkkajärvi. All the lakes in question are located in the Evo area (Figure 3).

The water was collected from each lake a bit of the shore to avoid picking up sand and other solid particles (Figure 3A). The water was collected into a bucket and filtered roughly through a net (Figure 3B) into a new clean bucket, to get rid of particles. After this, the water from each lake was poured into their own containers using a strainer (Figure 3 C) and marked accordingly. Before being added into the buckets, the water was once more filtered through a glass microfiber filter (0.7 µm pore size, 47 mm diameter, Whatman GF/F). Water color (absorbance), N and P were also measured from all water samples before adding it to buckets (Table 1)



Replicates 1	Replicates 2	Replicates 3
1	2	5
2	1	4
3	4	3
4	5	2
5	3	1

Figure 2: Experimental grid design next to the real-life grid. The different numbers represent the different colors of water in each bucket. Each bucket had all together three replicates made, placed in an organized order to account for

possible physical disturbances. Two of these grids were made, one containing added nutrients while the other was used as control (no nutrient additions). Picture: Emma Sinisalo.

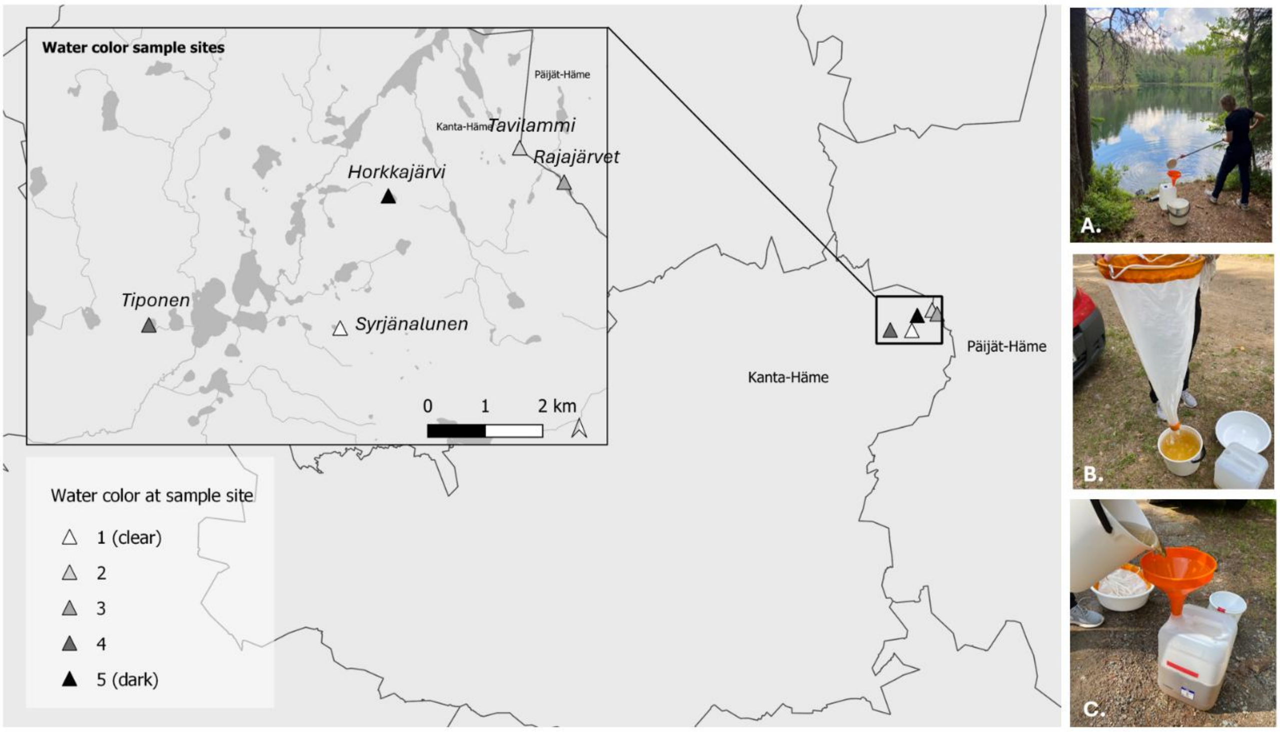
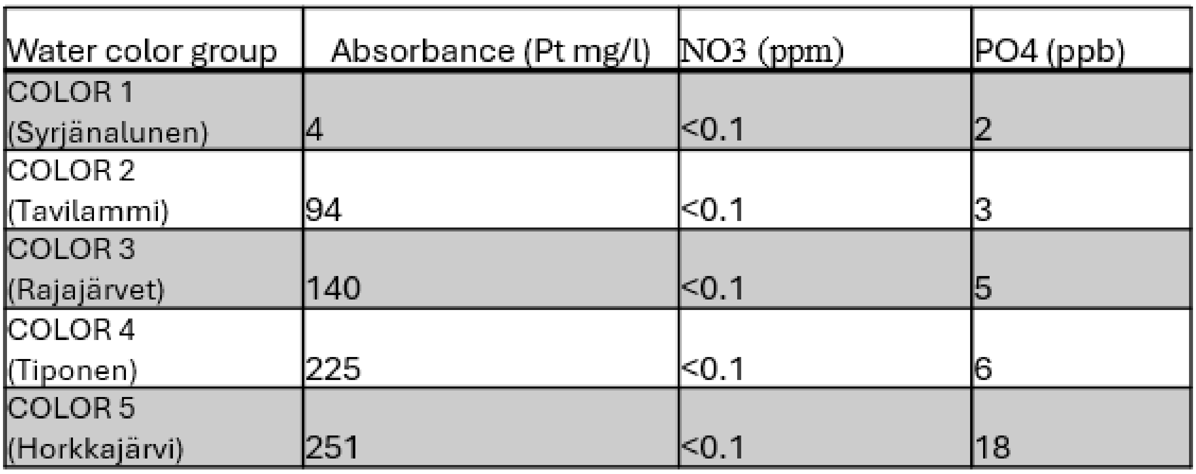


Figure 3: Experimental pool water sampling sites in Evo Hiking Area (Kanta-Häme), representing five different colors of water from clearest to darkest (1-5). After collecting the water (A.), the water was filtered once through a net (B.) and then stored in their respective containers (C.). Pictures: Emma Sinisalo.

Table 1: Water color (absorbance), NO3 (ppm) and PO4 (ppb) measurements of the water samples.



Water color group	Absorbance (Pt mg/l)	NO3 (ppm)	PO4 (ppb)
COLOR 1 (Syrjänalunen)	4	<0.1	2
COLOR 2 (Tavilammi)	94	<0.1	3
COLOR 3 (Rajajärvet)	140	<0.1	5
COLOR 4 (Tiponen)	225	<0.1	6
COLOR 5 (Horkkajärvi)	251	<0.1	18

The buckets were then placed into the wooden grids, at a flat, open, unshaded (near canopy to avoid extreme evaporation) spot at Lammi biological station (Figure 1, Figure 4). The two grids were placed close to each other to ensure similar environments. Into the buckets of the second grid, nutrients were added. To reach a level of 1600 ppb of N, 4,8 ml of NO3 was pipetted into each bucket in the second grid. Similarly, to reach 100 ppb P, 3 ml of PO4 was pipetted into each bucket in the second grid.

At the start of the experiment, tiles were empty and no initial diatom communities were inoculated. The grids were not covered by lids to allow airborne diatom dispersal. The whole grid was, however, covered by a net (grid size 1 cm x 1 cm) to guard against birds and other animals and larger debris. After this, the grids were left untouched to allow diatoms to freely colonize the buckets.

Figure 4: Setting up the experimental pools in each wooden grid. The buckets were filled with water colors 1-5. Pictures: Emma Sinisalo

6.1 Water color analysis

To ensure that the water color in the experimental pools were in the correct order, water color analysis was done. This was done by measuring absorbance according to EN-ISO 7887:2011. This technique measures the light absorption of dissolved matter in the water. About 20-30 ml of all pool water samples was first filtered through a 45µm syringe filter using a 10ml syringe (Figure 5B). This was done a few times to reach the desired amount of water. After this, the sample was put through a spectrophotometer at 410nm (Figure 5C, 5D), comparing the color level to the control samples (Figure 5A). The control samples were made specifically for this analysis, containing 30, 60 and 300 mg/L Pt (mg platinum per liter). These values were selected based on the range at which natural waterbodies usually land around.

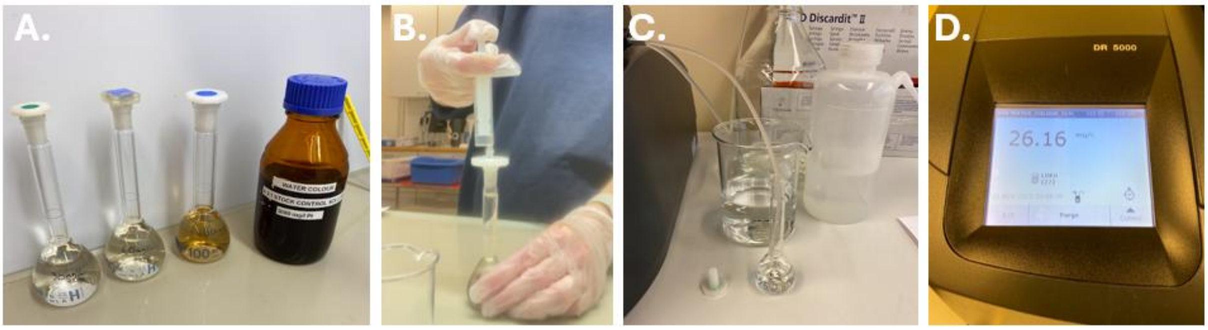


Figure 5: Water color analysis (EN ISO 7887:2011). First, control solutions (A.) were made using stock control solution containing 3000mg/l Pt. These controls were 30, 60 and 300 mg/l Pt. After this, a few syringes of each pool sample water was put through a 45 μm syringe filter (B.), after which they were tested with the spectrophotometer (C.) at 410nm. The spectrophotometer then displayed the measured absorbance level (water color) of each sample (D.). Pictures: Emma Sinisalo

6.2 Diatom sampling

The diatom and water samples from natural lakes and streams were collected at the end of June 2023 from the sites presented in figure 1. The diatom samples were collected by sampling randomly chosen suitable stones at the shore by scrubbing them with a brush, thus creating a composite sample from each site (Figure 6A). These samples were collected for the purpose of comparing the diatom communities in the field experiment to the natural communities in lakes and rivers. From each site in situ water measurements (YSI multiparameter tool) containing temperature, pH and conductivity (uS/cm) data were collected. Water samples were also collected and stored frozen.

Diatom samples were taken from each experimental pool on August 9. This was done by scraping two tiles from the bottom of each bucket with a small sponge (approximately 2 cm x 2 cm x 2 cm). The sponge was exchanged between sampling different pools (Figure 6B). For all diatom samples collected, both natural and experimental, a few drops of ethanol were added for preservation. The experimental pools were sampled using similar methods second time in September 2023, at the end of the experiment. Water temperature, conductivity and pH were also measured from each bucket with the YSI parameter tool. Water samples were also collected for nutrient analysis (NO3, PO4), which was done in Kumpula campus by HelLabs (Appendix 2). Nitrogen (NO3, NO3- ppm) values were not used in this study since they were undetectably low (<0.1) in most pools.

Figure 6: Diatom sampling in (A.) natural waterbodies versus (B.) experimental pools. In natural waterbodies (A.), 10 suitable rocks were collected and scrubbed with a toothbrush. For the experimental pools (B.), a soft sponge (~2cm x 2cm x 2cm) was used for each sample, to gently scrub the biofilm into the composite sample. Pictures: Emma Sinisalo

6.3 Diatom analysis

Diatom preparates for each experimental pool and natural site were made in the laboratory. Part of each diatom sample were pipetted into 15 ml test tubes and marked accordingly. Then organic material was removed from each sample by wet combustion using hydrogen peroxide (30% H_2O_2) (Figure 7A). After this, H_2O_2 residue was removed and the samples were cleaned by putting each sample through the centrifuge three times for 20 minutes (centrifuge speed 1250 rpm). Between each time, samples were washed by using distilled water. When this was done, a few drops of each sample were put onto glass slides, allowing them to dry before continuing (Figure 7B). The samples were then mounted covered with slips using Naphrax (Brunel Microscopes Ltd, Chippenham, UK), and inspected through x1000 magnification on a phase contrast microscope (Figure 7C).

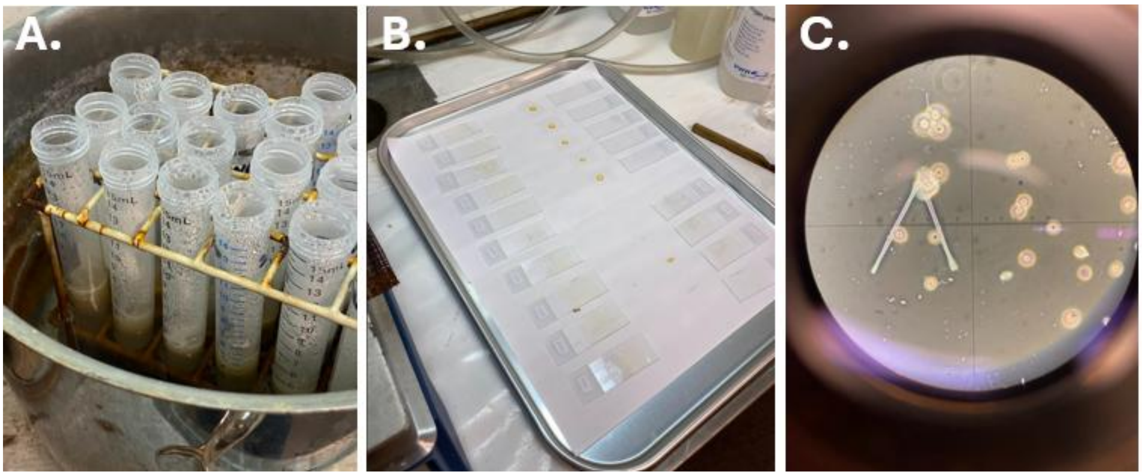


Figure 7: Making of the diatom preparates. (A.) The samples were first boiled with H_2O_2 added to allow for wet combustion. (B.) Using Naphrax, the preparates were mounted on glass slides marked with their respective names. (C.) Diatoms were then identified and counted with a microscope (1000x magnification). Pictures: Emma Sinisalo

In total 100 frustules were counted from each sample from the experimental pools, while 500 frustules were counted from the samples from lakes and streams. This difference in counted frustules stems from the low species richness and cell density in the pool samples. They were identified to the lowest possible taxonomic level according to Lange-Bertalot (Lange-Bertalot et al., 2017). In this study, only the first 100 cells counted are used for lakes and streams to allow for comparison with experimental pools (Appendix 1). In nine of the samples from the experimental pools, diatoms were very scarce, in which case duplicates were made and the count of both samples were summed up to get to 100. Six out of nine of these duplicates were made of samples from September.

