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Diatom species richness and community composition along an elevational gradient in subarctic ponds

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Subarctic ponds are important habitats for many freshwater species. The recent increase in global temperatures have stressed on the study of these habitats as rising water temperatures may have severe consequences to these cold and harsh ecosystems. Despite its importance, this topic has been largely overlooked in scientific research. Diatoms are microscopic, single-celled benthic algae, which are important indicators for environmental quality. Elevation is one of the main environmental variables controlling the composition and richness of diatom species as it shapes communities through several environmental variables such as temperature and water chemistry. The aim of this thesis was to illustrate the variability in diatom species richness and community composition along an elevational gradient in Kilpisjärvi and reveal the most important environmental drivers. As an additional focus, the applicability of the BenthoTorch sampling device was tested in measuring benthic algae biomass. Field and laboratory measurements were done using universal standards. Statistical analyses included multiple univariate and multivariate data analysis techniques. It was found that water pH, aluminium concentration and air temperature explained the variation in species richness and community composition the most. Elevation had only a secondary, non-significant role in shaping the diatom communities in subarctic ponds. Nearby sites showed similar compositions in terms of water chemistry and diatom communities. Biotope characterisation did not provide any further insight into the differences or similarities of diatom community composition or species richness. There were some differences in how genera responded to environmental variables. The centre of distributional range of many taxa was below the mid-point of the elevational gradient but species often occupied the whole elevational gradient. Rare taxa appeared at the ends of the elevational spectrum. The amount of singleton taxa was high (25.8%) and can be expected to increase with climate change. The BenthoTorch did provide reasonable results for benthic algae in the subarctic when compared to previous literature, but further research is required to grasp its full potential. More examination into the relationship between explanatory variables can be suggested (e.g. total phosphorus and ion balance) to gain better understanding on the changes in diatom species richness and community composition along elevational gradients.

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Global warming, benthic algae, multivariate analysis, BenthoTorch

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Tiivistelmä – Referat – Abstract

Subarktiset lammet ovat tärkeitä elinympäristöjä monille vesieliöille. Ilmaston lämpenemisen myötä subarktisiin vesiin liittyvät tutkimukset ovat lisääntyneet, sillä veden lämpötilan nousulla saattaa olla peruuttamattomia vaikutuksia karuihin tunturilampiekosysteemeihin. Silti ekosysteemien tuntemus on vielä melko vähäistä. Piilevät ovat mikroskooppisia ja yksisoluisia pohjaleviätärkeä. Ne ovat tärkeitä ympäristön laadun indikaattoreita. Korkeus on yksi merkittävimmistä muuttujista selittämään piileväyhteisöjen lajirikkautta ja lajistoa sen suoran lämpötilavaikutuksen sekä muiden muuttujien kanssa olevan epäsuoran vaikutuksen kautta. Tämän opinnäytetyön tarkoitus on havainnollistaa piilevälajiston koostumuksen sekä lajirikkauden vaihtelua korkeusgradientilla Kilpisjärvellä ja tutkia, mitkä muuttujat selittävät vaihtelua parhaiten. Tutkimuksessa käytettiin pohjalevien biomassaa mittaavaa laitetta, BenthoTorch:ia ja keskusteltiin laitteen mahdollisuuksista subarktisissa tutkimuksissa. Kenttä- ja laboratoriomittaukset tehtiin käyttäen universaaleja standardeja. Tilastollisina analyyseinä käytettiin regressionmalleja ja monimuuttuja-analyysejä. Veden pH-arvo, alumiinin määrä ja ilman lämpötila selittivät piilevälajirikkauden ja -lajiston vaihtelua parhaiten korkeusgradientilla. Korkeus ei selittänyt merkittävästi vaihtelua itsenäisesti, vaan muokkasi ympäristömuuttujia, jotka vaikuttivat yhteisöjen vaihteluun. Lähekkäin olevat lammet olivat hyvin samanlaisia vesikemian, lajirikkauden sekä piilevälajiston suhteen. Tutkimuslampien lajittelu paikallisen biotoopin mukaan ei antanut lisätietoa tutkimuslampien eroavaisuuksista tai samankaltaisuuksista. Piileväsukujen välillä oli eroja niiden vasteissa ympäristömuuttujien suhteen. Monien lajien suurin tiheys oli korkeusgradientin keskipisteen alapuolella, mutta monilla lajeilla oli myös maksimaalinen korkeusskaala. Harvinaiset lajit esiintyivät korkeusgradientin ääripäissä. Harvinaisten lajien määrä oli korkea (25.8%) ja sen on odotettu nousevan ilmaston lämpenemisen myötä. BenthoTorch-mittaukset olivat luotettavia subarktisella alueella, mutta enemmän tutkimusta tarvitaan, jotta laitteen kokonaispotentiaali saataisiin selville. Tulevissa tutkimuksissa tulisi selvittää enemmän muuttujien välistä suhdetta (esim. kokonaisfosfori ja ionibalanssi), jotta ymmärrettäisiin paremmin tärkeiden piilevälajirikkauteen ja -lajiston vaihteluun vaikuttavien muuttujien merkitys korkeusgradientilla.