6.4 Statistical analysis

To understand how browning and eutrophication respectively explained variation in diatom richness and composition and where the experimental pool diatoms originated from, statistical analysis was done using R Studio (R 4.4.2).

The analysis on water color and nutrient enrichment effects on community composition in experimental pools was done using NMDS (non-metric multidimensional scaling) and species count data (Appendix 1) and water quality data (Appendix 2). NMDS is suitable for species count data as it condenses the information into a multidimensional while not assuming linearity in relationships (Clarke, 1993). Distance matrix Bray-Curtis dissimilarity was chosen in this case, because of its suitability for count data. This was done using package vegan in R. Since the nutrient and water color effects changed with time, only August samples were used for diatom community analysis. The only time September diatom data was used, was when temporal changes were examined (7.3 Temporal changes in pools).

To examine how community composition responded to water color, two-dimensional NMDS plots were made using August control and nutrient enriched samples respectively. The control pool plot was further complemented with environmental factors, water color measured in absorbance level, and P (PO4) (function env.fit, package vegan). For examining how composition responded to nutrient additions, three-dimensional NMDS was chosen to minimize stress value. Another two-dimensional NMDS was made when examining the possible dispersal of diatoms. Also, Venn diagrams were used for visualizing similarities between diatom species composition in experimental pools in August and samples from lakes and streams.

ANOSIM (Analysis of similarities) was used to complement these other analyses and test for statistical significance. ANOSIM is a non-parametric test comparing dissimilarities between and within groups. The results explain if the differences are statistically greater than expected by chance. By rejecting the null hypothesis, the test suggests that there are differences between communities in different groups. Furthermore, ANOVA tests were conducted to determine if relationships between water color and treatment impacted species richness in a statistically significant way.

Species richness, diversity (Shannon-Wiener) and evenness (Pielou’s J) were chosen as complementary diversity indices to describe the structure of diatom communities. They were chosen because they capture different aspects of biodiversity; richness reflects the number of taxa present, Shannon-Wiener diversity combines richness with the relative abundances of species, and Pielou’s evenness standardizes diversity in relation to the maximum possible for a given richness. These indices have been found to be representative of the data even when cell count is low (Blanco et al., 2020; Nobre et al., 2025).

To further examine differences in diatom communities between different water color groups and enriched versus control pools, indicator species analysis was done using function *multipatt* from package indicspecies in R (De Cáceres et al.). This function finds species associated with specific groups, and when more than two groups are present it considers combinations of them too. The function returns all statistically significant species ($p<0.05$)

and lists out which groups they were associated with. Water color groups and nutrient enrichment were respectively used as explaining factor for changes in composition.

7. Results

7.1 Response of diatom communities to water color

The NMDS analysis showed statistically significant separation between the diatom communities of different water color groups in control pools in August. The different water color groups show some overlap (Figure 8), mainly the darker water groups 3–5. The clear water pools, groups 1–2, also overlap slightly. The clearest water pool (1) shows some variance within the group itself, highlighted by the size of the polygon, suggesting that the diatom species in the clearest water pools varied a lot compared to others. However, color group 4 representing the darkest pool also shows relatively high variance within the group. Although some of the groups vary, the ANOSIM R-value of 0.25 (p=0.03) shows that the groups are more dissimilar between each other than within themselves. Hence, color did play a role in the shaping of diatom communities. This is also evident by examining the environmental vectors explaining how color (absorbance, Pt mg/l) and P relate to diatom community structure (Figure 8). Color has a significant correlation to species composition, meaning that water color had a significant effect on communities.

When examining the diatom communities in water color groups in enriched pools, ANOSIM test results (R =0.15, p=0.09) show that there is no statistically significant difference between groups (Figure 9). Diatom communities show greater variability within groups than for control pools, meaning that diatom communities show great differences in enriched pools.

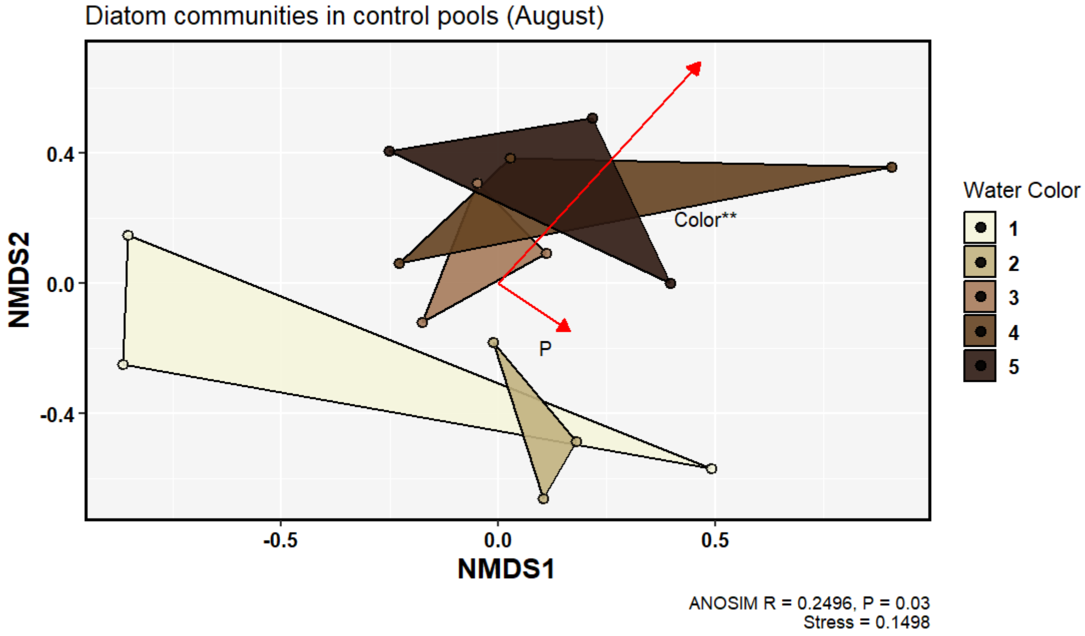


Figure 8: NMDS analysis showing differences in diatom communities based on water color group. Data is from the control pools (August). The different polygons represent variance within each of the color groups. ANOSIM R=0.25, p=0.03 shows that there are statistically significant differences between the diatom communities of different water color groups. Environmental vectors describe the effect of environmental variables color (absorbance Pt mg/l) and P (PO4) on diatom species composition. Color had a statistically significant strong correlation with composition (R^2=0.63, p=0.004), while P did not have a significant effect. The stress value for the NMDS is 0.15, meaning that the fit is decent.

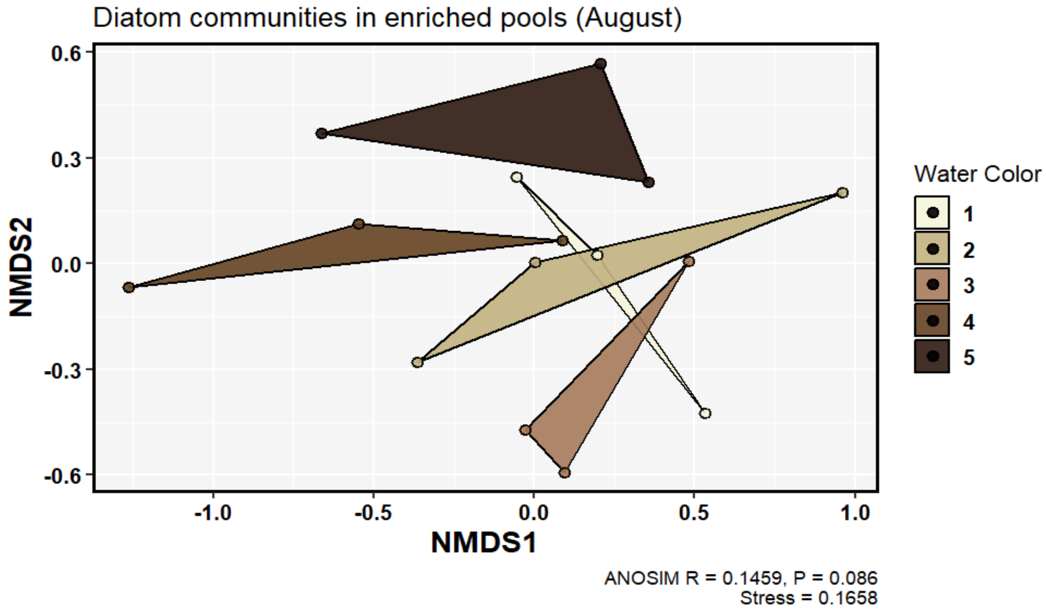


Figure 9: NMDS analysis showing differences in diatom communities based on water color group. Data is from the enriched pools (August). The different polygons represent variance within each of the color groups. ANOSIM R=0.15, p=0.09 shows that the difference between diatom communities is no longer significant when nutrient additions are considered. Stress value is 0.17, meaning that the fit is decent.

Species richness was impacted by water color group in a statistically significant way in the control pools (ANOVA: F= 4.51, p=0.05) (Figure 10). Water color group 4 had the most variance and also the maximum values of species richness. Group 3, in contrast, experienced the least amount of variance. Species diversity was impacted by water color group more than richness (ANOVA: F=14.28, p=0.002), with color group 1 experiencing the most variance and lowest species diversity. Groups 2 and 5 experienced very little variance within themselves. Species evenness was not impacted statistically significantly, but group 1 clearly experienced the lowest evenness out of all groups (Figure 10). The other water color groups experienced similar values of evenness, around 0.3-0.5.

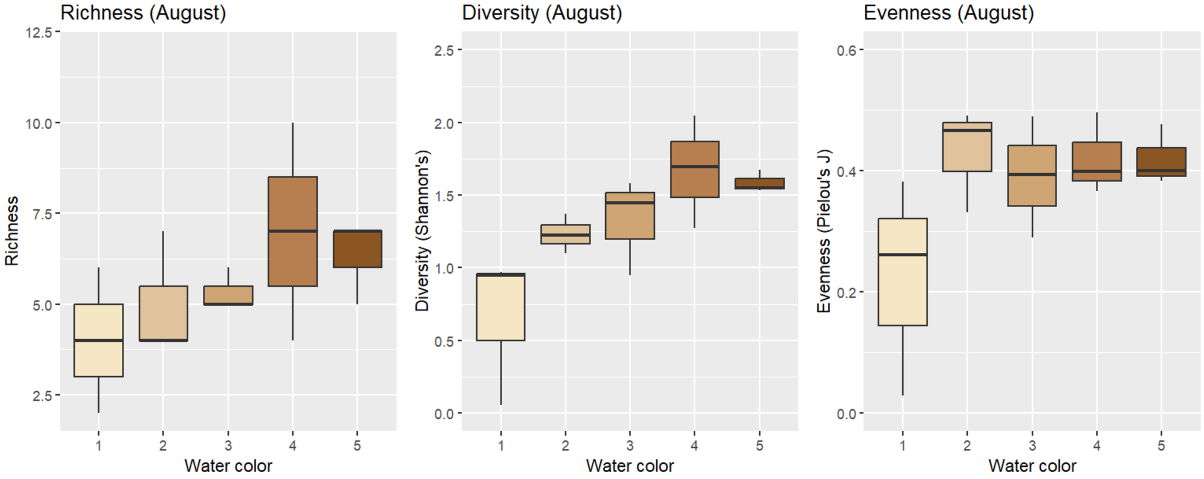


Figure 10: Species richness, diversity (Shannon-Wiener) and evenness (Pielou's J) in different water color groups. The colors represent the color of the pool. The data is from the august control pools. All were impacted by water color with varying statistical significance. Diversity was impacted the most by water color group.

In the enriched pools, no diversity measures were impacted statistically significantly (Figure 11). Water color had the strongest effect on diversity in enriched pools, in which diversity increased slightly in the darker colored pools (groups 3–5). Compared to the control pools, species diversity was higher for the clearest enriched water group (1) and medium colored pools (3). However, the control pools had slightly higher diversity values compared to the other enriched pools. Enriched pools were also higher in evenness values (0.4–0.5) than control pools, especially the clearest water color group.

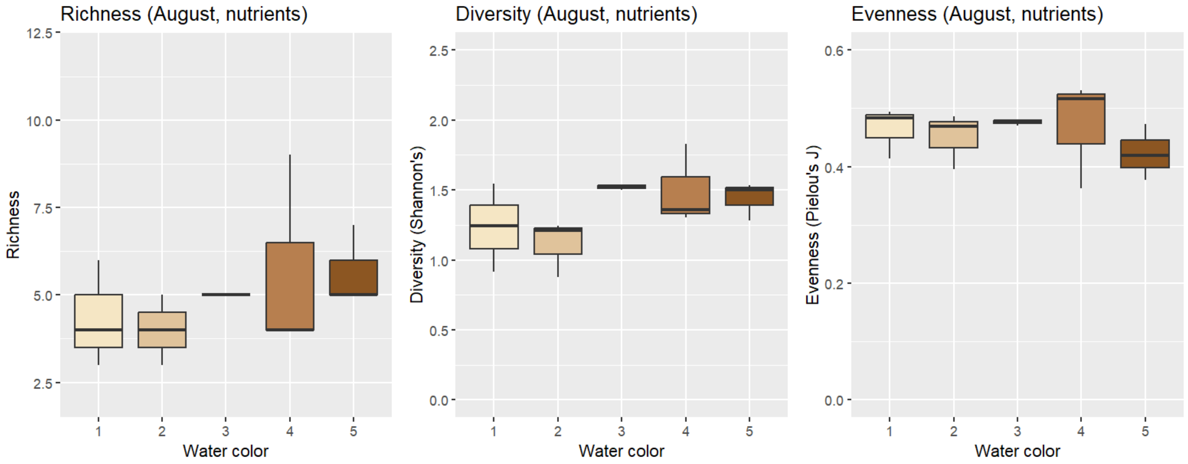


Figure 11: Species richness, diversity (Shannon-Wiener) and evenness (Pielou's J) in different water color groups in August. The colors represent the color of the pool. The data is from the august enriched pools. None were impacted by water color in a statistically significant way. However, species diversity was impacted the most by color in the enriched pools (ANOVA: F=3.65, p=0.08).

When inspecting the water color groups at species level, *Nitzschia brevissima* emerged as an indicator species for three water color groups, being 3, 4 and 5 (Indicator value: 0.81, p=0.01) in the control pools. This was the only statistically significant indicator species found in control pools. *N. brevissima* is rare in water color group 1 and 2 (Figure 12), while increasing for groups 3-5, with group 3 being the most abundant in this species. In the enriched pools, *Asterionella formosa* emerged as an indicator of water color group 3 (Ind.val.: 0.88, p=0.001). The distribution of *A. formosa* (Figure 13) is very clearly higher in the third color group, while being lower in the other color groups.

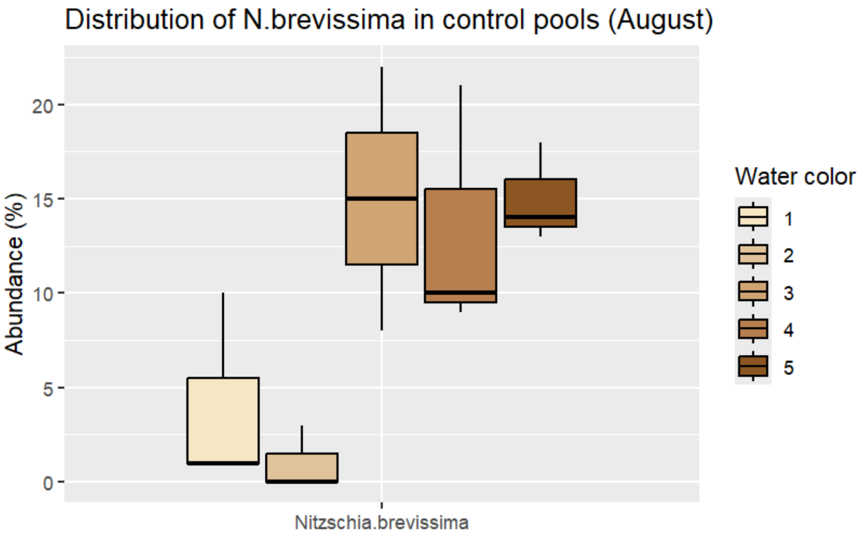


Figure 12: Indicator species analysis results showing the distribution of Nitzschia brevissima in all water color groups. N.brevissima had a statistically significant indicator value of 0.81 (p=0.01) for groups 3-5. This means that the species was a strong indicator for the darker water colors. August control pool data was used here.*

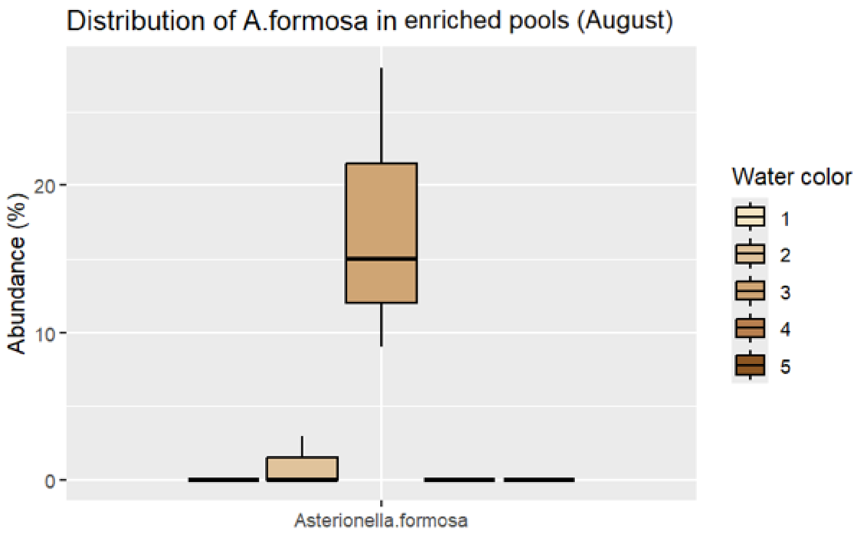


*Figure 13: Indicator species analysis results showing the distribution of Asterionella formosa in all water color groups. A.formosa had a statistically significant indicator value of 0.88 (p=0.001**) for group 3. This means that the species was a strong indicator of medium colored water (group 3). August enriched pool data was used here.*

7.2 Response of diatom communities to nutrients

When comparing the nutrient enriched and control pools, the ANOSIM test (r=0.03, p= 0.18) showed no statistically significant dissimilarities, suggesting no differences in community composition between enriched and control groups. The enriched and control pool diatom communities do overlap with each other, with the nutrient enriched communities showing greater variance within the group compared to communities in control pools (Figure 14). The lack of statistical significance means that nutrient enrichment did not impact diatom communities in this experiment.

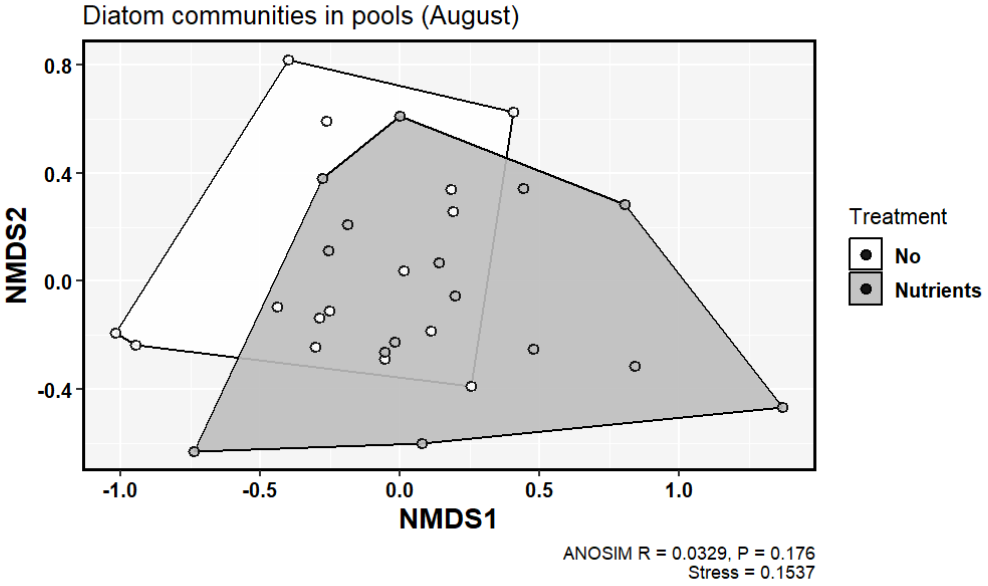


Figure 14: NMDS analysis showing differences in diatom communities based on treatment. Data is from all pools (August). The enriched pools were enriched with nutrients P and N. The polygons represent enriched (Treatment = "Nutrients") and control (Treatment = "No") diatom communities. ANOSIM R=0.03, p=0.18 shows that there are no statistically significant differences between the diatom communities of control and enriched pools. The stress value is 0.15, meaning that the fit is decent.

In August, both species richness (ANOVA: F=0.89, p=0.35) and species diversity (ANOVA: F=0.35, p=0.56) similarly to each other showed no statistically significant changes based on treatment (Figure 15). Species richness showed higher variance in control pools than enriched ones. In contrast, enriched pool diatom communities showed greater dissimilarities within than communities in control pools. Species evenness was also not statistically significant but held the most explanatory power out of them all (ANOVA: F=3.76, p=0.06), with enriched pools showing slightly higher values than control groups. In general, the control pools scored higher values than enriched pools at all diversity measurements excluding evenness. The differences were, however, minimal.

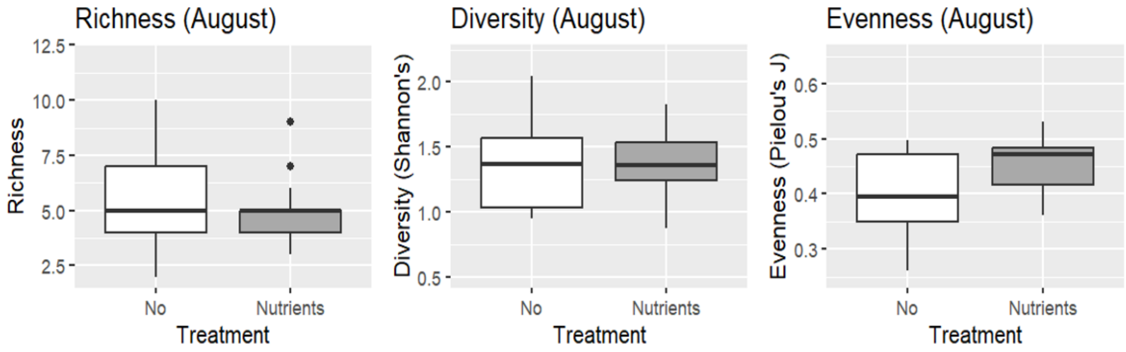


Figure 15: Species richness, diversity (Shannon-Wiener) and evenness (Pielou's J) in nutrient enriched (gray) and untreated control (white) pools. None were statistically significant, but species evenness had the strongest effect on communities, with enriched pools scoring slightly higher.

When examining which diatom species preferred enriched and control pools, *Pinnularia sinistra* emerged as an indicator of nutrient enriched pools. This species was the only indicator species found for both control and enriched pools. *P. sinistra* has a relatively low indicator value of 0.39 (p=0.04). *P. sinistra* is more abundant in enriched pools (Figure 16) but is also found in control pools in lower abundances. *P. sinistra* shows high variability in enriched pools, as can be seen by the size and placement of the boxplot in figure 16.

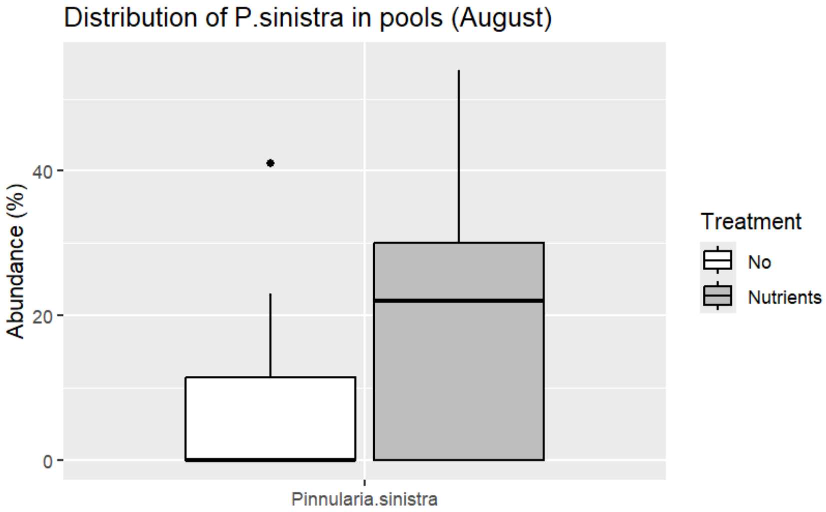


Figure 16: Indicator species analysis results showing the distribution of P. sinistra (0.39, p=0.04). P. sinistra is an indicator for nutrient enrichment in the pools.*

7.3 Temporal changes in pools

Over the course of the experiment, the pools experienced changes in water color. These changes led to diatom communities in the different water color groups becoming more similar to each other, and less similar within groups (Figure 17). ANOSIM showed similarities between and within diatom communities of different water color groups no longer were statistically significant in September with a low R-value of about 0.10 (p =0.20). Changes were also visible to the eye when comparing pool colors of different sample times (Figure 18), with the water getting cloudier and losing color with time. ANOVA tests showed that these changes in water color were explained by time, and highly significant with an F-value of 32.73 (p=0.001) (Figure 19). The darker colored pools lost more of their color than their clearer counterparts.

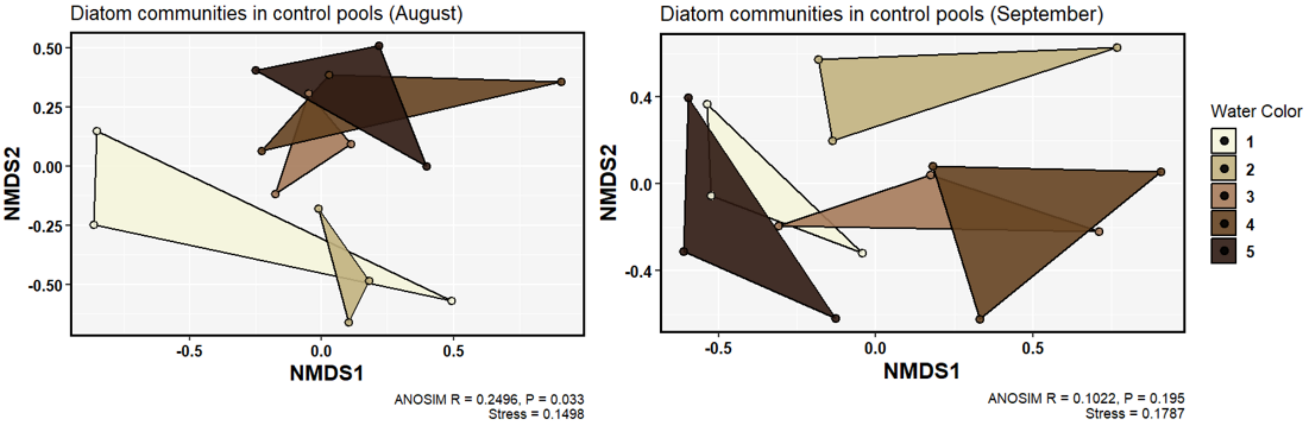


Figure 17: NMDS plots comparing the communities in August and September. The plots depict the same diatom communities sampled at different times. In September, the results are no longer statistically significant because of changes in diatom communities.