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Ilmaston lämpeneminen, pohjalevät, monimuuttuja-analyysi, BenthoTorch

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1. Introduction

The planet has gone through billions of years of change with the most drastic anthropogenically caused changes occurring in the last 100 years in the form of climate change. Some of the mainstream images for global warming include pictures of melting glaciers and arctic species dying. The question is, what do these descriptions have in common? They all occur in the arctic/subarctic. The arctic can be described as the transition from continuous boreal forest to open tundra, whereas the subarctic can be defined as the area between the closed-canopy boreal forest and the treeline and is often related to the southern limits of the discontinuous permafrost (Forsström, 2006). Subarctic freshwater systems consist of very adaptive and variable community structures and are thus a key focus point for researchers. These habitats consist of a variety of species ranging from macro to micro-scale. Both have been a key to understand climatic patterns, but it is the more vulnerable micro-organisms (eukaryotes and prokaryotes) that supply researchers with the most accurate information because of their location in harsh conditions. Diatoms are microalgae found in a variety of aquatic settings. Due to their unique shape and easy accessibility, diatoms are a key component when it comes to aquatic research. They can attach to a variety of surfaces and can typically be identified down to species level. Diatoms respond fast to environmental changes due to their short life cycle. Thus, diatoms have become an important indicator for environmental change (Lotter et al., 2010).

Ongoing rapid environmental change needs also integrative solutions to monitor them. Since the EU water framework directive was established in 2000, the use of biological organism groups as indicators for water quality and monitoring has increased (Fisher et al., 2010). Through water quality indices, ecological statuses can be universally assessed (King et al., 2006), while providing valuable information to local authorities and agencies on water quality (Quiblier et al., 2013). These changes are perhaps easiest to measure in arctic or subarctic regions, where the impact of any slight environmental disturbance will trigger changes in the diatom communities (Wrona and Reist, 2013). The Intergovernmental Panel on Climate Change (2014) has reported that due to the increasing concern of global warming, these high latitude environments should be evaluated with care and caution and thus latest EU funded projects such as EMERGE and MOLAR have concerned high-mountain and high-latitude lakes to examine their environmental and biotic features (Forsström, 2006). As noted in the State of the arctic freshwater biodiversity report (SAFBR, Lento et al., 2019), diatom taxa are consistent and dominant throughout the global arctic. This means that even though temperature has a great effect on diatom diversity, the distribution of species is also driven by local conditions of geology and water chemistry.

According to the SAFBR by Lento et al. (2019) subarctic and arctic lakes and ponds are generally not included as part of routine programmes (e.g. circumpolar biodiversity monitoring program). Therefore, many programmes and reports heavily rely on academic research which focuses on arctic and subarctic lakes and ponds. Together these areas occupy the circumpolar (Figure 1) and the aquatic system within it are now considered as biodiversity hotspots (Zaharescu et al., 2016). However, research in this field is sparse and more should be explored to fully understand the potential of freshwater lake and pond diatoms and their biodiversity. Shallow, arctic ponds may be the most sensitive to climate change and thus might show the most pronounced community shifts (Pienitz et al., 2004). The impact from climate change will directly influence the subarctic pond ecosystem and thus indirectly shape diatom communities. Increasing temperatures and shortened

Figure 1. The different shades of purple cover both the arctic and subarctic and together the area is referred to as the circumpolar in this research. The red line indicates the boundary of the Conservation of arctic flora and fauna (modified from Lento et al. 2019).

ice cover will increase light and nutrient availability in the subarctic ponds causing an increase in diatom species diversity in the circumpolar (Wrona and Reist, 2013).

Across the circumpolar, diatoms generally dominate over other benthic algal groups at low nutrient conditions and neutral water pH levels, while showing high diversities in nutrient rich waters. Diatom species richness varies across space and time according to several environmental factors such as pH, which is heavily regulated by air and pond temperatures (Teittinen et al., 2016). On top of these abiotic factors, diatom communities are shaped by biotic factors such as competition from other eukaryotic algae for instance cyanobacteria and green algae. However, it has been found that the most important environmental factor varying with elevation is temperature (McCain and Grytnes, 2010). Thus elevational gradient studies are excellent in mimicking rising temperatures due to global warming. Furthermore, the rising temperatures from global warming will increase precipitation and promote permafrost thawing, having an additional, indirect impact on subarctic ponds and their communities (Forsström, 2006). The role of temperature will have a large range of consequences on the circumpolar. Everything affects everything, making research in the circumpolar tedious and extensive. A high correlation between terrestrial and aquatic ecosystems in diatom diversity and richness research provides valuable information on modelling long term environmental conditions (Soininen et al., 2015). Giving the small spatial area occupied by the subarctic ponds, they are especially sensitive to changes in the environment (Forsström, 2006).