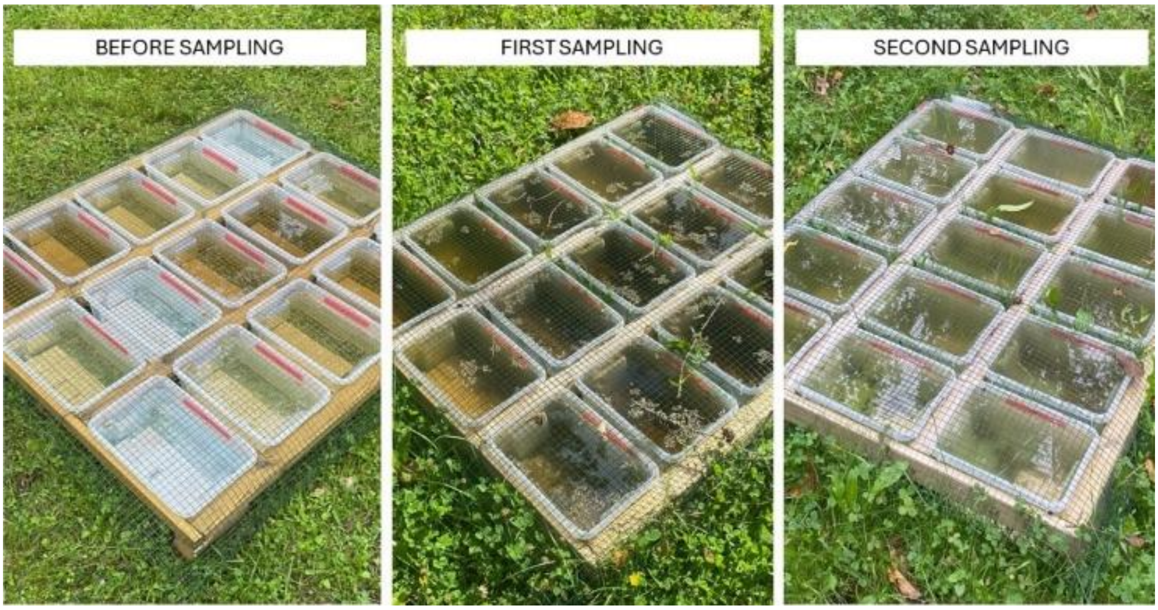


Figure 18: Visual changes in pool water color. The first picture is from when the experiment first was set up, the second from August, and the last one from September. At the first sampling, water color groups were somewhat distinguishable from each other. The pools were indistinguishable from each other by the last sampling. Picture is of the control (untreated) pools. Picture: Emma Sinisalo

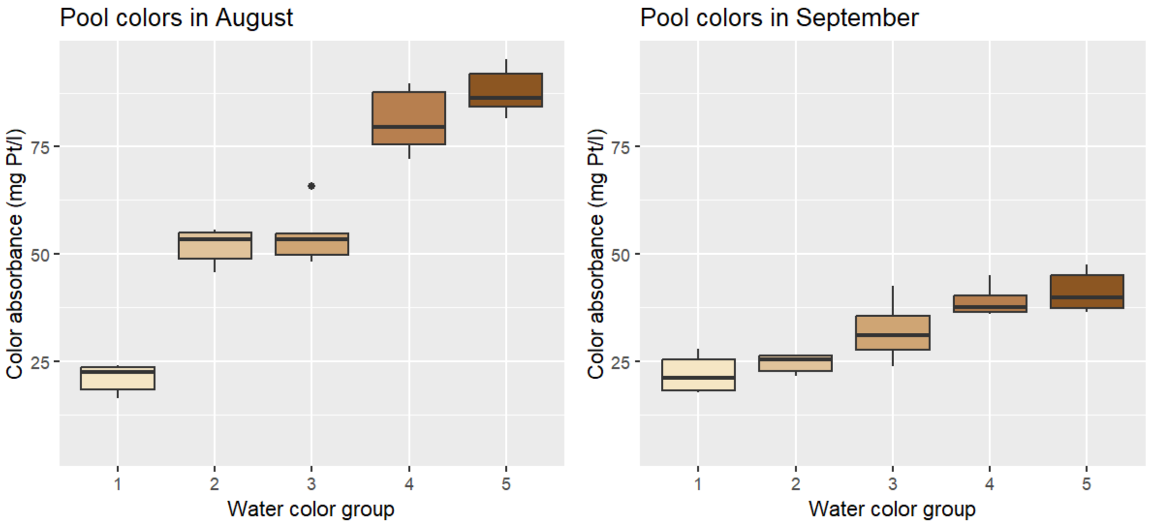


Figure 19: Changes in pool water color over time. Samples from August (left) and September (right) show clear differences in absorbance (mg Pt/l). All but one group (water color group 1) lost color. However, colors still stayed in the same order as before, with color group 5 being the darkest of them. Time had a statistically significant effect on water color (ANOVA: F=32.73, p=0.001). Data is from control pools in August and September.

The effect of nutrient enrichment on diatom communities was not apparent in August (ANOSIM: R=0.03, p= 0.18). The diatom communities were similar to each other in August, put showed differentiation between the two groups in September. However, the groups are still showing a lot of variation within groups (Figure 20). The diatom communities differed in the two groupings based on diatom growth limiting factor P in August (ANOVA: F=3.27, p=0.08) (Figure 21). In August, the amount of P was higher in the groups with added nutrients, which became statistically insignificant in September (ANOVA: F=0.11, p=0.75). Hence, September data was not used in nutrient treatment analysis. Similarly, conductivity also experienced decreases in treated and untreated pools with time (Figure 20). In August, conductivity was higher in enriched pools (ANOVA: F=7.85, p=0.001). These values decreased in September and became statistically insignificant (ANOVA: F=2.41, p= 0.13). The diatom communities in control pools showed less variation within the group compared to enriched pool communities in both August and September.

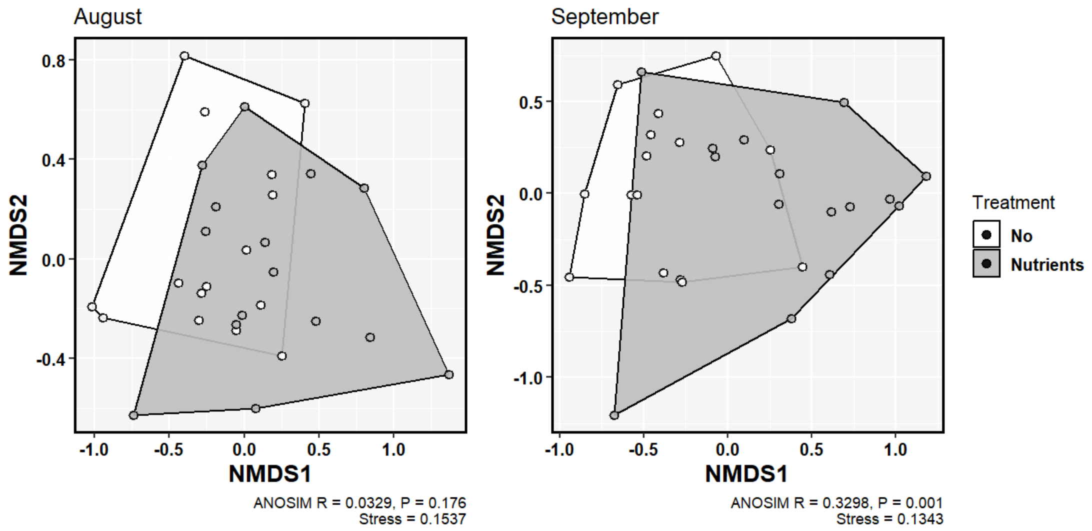


Figure 20: NMDS plots comparing diatom communities in August and September based on treatment. The plots depict the same diatom communities sampled at different times. In August, treatment did not have a statistically significant impact on diatom communities (ANOSIM p=0.18), but impact was significant in September (p=0.001).

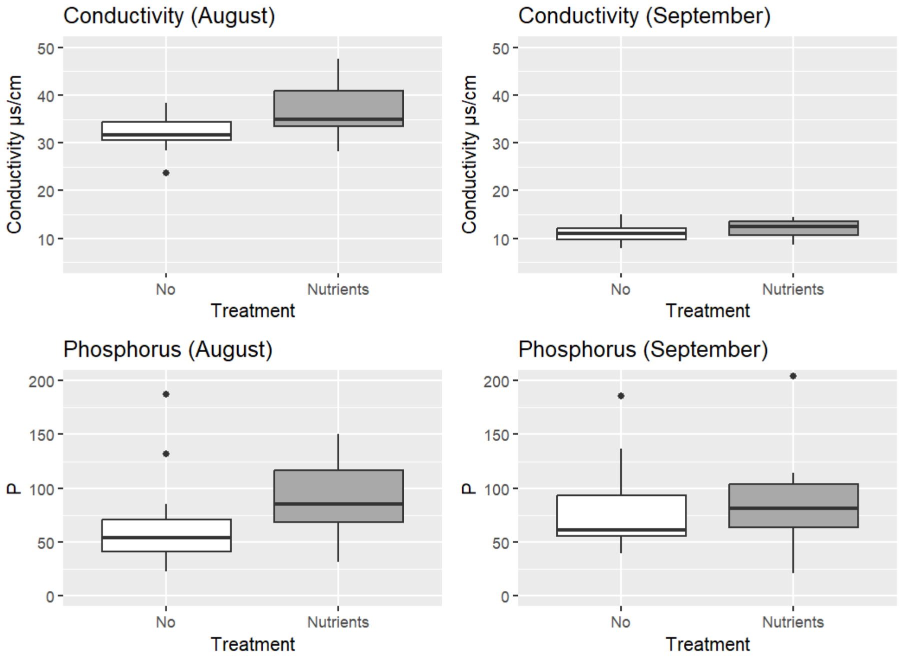


*Figure 21. P and Conductivity values changed with time (August to September). Both were statistically significant in August, but insignificant in September. Conductivity (ANOVA: F=7.854, p=0.01**) and P (ANOVA: F=3.269, p=0.0813) were both higher for the enriched group in August.*

7.4 Diatom dispersal into pools

Regardless of pool treatment and sampling time, the pool diatom communities differed substantially from the natural samples (Figure 22). Enriched and control groups were overlapping and both similar within and between groups. Enriched pools show greater dissimilarities between diatom communities within the group, represented by the size of the polygon in figure 22. Lakes and streams also overlap with each other. Lake and stream samples were collected in June (2023) and used as reference communities. While sampled earlier than the experimental pools, these natural communities are assumed to represent relatively stable baselines for comparison. Diatom communities between control pools and streams were the most dissimilar (Table 2), and diatom communities between enriched pools and streams were the least dissimilar. However, all values are over 0.90, meaning that although there is variation between pool samples, they are still, despite treatment, highly dissimilar from lakes and streams.

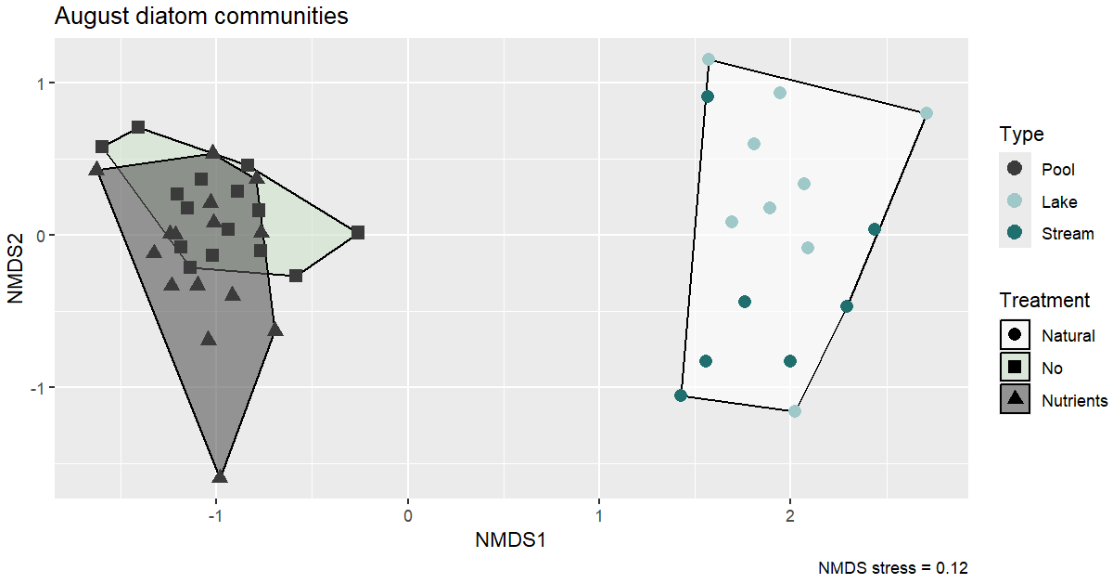


Figure 22: NMDS plots comparing the diatom communities in August to the natural (lake & stream) communities. Diatom community origin is described by different colors ("Type") and treatment by hulls and point shapes. Pool communities are similar to each other despite treatment. NMDS stress value is 0.12, indicating decent fit.

Table 2: ANOSIM values depicting dissimilarity between pools (control/enriched) and natural reference waterbodies (lake/stream). Value 1 indicates total dissimilarity between pool type and natural waterbody, while lower values indicate more similarity. All results are statistically significant (p=0.001).

Pool Type	**Reference**	**ANOSIM R**	**p-value**
Control	Lake	0.9994	0.001
Control	Stream	0.9896	0.001
Enriched	Lake	0.9543	0.001
Enriched	Stream	0.9448	0.001

Since diatom community composition did not reveal possible dispersal from lakes or streams, diatom communities were analyzed by inspecting indicator species. The same group comparisons were used as in the ANOSIM tests (Table 2). Several species emerged as indicators for lakes, streams and pools. In lake samples, *Tabellaria flocculosa*, *Achnanthidium minutissimum* and *Tabellaria quadriseptata* were consistently found as significant indicators (Table 3). For stream samples, *A. minutissimum* and *Gomphonema cymbelliclinum* emerged as indicators. For the experimental pools, *Mayamaea permitis* and *Mayamaea atomus* were consistently found as indicators. *N. brevissima* and *P. sinistra* were also indicators in many of the comparisons (Table 3). The indicator species found in pools were rarely found in lakes and streams vice versa (Figure 23). Out of all the indicators, *P. sinistra* was found in a few lake samples in low abundance.

*Table 3: Comparison of indicator species based on control pools and enriched pools from August and September. The table contains all statistically significant (p<0.05) species ('**' p=0.001, '***' p=0.000). Indicator values are stated after each species, in order of highest to lowest.*

Comparison	**Lake indicator species**	**Stream indicator species**	**Pool indicator species**
Control pools vs Lake	Tabellaria flocculosa (0.706)***, Achnanthidium minutissimum (0.626)***, Tabellaria quadriseptata (0.622)***, Cyclotella radiosa (0.536)***		Mayamaea permitis (0.772)***, Mayamaea atomus (0.691)***, Nitzschia brevissima (0.685)***
Control pools vs Stream		A. minutissimum (0.908)***, Gomphonema cymbelliclinum (0.675)***	M. permitis (0.772)***, M. atomus (0.693)**, N. brevissima (0.664)**
Enriched pools vs Lake	T. flocculosa (0.706)***, A. minutissimum (0.622)***, T. quadriseptata (0.622)***		M. permitis (0.798)***, M. atomus (0.696)**, P. sinistra (0.631)**
Enriched pools vs Stream		A. minutissimum (0.899)***, G. cymbelliclinum (0.675)***, Eunotia incisa (0.646)***	M. permitis (0.798)***, P. sinistra (0.651)**, M. atomus (0.609)**

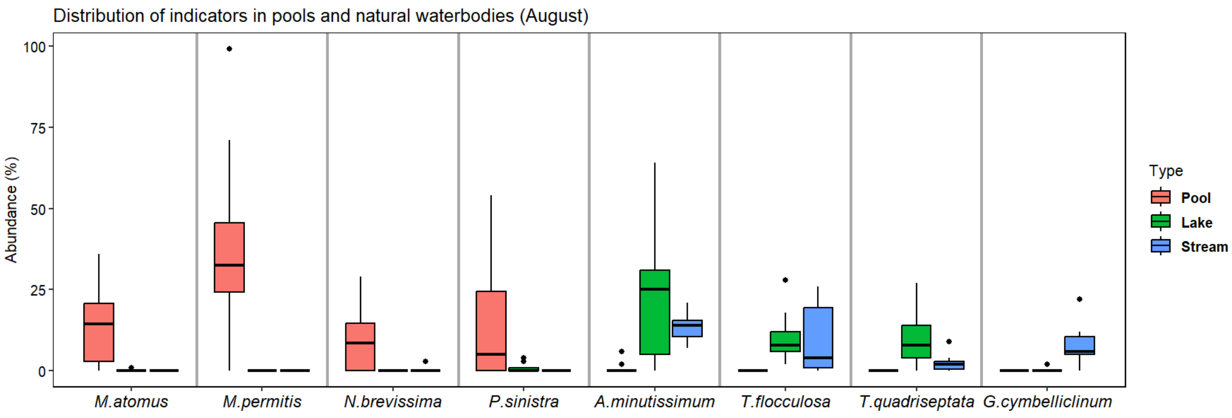


Figure 23: Indicator species abundances in different types of samples. All species are statistically significant indicators (Table 3). Mayamaea atomus, Mayamaea permitis, Nitzschia brevissima and Pinnularia sinistra are indicators for pools. Achnanthidium minutissimum is an indicator for both lake and stream samples. Tabellaria

flocculosa and Tabellaria quadriseptata are indicators for lakes. Gomphonema cymbelliclinum is an indicator for stream samples.

Since these analyses did not show how many species the groups shared with each other, Venn diagrams were made to visualize these similarities (Figure 24). The Venn diagrams illustrate which species are individual to which groups, and which are shared between groups. Lakes had the highest species richness. Species richness is low in the pools, making comparisons hard and possibly unreliable. However, there are statistically significant differences between the groupings based on previous ANOSIM results (Table 2). The most species shared by all the groups is found in the control pools, scoring eight shared species in total. Control pools share five species with just lakes and two with streams. The enriched pools had only five species shared between pools, lakes and streams. Enriched pools also shared three different species with both lakes and streams.

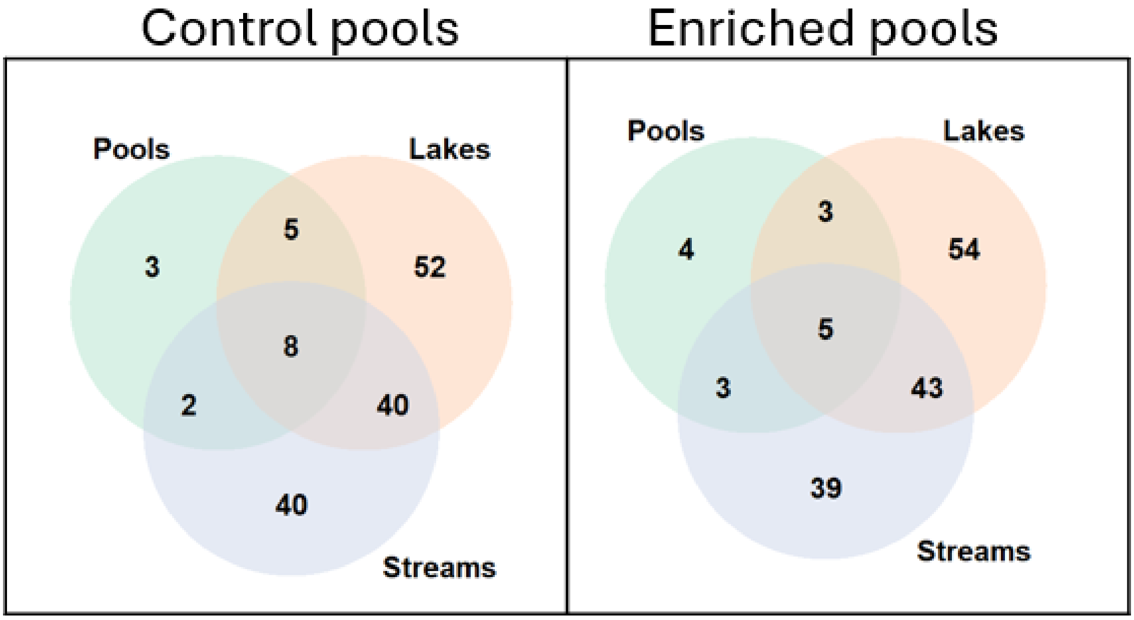


Figure 24: Venn diagrams of shared species in pools, lakes and streams. The numbers signify the number of diatom species shared by groups. Control pools are on the left side of the figure, enriched on the right. Diagrams show the big differences in species richness between groups, with lakes having 105 species and streams 90 species total. Pools had 18 (Control) and 15 (Treated) species. Control pools shared slightly more species with natural waterbodies.

8. Discussion

8.1 How do diatom species richness and community composition respond to browning and eutrophication?

Diatom community composition was dissimilar based on water color in a statistically significant way in the control pools. These pools untreated were expected to be impacted mainly by water color. The diatom communities showed clear differences, with medium and dark-color pools supporting communities with higher species richness, diversity and evenness. Diversity was the most affected by water color. The clearest water color supported the least amount of different species, and was also low in diversity and evenness, suggesting dominance of one or a few species. The darker colored pools showed the highest species richness and diversity values, suggesting that these conditions supported the most diverse diatom communities. This could be attributed to the light conditions providing habitat for diatom adapted to humic-water species, which are absent in the clearest pools. The unique conditions could therefore maintain a more diverse community. For the species that require a lot of light, water color could have acted as an environmental filter (Finkel et a., 2010). This supports the idea that biodiversity may decline in very clear water in response to very strong browning. In the control pools, *N. brevissima* emerged as an indicator of darker water colors. *N. brevissima* showed clear preferences to darker water colors than clearer, indicating possible preferences for humic waters.

In the nutrient enriched pools, water color did not impact diatom community composition in a statistically significant way. These pools were impacted by nutrients, and these combined effects made for less dissimilar diatom communities. Species richness was lower than in control pools, and slightly elevated in darker water colors. Meanwhile, diversity was relatively even in all pools, with the clearest water pools showing greater diversity values than for control pools. Evenness also increased for the clearest water groups, meaning that dominance of a few species decreased in enriched environments. Therefore, the community composition was more similar between enriched pools. This hints at nutrient additions dampening the effects of water color, possibly due to complex interactions impacting light availability. In the nutrient enriched pools, *A. formosa* emerged as an indicator of medium water color. *A. formosa* is almost only found in this water color group, indicating preferences for nutrient-rich water.

When examining dissimilarities in diatom community composition based on nutrient treatment, the communities showed no statistically significant differences between each other. Nutrient treated pools showed greater dissimilarities within the group than control pools, suggesting that enrichment did not affect community composition in a simple way. Species richness was higher in the control groups, indicating that the effects of nutrients were buffered by something else. This could be the browning effect coming through, limiting light penetration and therefore primary production while nutrients may increase pH of the humic waters by increasing production (Lyche Solheim et al., 2023). Nutrient additions, in theory, should increase species richness until a threshold above which richness starts to decline (Bergström & Karlsson, 2019; Isles et al., 2021). However, this did not seem to happen in the enriched pools. Diversity and evenness were similar in enriched and control pools, with enriched pools being slightly more even. *P. sinistra* emerged as an indicator of nutrient enriched pools. *P. sinistra* can also be found in control pools in lower abundances. This indicates a possible preference towards eutrophic water.

The nutrient-enriched pools might also have been impacted by the growth of other algae, including cyanobacteria and green algae. Cyanobacteria have been found to outcompete other algae, like diatoms, in P-rich humic water (Lyche Solheim et al., 2023).Their ability to fix nitrogen would have provided them with an advantage in the N-poor pools. Cyanobacteria would then have impacted light penetration by forming a layer at the surface of the pool, reducing the perceived effects of both water color and nutrient enrichment. This explanation would also account for the green hue present in the experimental pools at the September sampling (Figure 17).

With time, water color in the pools became more uniform, and the water chemistry changed. In September, the difference in P had decreased between nutrient-enriched and control pools. However, community composition showed statistically significant dissimilarities between the two groups. This is interesting considering the decreased effect of water color on community composition in September. The differences in water color groups decreased and got more uniform, while dissimilarities based on nutrient enrichment increased. This hints at a possibility of browning buffering eutrophication, since the effect was only visible after browning conditions stabilized. However, because of the drastic changes in water color and other physico-chemical factors, it is likely that multiple variables impacted these results.

8.2 From where did the diatoms most likely disperse into the experimental pools?

The diatom communities in the experimental pools did not resemble the communities found in nearby lakes or streams, although becoming slightly less dissimilar with time. The pools remained low in species richness throughout the whole experiment, making diatom analysis and conclusions indecisive. Some species could have dispersed by air or other vectors from nearby Lake Pääjärvi, a major lake located approximately 400 meters from the experiment site (Figure 1). It is reasonable to assume that some species could have originated from there. ANOSIM results suggested that the closest resemblance to natural waterbodies was in diatom communities in the nutrient-treated pools in September. This, however, seemed to be a function of time instead of environment, since all pools decreased in dissimilarity to natural waterbodies with time.

Most species found abundant in the pools were not abundant in any of the lake and streams samples. This suggests that dispersal could have happened from elsewhere than the natural waterbodies sampled. Some species likely came from the vegetation and soil at the experimental pool plot. Additionally, aerophilic diatoms, well-adapted to airborne dispersal may have played a role in colonization. Species abundant in some of the pools, such *as M. atomus*, *M. permitis* and *L. mutica* can be found in aerial habitats (Johansen, 2010). Some species of *Mayamaea* are also present in soils (Kezlya et al., 2020).These are small species with quick reproduction, making them common as colonizers (Foets et al., 2020). Wind and rain are known to transport diatom cells over considerable distances, and aerophilic diatoms are adapted to surviving in transient habitats (Kristiansen, 1996).

Given that the pools were relatively isolated, it is likely that dispersal limitations played a role in structuring the communities. Some species may have been unable to reach the pools, while others arrived but failed to establish themselves due to stressful and unpredictable conditions. This highlights the importance of both dispersal and environmental filtering in shaping diatom communities (Passy et al., 2017). Simply arriving in a new habitat is not enough, species must be able to survive and compete under the conditions.

8.3 Possible sources of error

While this study provides insight into diatom community responses to browning and nutrient enrichment, several limitations should be considered. One important factor is the relatively short duration of the experiment. Diatom communities shift over time, and a longer study period could have provided a more comprehensive understanding of successional changes and long-term adaptations. Seasonal variations were not accounted for, which may influence species composition in natural settings.

The experimental pools, while useful for testing specific environmental conditions, were still subject to natural variability. Rainfall events may have altered nutrient concentrations and introduced additional organic matter, possibly masking or amplifying treatment effects. Debris accumulation, fluctuating water levels, and wind-driven mixing could also have influenced diatom distribution within the pools. These factors make it difficult to fully isolate the effects of browning and nutrient addition. Future studies could minimize these uncertainties by using a more controlled pool setup.

The experimental setup does not fully replicate complex natural aquatic environments. In lakes and streams additional factors such as connectivity, sediment, grazing by invertebrates and competition play a role in structuring diatom communities. In flowing water, for example, organic matter does not accumulate in the same way as in stagnant pools, therefore possibly causing different diatom responses. While the pools provided valuable insight into isolated environmental factors, future studies should compare experimental results with in situ observations to better understand how these processes work in real ecosystems. The small buckets experienced high pH and temperature values and were possibly only little affected by shading by dark water due to shallowness of the pools, likely impacting the results of this study. Additionally, some uncertainty is always present in the measurement of environmental variables. While measurements were done carefully, natural fluctuations and analytical errors may have influenced the results. The relationship between browning, nutrients, and diatom responses is complex, and while statistical analyses helped identify patterns, further research on waterbodies in their natural state is needed to fully capture these interactions.

Finally, a possible source of potential error lies in species identification. Many diatom species are morphologically similar, making it harder to distinguish under light microscopy, especially considering the author’s limited experience. Methods such as DNA-analysis could improve taxonomic resolution in future studies, though challenges of course remain in the interpretation of the genetic data (Bailet et al., 2019). The low cell density of diatoms in the experimental pools also provided possible sources of errors, causing adjustments to lower frustule counts while microscoping, with only 100 frustules being counted.