Elevational gradients are a key to understanding global biodiversity patterns as in small spatial extent, environmental conditions vary substantially along elevation resulting in similar community segregations as in latitudinal gradient (McCain and Grytnes, 2010). Elevation itself plays a crucial role in shaping the surrounding environment by affecting temperature, precipitation and productivity either directly or indirectly (Figure 2). It is essential to include a set of more specific variables into a conceptual model depending on the study. Such variables could include above ground biomass or shading to enhance the knowledge on local diatom species diversity and richness, but the variables should not overlook the greater impact that comes from the environment such as climate (Soininen, 2007). Both biotic and abiotic factors should be evaluated closely and discussed in depth when assessing species composition and richness in a changing environment. Using natural gradients such as elevation will help scientists understand long-term and large-scale community and ecosystem responses to environmental change (Sundqvist et al., 2013).

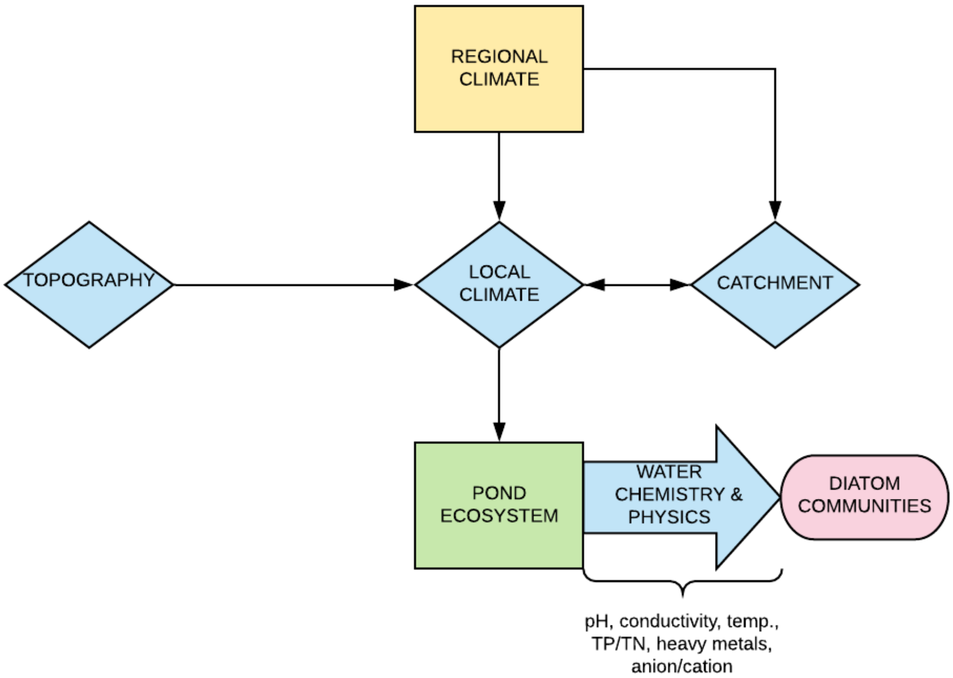


Figure 2. A basic flow chart outlining the large-scale and smaller-scale variables affecting diatom communities in subarctic ponds.

The relationship of elevation and different organism groups will provide more information on the effect of temperature variation on ecosystem properties and processes (Sundqvist et al., 2013). In general, species richness for microorganisms shows a contrasting relationship with macro-organisms along elevation (Taxböck et al., 2020). For example, plant and mammalian richness tends to decrease with higher elevation, whereas diatom species richness was found to weakly increase or show variable patterns (Wang et al., 2017). Despite having contradicting results to other diatom-altitude relationship assessments, there are several drivers of species richness that might vary independently of elevation such as competition. Due to their extensive study spectrum, benthic algae ecologies are well known but their interactions remain an uncertainty. Therefore, a benthic algal sampling device, the BenthoTorch could potentially bring further knowledge in understanding the interactions between benthic algae groups through various types of research (Covich et al., 2004). The BenthoTorch is an *in-vivo* fluorescence chlorophyll-a measurement tool of cyanobacteria, green algae and diatoms (bbe-Moldaenke, 2020). The BenthoTorch uses different wavelengths during LED-measurements allowing distinction between the three algal groups (Figure 3). The BenthoTorch has a large range of capabilities in a spectrum of environments and has gained popularity in various types of research over the past decade (Figure 4). Harris and Graham (2015)

Figure 3. The LED's equipped on BenthoTorch. The "empty" spot indicates the location of the additional LED (wavelength 700nm). The wavelengths are 470nm (=blue), 525nm (=green) and 610nm (=yellow; bbe-Moldaenke, 2014).

assessed the reliability of the BenthoTorch in relation to measurements acquired from the laboratory and *in-vivo* BenthoTorch values of below 4 µg/cm² are reliable.