Despite these limitations, this study successfully tested multiple environmental stressors in an experimental setting, contributing to the understanding of how diatom communities respond to browning and nutrient enrichment. The findings provide a basis for future research on long-term ecological shifts in freshwater systems.

8.4 Conclusions and future research

Increasing browning impacted diatom community composition and diversity (Figure 25). Clear waters supported less diverse communities compared to darker ones, suggesting that medium browning conditions support a multitude of species. The effects of eutrophication were weaker, mainly lowering species diversity, hinting at complex relationships between browning and nutrients. Diatom communities in experimental pools were distinct from nearby lakes and streams, suggesting colonization from other sources.

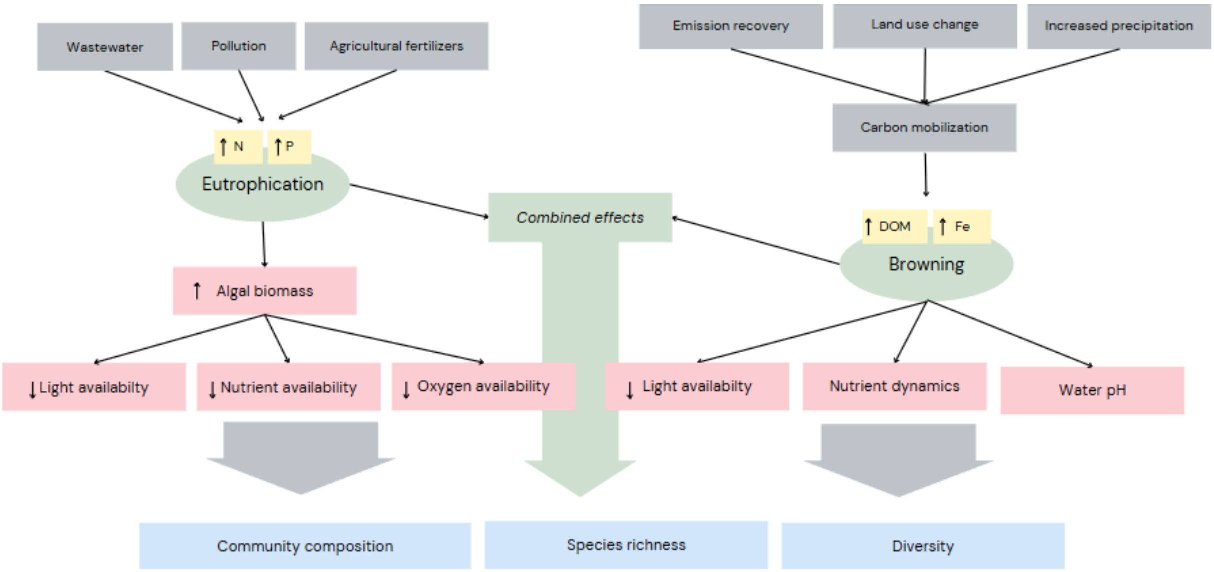


Figure 25: Flowchart depicting main findings coupled with the theoretical background of this study. Arrows show the direction of impacts. Drivers of each phenomnenon are stated above. Brownification decreases light availability, and causes changes in water pH and nutrient dynamics, which impacts community composition, species richness and

diversity. Eutrophication increases algal biomass causing decreases in light, nutrients and oxygen, which impacts diatom communities. The combined effects of both also likely impacted diatom communities.

The observed responses of diatom communities to browning and nutrient enrichment have broad implications for freshwater ecosystems, particularly in northern lakes where climate-driven increases in water color are expected to alter aquatic productivity and diversity (Vyverman et al., 2007). As this study found, the interactive effects of browning and eutrophication are not simple, and predicting community responses requires considering not only nutrient availability but also light limitation and the amount of organic matter. The dominance of a few highly adapted taxa raises concerns about more long-term shifts in species composition. These conditions also cause a monotone diatom community, with only a few tolerant species being able to persist. High-DOM environments also promote bacterial growth, outcompeting diatoms and other algae (Jyväsjärvi et al., 2022). When more diverse diatom assemblages are replaced by a small number of tolerant species, primary production and therefore ecosystem productivity could be impacted. Reduced light levels limit primary production, altering energy transfer between trophic levels impacting food webs.

Beyond responses in diatom communities, browning also influences nutrient cycling. Investigating these mechanisms across different aquatic ecosystems could improve predictions of the impact if browning on eutrophication. The role of iron should also be considered in this research suggestion. Future research could also examine how shifts in diatom communities affect higher trophic levels. Some studies focusing on higher trophic levels such as benthic invertebrates show a predicted decline because of browning (Brüsecke et al., 2023). Browning also alters productivity, supported by studies showing decreases in fatty acids and sterols (Brüsecke et al., 2023). This will affect food webs in unpredictable ways. Researching traits such as light absorption efficiency could help with understanding and predicting diatom community responses to climate change. Finally, the role of dispersal for structuring diatom communities could be explored through DNA-based tracking. This could clarify whether browning influences distribution by altering ecosystem connectivity, providing information on how diatoms will establish and adapt.

These findings also have implications for water management and restoration. Restorations of peatland and forests could decrease anthropogenic browning. Eutrophication mitigation often focuses on reducing nutrient inputs, while this study points out that high DOM levels might counteract the expected benefits of nutrient reductions. If browning intensifies due to terrestrial runoff and climate change, nutrient reduction alone may not be sufficient to restore aquatic ecosystems. Instead, a more integrated approach considering the interactions between DOM, light availability and nutrient cycling is needed. It is crucial to understand these interactions for freshwater management and to be able to find efficient ways of securing biodiversity and ecosystem health, especially considering the impact of climate change on shifts in DOM levels.

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Appendix

Appendix 1: Diatom count data (100 frustules) from each sample used in this study. Experimental pool samples are marked by their water color group (C:1-5), replicate number (R:1-3), possible nutrient enrichment (E), and sampling time (B for September samples)

Vesijärvi		**Alinen Rautjärvi**		**Ylinen Rautjärvi**		**Ekojärvi**		**Evojoki**		**Haarajoki**	
species	count	species	count	species	count	species	count	species	count	species	count
Achnanthidium minutissimum	36	Achnanthidium minutissimum	4	Achnanthidium minutissimum	5	Achnanthidium minutissimum	25	Achnanthidium minutissimum	16	Achnanthidium minutissimum	7
Brachysira neoexilis	2	Brachysira neoexilis	1	Cavinula pseudoscutiformis	1	Amphora eximia	1	Brachysira intermedia	3	Aulacoseira iltalica	2
Cavinula pseudoscutiformis	1	Cavinula pseudoscutiformis	1	Cyclotella radiosa	1	Cyclotella atomus	4	Cymbella cymbiformis	2	Cavinula jaernefeltii	2
Craticula bruderi	5	Cyclotella atomus	1	Cymbella affinis	1	Cymbella excisiformis	4	Cymbella helvetica	2	Cyclotella radiosa	1
Cyclotella radiosa	3	Cymbella affinis	1	Cymbella helvetica	1	Cymbella proxima	7	Cymbella minuta	1	Eunotia incisa	1
Cymbella silesiaca	3	Cymbella proxima	2	Diploneis finnica	1	Cymbella silesiaca	6	Diatoma tenuis	1	Eunotia minor	1
Cymbopleura amphicephala	4	Cymbopleura amphicephala	1	Discostella asterocostata	2	Diatoma problematica	8	Encyonema ventricosum	2	Eunotia mucophila	5
Eucocconeis laevis	2	Diploneis finnica	1	Eucocconeis laevis	1	Epithemia turgida	1	Encyonema vulgare	1	Eunotia pectilis	4
Fragilaria capucinana	5	Encyonema neogracile	3	Eunotia diadema	1	Fragilaria austriaca	1	Eunotia implicata	3	Fragilaria capucina	2
Fragilaria pectilis	7	Eunotia diadema	5	Eunotia incisa	1	Fragilaria tenera	1	Eunotia minor	6	Frustulia saxonica	1
Fragilaria radians	1	Eunotia incisa	2	Eunotia minor	1	Frustulia erifuga	2	Fragilaria capucina	3	Gomphonema corotum	1
Frustulia erifuga	1	Eunotia minor	2	Frustulia erifuga	2	Gomphonema acumitum	3	Fragilaria construens	1	Gomphonema cymbelliclinum	5
Gomphonema acumitum	2	Eunotia mucophila	5	Gomphonema corotum	1	Gomphonema brebissonii	1	Frustulia erifuga	1	Hantzschia amphioxys	2
Gomphonema exilissimum	4	Fragilaria tenera	3	Gomphonema cymbelliclinum	2	Gomphonema corotum	4	Gomphonema cymbelliclinum	22	Luticola mutica	26
Luticola mutica	1	Gomphonema acumitum	8	Gomphonema parvulum	4	Gomphonema parvulum	4	Gomphonema exilissimum	12	Meridion circulare	2
Navicula rhynchocephala	1	Gomphonema corotum	3	Mayamaea fossalis	1	Gomphonema truncatum	1	Nitzschia dissipata	1	Navicula rhynchocephala	10
Nitzschia paleaformis	1	Gomphonema parvulum	1	Navicula cryptocephala	3	Melosira varians	3	Nitzschia microcephala	1	Nitzschia dissipata	4
Nitzschia recta	2	Navicula cryptocephala	4	Nitzschia paleaformis	2	Navicula cryptocephala	2	Nitzschia paleaformis	1	Nitzschia microcephala	3
Placoneis elginensis	2	Nitzschia paleaformis	4	Nitzschia recta	1	Navicula gregaria	1	Sellaphora stroemii	2	Nitzschia paleaformis	2
Planothidium rostratoholarcticum	1	Pinnularia lundii	3	Pinnularia acidoclitum	1	Navicula striolata	2	Tabellaria flocculosa	15	Pinnularia gibba	1
Rhoicosphenia abbreviata	1	Pinnularia sinistra	3	Pinnularia elginensis	2	Tabellaria flocculosa	8	Tabellaria quadriseptata	4	Pinnularia saprophilia	1
Sellaphora bacillum	2	Placoneis elginensis	7	Pinnularia lundii	2	Tabellaria quadriseptata	11			Sellaphora schadei	2
Sellaphora laevissima	1	Tabellaria flocculosa	18	Pinnularia rossii	4					Stauroneis kriegeri	1
Staurosira construens	1	Tabellaria quadriseptata	14	Pinnularia subcapitata	4					Stauroneis smithii	2
Staurosirella martyi	1	Tetracyclus glans	1	Pinnularia subrupestris	1					Staurosirella leptostauron var. dubia	1
Tabellaria flocculosa	6	Trybionella angustata	1	Pinnularia undula	1					Staurosirella venter	1
Tabellaria quadriseptata	4	Ulnaria ulna	1	Sellaphora laevissima	2					Tabellaria flocculosa	4
				Tabellaria fenestrata	9					Ulnaria ulna	6
				Tabellaria flocculosa	28						
				Tabellaria quadriseptata	14						
sp. total:											
	27		27		30		22		21		28

Iso Ruuhijärvi		**Koiransuolenoja**		**Kuohijärvi**		**Luutajoki**		**Majajoki**		**Mustajoki**	
species	count	species	count	species	count	species	count	species	count	species	count
Caloneis silicula	4	Achnanthidium minutissimum	14	Achnanthidium minutissimum	8	Achnanthidium minutissimum	21	Achnanthidium minutissimum	10	Achnanthidium minutissimum	15
Cyclotella radiosa	8	Diatoma moniliformis	4	Asterionella formosa	3	Eunotia incisa	3	Asterionella formosa	1	Achnanthidium saprophilum	1
Cymbella helvetica	12	Eunotia incisa	1	Brachysira lilia	2	Eunotia meisterioides	1	Brachysira calcicola	5	Eunotia curtagrunowii	1
Cymbella minuta	1	Eunotia minor	1	Brachysira neoexilis	9	Fragilaria capucina	1	Cyclotella pseudostelligera	9	Eunotia incisa	5
Eunotia implicata	1	Eunotia praerupta	1	Caloneis silicula	2	Fragilaria construens	1	Cyclotella radiosa	2	Eunotia minor	1
Eunotia minor	1	Fragilaria capucina	3	Craticula bruderi	6	Fragilaria gracilis	1	Cymbella helvetica	2	Eunotia mucophila	1
Frustulia erifuga	5	Fragilaria henryi	2	Cyclotella radiosa	5	Fragilaria tenera	2	Eunotia incisa	2	Fragilaria pararumpens	1
Luticola mutica	1	Fragilaria tenera	1	Cymbella dorsenotata	10	Frustulia erifuga	5	Eunotia minor	2	Fragilaria radians	5
Navicula cryptocephala	5	Gomphonema cymbelliclinum	12	Cymbella helvetica	1	Gomphonema capitatum	3	Frustulia erifuga	1	Gomphonema acumitum	1
Nitzschia paleaformis	1	Hantzschia amphioxys	1	Cymbella proxima	3	Gomphonema exilissimum	4	Frustulia saxonica	3	Gomphonema cymbelliclinum	6
Pinnularia sinistra	4	Luticola mutica	16	Encyonopsis microcephala	5	Pinnularia rhenohassiaca	3	Gomphonema cymbelliclinum	9	Gomphonema parvulum	3
Pinnularia subcapitata	3	Meridion circulare	13	Fragilaria biceps	1	Psammothidium bioretii	4	Luticola mutica	3	Hannaea arcus	1
Pinnularia viridis	6	Navicula rhynchocephala	12	Fragilaria capucina	3	Psammothidium subatomoides	2	Melosira Carians	9	Meridion circulare	1
Rhoicosphenia abbreviata	1	Nitzschia dissipata	4	Fragilaria construens	5	Sellaphora bacillum	12	Meridion circulare	2	Navicula cryptocephala	4
Staurosira construens	2	Nitzschia paleaformis	1	Fragilaria gracilis	2	Tabellaria flocculosa	26	Navicula rhynchocephala	12	Navicula gregaria	6
Tabellaria fenestrata	6	Nitzschia recta	1	Gomphonema truncatum	1	Tabellaria quadriseptata	9	Sellaphora pseudoCentralis	1	Navicula quadriseptata	6
Tabellaria flocculosa	12	Odontidium anceps	1	Navicula rhynchocephala	3	Ulnaria ulna	2	Sellaphora stroemii	1	Navicula trivialis	3
Tabellaria quadriseptata	27	Pinnularia borealis	1	Nitzschia dissipata	5			Tabellaria flocculosa	24	Nitzschia brevissima	3
		Pinnularia gibba	1	Pinnularia gibba	4			Tabellaria quadriseptata	2	Nitzschia paleaformis	15
		Planothidium rostratoholarcticum	1	Planothidium rostratoholarcticum	6					Nitzschia recta	2
		Staurosira anceps	1	Sellaphora bacillum	1					Pinnularia braunii	2
		Surirella angusta	2	Surirella amphioxys	2					Pinnularia grunowii	1
		Surirella brebissonii	5	Tabellaria flocculosa	11					Staurosirella leptostauron var. dubia	1
		Ulnaria ulna	1	Trybionella angustata	2					Surirella angusta	4
										Surirella brebissonii	5
										Tabellaria flocculosa	1
										Tabellaria quadriseptata	2
										Ulnaria ulna	3
sp. total											
	18		24		24		17		19		28

Ormajärvi		Pääjärvi		Pääjärvi Uimaranta		Teuronjoki	
species	count	species	count	species	count	species	count
Achnanthidium minutissimum	27	Achnanthidium minutissimum	31	Achnanthidium minutissimum	64	Achnanthidium minutissimum	11
Amphora eximia	2	Brachysira neoexilis	7	Anomoeneis sellaphora	2	Amphipleura pellucida	1
Cyclotella atomus	1	Cyclotella radiosa	11	Anomoeneis spaerophora	3	Brachysira lilia	4
Cymbella minuta	14	Cymbella affinis	3	Caloneis alpestris	1	Brachysira neoexilis	5
Cymbella silesiaca	8	Cymbella minuta	2	Cyclotella radiosa	2	Cocconeis lineata	9
Encyonema lacustre	1	Diatoma tenuis	1	Cymbella minuta	2	Cyclotella radiosa	9
Fragilaria capucina	2	Fragilaria capucina	2	Diatoma problematica	2	Cymbella affinis	1
Fragilaria perminuta	4	Fragilaria perminuta	1	Eucocconeis laevis	1	Cymbella minuta	10
Hannaea arcus	1	Gomphonema angustatum	2	Fragilaria austriaca	2	Diploneis finnica	4
Luticola mutica	3	Gomphonema exilissimum	4	Fragilaria radians	3	Eunotia implicata	2
Navicula gregaria	2	Gomphonema parvulum	3	Gomphonema elegantissimum	2	Eunotia incisa	1
Nitzschia paleaformis	5	Hippodonta capitata	2	Gomphonema parvulum	1	Fragilaria austriaca	2
Placoneis gastrum	7	Hippodonta linearis	3	Luticola mutica	2	Frustulia saxonica	1
Planothidium rostratoholarcticum	3	Navicula gregaria	4	Mayamaea atomus	1	Gomphonema cymbelliclinum	5
Staurosirella venter	2	Navicula vulpi	1	Navicula vulpi	1	Navicula rhynchocephala	7
Tabellaria flocculosa	5	Nitzschia brebissonii	3	Nitzschia paleaformis	1	Nitzschia dissipata	3
Tabellaria quadriseptata	8	Nitzschia microcephala	1	Psammothidium bioretii	2	Nitzschia microcephala	1
Ulnaria grunowii	5	Nitzschia paleaformis	1	Psammothidium ventrale	1	Nitzschia paleaformis	2
		Odontidium anceps	1	Tabellaria flocculosa	6	Nitzschia recta	2
		Pinnularia bioretii	2	Ulnaria ulna	1	Paraplaconeis minor	2
		Pinnularia sinistra	1			Pinnularia borealis	1
		Planothidium minutissimum	2			Pinnularia rhenohassiaca	4
		Stauroneis anceps	3			Pinnularia saprophilia	2
		Tabellaria angustata	1			Rhoicosphenia abbreviata	1
		Tabellaria flocculosa	2			Sellaphora schadei	3
		Tabellaria quadriseptata	4			Staurosirella martyi	1
		Ulnaria ulna	2			Surirella brebissonii	2
						Surirella helvetica	1
						Tabellaria flocculosa	1
						Tabellaria quadriseptata	1
						Trybionella angustata	1
sp. total							
	18		27		20		31

C1R1		C1R1B		C1R1E		C1R1EB		C1R2		C1R2B	
species	count	species	count	species	count	species	count	species	count	species	count
Fistulifera saprofilia	25	Mayamaea atomus	46	Luticola goeppertia	12	Achnanthidium minutissimum	2	Eunotia incisa	3	Luticola mutica	41
Mayamaea permitis	63	Mayamaea permitis	41	Mayamaea atomus	20	Hantzschia amphioxys	1	Hantzschia amphioxys	10	Mayamaea atomus	22
Nitzschia brevissima	10	Nitzschia brevissima	11	Mayamaea permitis	49	Luticola mutica	33	Luticola mutica	13	Mayamaea permitis	18
Planotidium minutissimum	2	Pinnularia sinistra	2	Pinnularia sinistra	19	Mayamaea atomus	12	Mayamaea permitis	71	Nitzschia paleaformis	5
						Mayamaea permitis	14	Nitzschia brevissima	1	Nitzschia recta	14
						Nitzschia brevissima	1	Tabellaria fenestrata	2		
						Nitzschia paleaformis	1				
						Pinnularia subcapitata	31				
						Tabellaria fenestrata	5				
sp. total											
	4		4		4		9		6		5

C1R2E		C1R2EB		C1R3		C1R3B		C1R3E		C1R3EB	
species	count	species	count	species	count	species	count	species	count	species	count
Mayamaea atomus	26	Asterionella formosa	1	Mayamaea permitis	99	Eunotia minor	11	Achnanthidium minutissimum	6	Achnanthidium minutissimum	17
Mayamaea permitis	61	Craticula minuscoloides	63	Nitzschia brevissima	1	Mayamaea atomus	12	Luticola mutica	5	Luticola mutica	21
Nitzschia recta	13	Luticola mutica	10			Mayamaea permitis	63	Mayamaea atomus	15	Mayamaea atomus	10
		Mayamaea atomus	14			Nitzschia minuta	11	Mayamaea permitis	41	Mayamaea permitis	11
		Mayamaea permitis	11			Pinnularia subcapitata	3	Nitzschia brevissima	11	Nitzschia brevissima	2
		Pinnularia subcapitata	1					Pinnularia sinistra	22	Pinnularia borealis	1
										Pinnularia subcapitata	38
sp. total											
	3		6		2		5		6		7

C2R1		C2R1B		C2R1E		C2R1EB		C2R2		C2R2B	
species	count	species	count	species	count	species	count	species	count	species	count
Asterionella formosa	12	Luticola mutica	3	Mayamaea permitis	25	Eunotia incisa	3	Luticola mutica	21	Mayamaea atomus	15
Mayamaea atomus	16	Mayamaea atomus	41	Navicula menisculus	64	Hantzschia amphioxys	2	Mayamaea atomus	34	Mayamaea permitis	23
Mayamaea permitis	55	Mayamaea permitis	32	Nitzschia brevissima	11	Luticola mutica	12	Mayamaea permitis	44	Pinnularia borealis	12
Nitzschia brevissima	3	Nitzschia paleaformis	18			Mayamaea atomus	17	Ulnaria ulna	1	Pinnularia sinistra	33
Pinnularia sinistra	2	Pinnularia subcapitata	6			Mayamaea permitis	23			Pinnularia subcapitat	17
Tabellaria fenestrata	2					Nitzschia brevissima	14				
Ulnaria ulna	10					Pinnularia subcapitata	29				
sp. total											
	7		5		3		7		4		5

C2R2E		C2R2EB		C2R3		C2R3B		C2R3E		C2R3EB	
species	count	species	count	species	count	species	count	species	count	species	count
Asterionella formosa	3	Mayamaea permitis	2	Luticola mutica	6	Hantzschia amphioxys	2	Mayamaea atomus	6	Asterionella formosa	1
Mayamaea atomus	11	Nitzschia brevissima	4	Mayamaea atomus	21	Mayamaea permitis	1	Mayamaea permitis	43	Luticola mutica	2
Mayamaea permitis	22	Nitzschia microcephala	11	Mayamaea permitis	32	Nitzschia brevissima	7	Nitzschia brevissim	19	Mayamaea permitis	2
Nitzschia minuta	10	Pinnularia sinistra	83	Pinnularia sinistra	41	Nitzschia paleaformis	23	Pinnularia sinistra	32	Nitzschia brevissima	3
Pinnularia sinistra	54					Pinnularia borealis	4			Nitzschia microcephala	1
						Pinnularia subcapitata	63			Pinnularia borealis	3
										Pinnularia sinistra	17
										Pinnularia subcapitata	71
sp. total											
	5		4		4		6		4		8

C3R1		C3R1B		C3R1E		C3R1EB		C3R2		C3R2B	
species	count	species	count	species	count	species	count	species	count	species	count
Mayamaea atomus	7	Asterionella formosa	9	Asterionella formosa	9	Asterionella formosa	22	Hantzschia amphioxy	14	Achnanthidium minutissimum	2
Mayamaea permitis	71	Cymbella minuta	2	Luticola mutica	14	Mayamaea atomus	15	Mayamaea atomus	19	Eunotia minor	2
Nitzschia brevissima	15	Luticola mutica	7	Mayamaea permitis	33	Nitzschia brevissima	5	Mayamaea permitis	28	Luticola mutica	15
Pinnularia sinistra	4	Mayamaea atomus	32	Nitzschia recta	15	Pinnularia borealis	3	Nitzschia brevissima	22	Mayamaea atomus	33
Tabellaria fenestrata	3	Mayamaea permitis	33	Pinnularia sinistra	29	Pinnularia sinistra	55	Pinnularia sinistra	17	Mayamaea permitis	11
		Nitzschia brevissima	3							Nitzschia minuta	19
		Nitzschia paleaformis	14							Pinnularia subcapitata	18
sp. total											
	5		7		5		5		5		7

C3R2E		C3R2EB		C3R3		C3R3B		C3R3E		C3R3EB	
species	count	species	count	species	count	species	count	species	count	species	count
Asterionella formosa	15	Mayamaea atomus	7	Asterionella formosa	46	Achnanthidium minutissimum	28	Asterionella formosa	28	Asterionella formosa	4
Luticola mutica	9	Mayamaea permitis	8	Mayamaea atomus	10	Asterionella formosa	11	Mayamaea permitis	29	Gomphonema parvulum	8
Mayamaea atomus	23	Nitzschia brevissima	23	Mayamaea permitis	11	Hantzschia amphioxys	2	Nitzschia brevissima	8	Mayamaea permitis	11
Mayamaea permitis	24	Pinnularia borealis	1	Nitzschia brevissim	8	Mayamaea atomus	5	Nitzschia recta	19	Navicula gregaria	1
Nitzschia brevissima	29	Pinnularia sinistra	22	Pinnularia sinistra	23	Mayamaea permitis	4	Pinnularia sinistra	16	Pinnularia sinistra	25
		Pinnularia subcapitata	39	Pinnularia subcapit	2	Nitzschia brevissima	1			Pinnularia subcapitata	51
						Nitzschia minuta	7				
						Nitzschia paleaformis	15				
						Nitzschia recta	9				
						Pinnularia sinistra	9				
sp.total						Pinnularia subcapitata	9				
	5		6		6		11		5		6

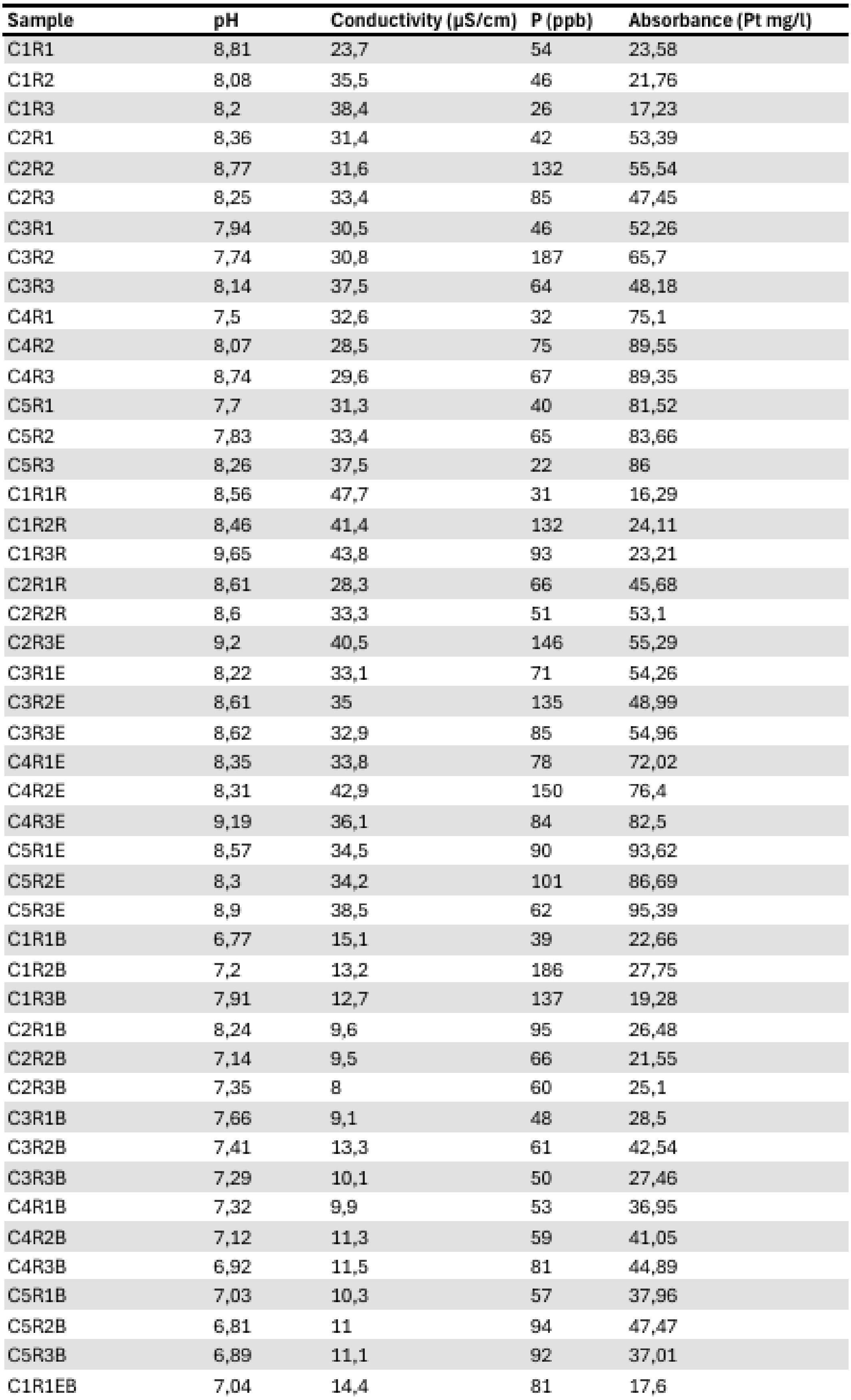
C4R1		C4R1B		C4R1E		C4R1EB		C4R2		C4R2B	
species	count	species	count	species	count	species	count	species	count	species	count
Asterionella formosa	19	Asterionella formosa	5	Mayamaea atomus	16	Nitzschia brevissima	1	Asterionella formosa	12	Asterionella formosa	4
Mayamaea atomus	14	Hantzschia amphioxys	2	Mayamaea permitis	29	Pinnularia sinistra	29	Hantzschia amphioxys	6	Luticola mutica	2
Mayamaea permitis	46	Mayamaea atomus	8	Nitzschia brevissima	24	Pinnularia subcapitata	70	Mayamaea atomus	35	Mayamaea atomus	58
Nitzschia brevissima	21	Mayamaea permitis	5	Pinnularia sinistra	31			Mayamaea permitis	26	Mayamaea permitis	13
		Nitzschia brevissima	13					Nitzschia brevissima	9	Nitzschia brevissima	3
		Nitzschia minuta	12					Nitzschia paleaformis	5	Nitzschia minuta	3
		Nitzschia paleaformis	24					Pinnularia subcapitata	7	Nitzschia paleaformis	17
		Pinnularia subcapitata	3								
		Staurosirella leptostauron var. dubia	17								
		Tabellaria fenestrata	11								
sp. total											
	4		10		4		3		7		7

C4R2E		C4R2EB		C4R3		C4R3B		C4R3E		C4R3EB	
species	count	species	count	species	count	species	count	species	count	species	count
Hantzschia amphioxys	6	Nitzschia brevissima	2	Achnanthidium minutissimum	2	Eunotia incisa	1	Hantzschia amphioxys	30	Hantzschia amphioxys	15
Luticola mutica	1	Nitzschia paleaformis	86	Eunotia incisa	4	Luticola mutica	1	Nitzschia minuta	10	Luticola mutica	7
Mayamaea atomus	6	Nitzschia recta	12	Eunotia minor	11	Mayamaea atomus	3	Pinnularia borealis	25	Nitzschia brevissima	2
Mayamaea permitis	13			Luticola mutica	3	Mayamaea permitis	4	Pinnularia sinistra	35	Pinnularia sinistra	16
Nitzschia paleaformis	33			Mayamaea atomus	10	Nitzschia minuta	7			Pinnularia subcapitata	60
Nitzschia recta	5			Mayamaea permitis	7	Nitzschia paleaformis	54				
Pinnularia borealis	6			Nitzschia brevissima	10	Nitzschia recta	30				
Pinnularia sinistra	25			Nitzschia paleaformis	21						
Pinnularia subcapitata	5			Nitzschia recta	26						
				Pinnularia sinistra	6						
sp. total											
	9		3		10		7		4		5

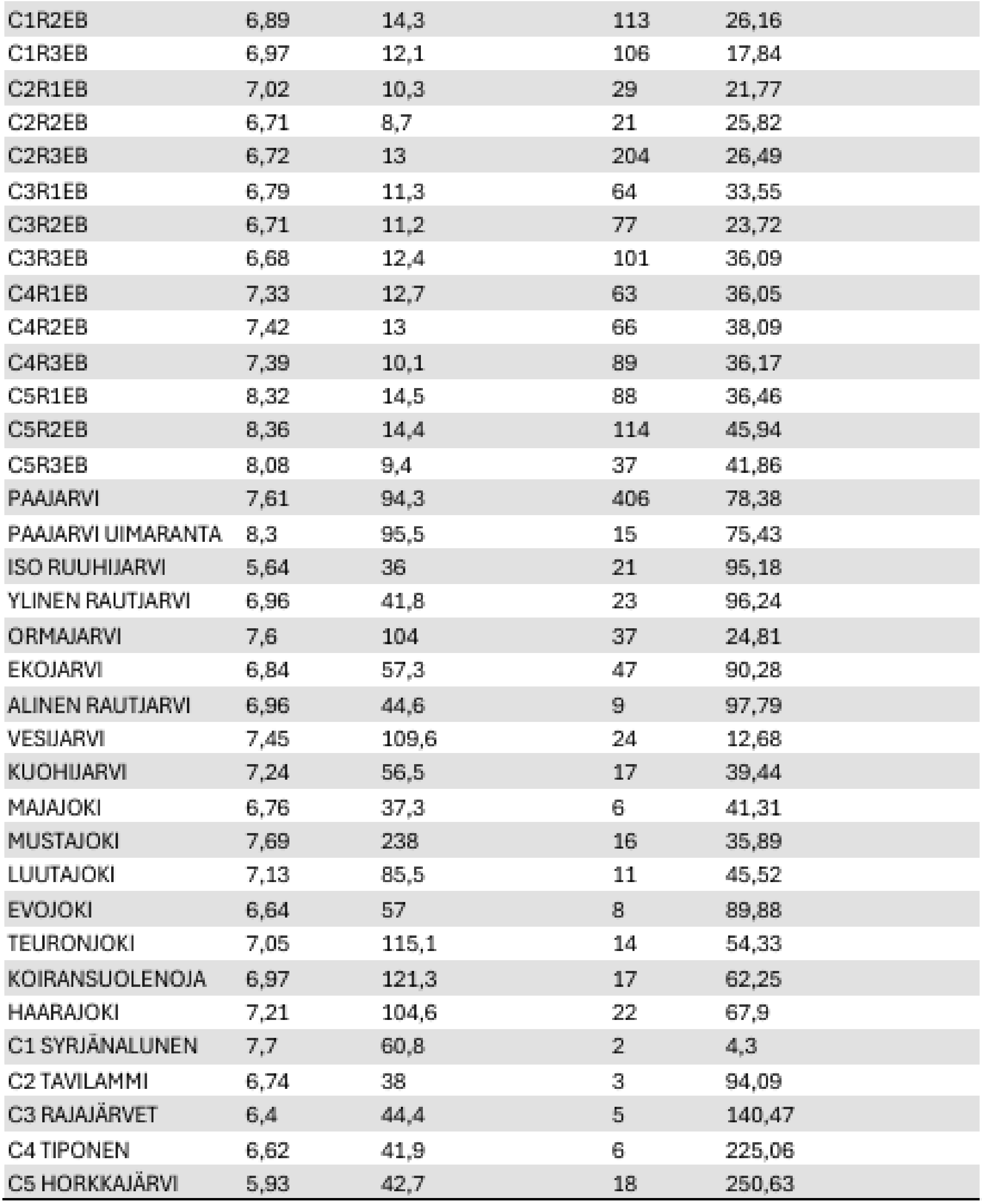
C5R1		C5R1B		C5R1E		C5R1EB		C5R2		C5R2B	
species	count	species	count	species	count	species	count	species	count	species	count
Achnanthidium minutissimum	2	Mayamaea atomus	42	Luticola mutica	10	Hantzschia amphioxys	26	Eunotia minor	21	Eunotia incisa	2
Asterionella formosa	1	Mayamaea permitis	25	Mayamaea atomus	31	Mayamaea permitis	27	Mayamaea atomus	18	Eunotia minor	7
Mayamaea atomus	19	Nitzschia brevissima	33	Mayamaea permitis	26	Nitzschia paleaformis	11	Mayamaea permitis	35	Fragilaria capucina	6
Mayamaea permitis	40			Nitzschia brevissima	24	Pinnularia borealis	15	Nitzschia brevissima	14	Mayamaea atomus	28
Nitzschia brevissima	18			Pinnularia borealis	9	Pinnularia sinistra	21	Pinnularia subcapitata	12	Mayamaea permitis	25
Nitzschia minuta	15									Pinnularia sinistra	9
Nitzschia paleaformis	5									Pinnularia subcapitata	23
sp. total											
	7		3		5		5		5		7

C5R2E		C5R2EB		C5R3		C5R3B		C5R3R		C5R3EB	
species	count	species	count	species	count	species	count	species	count	species	count
Luticola mutica	4	Gomphonema parvulun	62	Eunotia incisa	2	Mayamaea atomus	31	Hantzschia amphioxys	12	Pinnularia sinistra	31
Mayamaea atomus	36	Hantzschia amphioxys	16	Luticola mutica	12	Mayamaea permitis	8	Mayamaea atomus	2	Pinnularia subcapitata	69
Mayamaea permitis	42	Pinnularia borealis	1	Mayamaea atomus	36	Melosira varians	5	Mayamaea permitis	5		
Nitzschia paleaformis	12	Pinnularia subcapitata	21	Mayamaea permitis	12	Nitzschia microcephala	9	Nitzschia brevissima	2		
Pinnularia subcapitata	6			Nitzschia brevissima	13	Nitzschia paleaformis	7	Pinnularia borealis	12		
				Nitzschia recta	4	Nitzschia recta	20	Pinnularia sinistra	27		
				Pinnularia sinistra	21	Pinnularia sinistra	5	Pinnularia subcapitata	40		
						Pinnularia subcapitata	15				
sp. total											
	5		4		7		8		7		2

Appendix 2: Water chemistry table for each sample used in this study



Sample	pH	Conductivity (µS/cm)	P (ppb)	Absorbance (Pt mg/l)
C1R1	8,81	23,7	54	23,58
C1R2	8,08	35,5	46	21,76
C1R3	8,2	38,4	26	17,23
C2R1	8,36	31,4	42	53,39
C2R2	8,77	31,6	132	55,54
C2R3	8,25	33,4	85	47,45
C3R1	7,94	30,5	46	52,26
C3R2	7,74	30,8	187	65,7
C3R3	8,14	37,5	64	48,18
C4R1	7,5	32,6	32	75,1
C4R2	8,07	28,5	75	89,55
C4R3	8,74	29,6	67	89,35
C5R1	7,7	31,3	40	81,52
C5R2	7,83	33,4	65	83,66
C5R3	8,26	37,5	22	86
C1R1R	8,56	47,7	31	16,29
C1R2R	8,46	41,4	132	24,11
C1R3R	9,65	43,8	93	23,21
C2R1R	8,61	28,3	66	45,68
C2R2R	8,6	33,3	51	53,1
C2R3E	9,2	40,5	146	55,29
C3R1E	8,22	33,1	71	54,26
C3R2E	8,61	35	135	48,99
C3R3E	8,62	32,9	85	54,96
C4R1E	8,35	33,8	78	72,02
C4R2E	8,31	42,9	150	76,4
C4R3E	9,19	36,1	84	82,5
C5R1E	8,57	34,5	90	93,62
C5R2E	8,3	34,2	101	86,69
C5R3E	8,9	38,5	62	95,39
C1R1B	6,77	15,1	39	22,66
C1R2B	7,2	13,2	186	27,75
C1R3B	7,91	12,7	137	19,28
C2R1B	8,24	9,6	95	26,48
C2R2B	7,14	9,5	66	21,55
C2R3B	7,35	8	60	25,1
C3R1B	7,66	9,1	48	28,5
C3R2B	7,41	13,3	61	42,54
C3R3B	7,29	10,1	50	27,46
C4R1B	7,32	9,9	53	36,95
C4R2B	7,12	11,3	59	41,05
C4R3B	6,92	11,5	81	44,89
C5R1B	7,03	10,3	57	37,96
C5R2B	6,81	11	94	47,47
C5R3B	6,89	11,1	92	37,01
C1R1EB	7,04	14,4	81	17,6



C1R2EB	6,89	14,3	113	26,16
C1R3EB	6,97	12,1	106	17,84
C2R1EB	7,02	10,3	29	21,77
C2R2EB	6,71	8,7	21	25,82
C2R3EB	6,72	13	204	26,49
C3R1EB	6,79	11,3	64	33,55
C3R2EB	6,71	11,2	77	23,72
C3R3EB	6,68	12,4	101	36,09
C4R1EB	7,33	12,7	63	36,05
C4R2EB	7,42	13	66	38,09
C4R3EB	7,39	10,1	89	36,17
C5R1EB	8,32	14,5	88	36,46
C5R2EB	8,36	14,4	114	45,94
C5R3EB	8,08	9,4	37	41,86
PAAJARVI	7,61	94,3	406	78,38
PAAJARVI UIMARANTA	8,3	95,5	15	75,43
ISO RUUHIJARVI	5,64	36	21	95,18
YLINEN RAUTJARVI	6,96	41,8	23	96,24
ORMAJARVI	7,6	104	37	24,81
EKOJARVI	6,84	57,3	47	90,28
ALINEN RAUTJARVI	6,96	44,6	9	97,79
VESIJARVI	7,45	109,6	24	12,68
KUOHIJARVI	7,24	56,5	17	39,44
MAJAJOKI	6,76	37,3	6	41,31
MUSTAJOKI	7,69	238	16	35,89
LUUTAJOKI	7,13	85,5	11	45,52
EVOJOKI	6,64	57	8	89,88
TEURONJOKI	7,05	115,1	14	54,33
KOIRANSUOLENOJA	6,97	121,3	17	62,25
HAARAJOKI	7,21	104,6	22	67,9
C1 SYRJÄNALUNEN	7,7	60,8	2	4,3
C2 TAVILAMMI	6,74	38	3	94,09
C3 RAJAJÄRVET	6,4	44,4	5	140,47
C4 TIPONEN	6,62	41,9	6	225,06
C5 HORKKAJÄRVI	5,93	42,7	18	250,63