It has been hypothesised that the patterns of species composition and richness vary according to local pond variables, which are shaped by regional-scale variables such as land cover and climatic variables (Teittinen et al., 2017). In terms of land cover variation, it is unlikely that a significant pattern can be seen based on the influence of local pond variables rather than regional-scale variables. When examining biodiversities in the subarctic; physical, geographical and chemical factors should be measured to gain knowledge of the richness and diversity of diatoms (Bouchard et al., 2004). Understanding the biodiversity patterns in a subarctic region, provides valuable information about the environmental, chemical and physical conditions of the local area but also in a global context. This thesis aims (1) to gain further knowledge on how diatom species richness and composition vary with elevation in the subarctic northern Norway and Finland. This thesis will further aim (2) to find the most influential explanatory variables for diatom communities consisting of environmental variables (i) such as air temperature, and abiotic pond variables (ii) such as water chemistry affecting richness and composition along the elevational gradient. Further aim was to understand the differences and

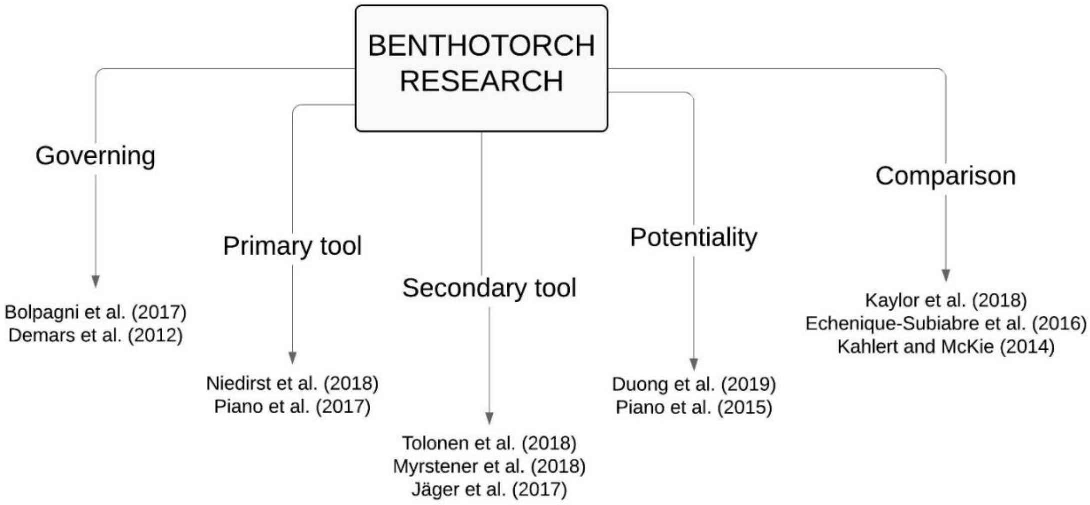


Figure 4. An outline of the research that have focused on and around the BenthoTorch from 2012 to 2019.

similarities between the studied subarctic ponds, and thus a characterisation based on local biotope was made (3). As an additional aim, the performance of the BenthoTorch device was tested and discussed (4).

2. Theoretical background

Subarctic aquatic habitats are important to study as they may act as biochemical sources for several species of zooplankton and phytoplankton and may thus have a significant role in global carbon fluxes contributing to global temperature changes (Rautio et al., 2011). Diatoms have a crucial role in global carbon dioxide and silica cycles, perhaps even as important as the contribution from soils (Field et al., 1998). Subarctic regions are vulnerable to small changes in abiotic and biotic conditions and therefore the examination of diatom biodiversity is of great importance when trying to conceptualize the role of the changing environment in a global scale. Diatoms typically indicate local environmental conditions accurately and are thus considered a valuable tool in modelling local climate (Fritz et al., 1991). Studying microbial communities in subarctic regions provide scientists with insights into changing biodiversities under shifting climatic conditions. Once such models have been created, it is possible to distinguish the relative vulnerability of subarctic habitats in relation to climate change. Smol et al. (2005) found an increase in beta diversity (i.e. compositional difference between at least two sites) in the arctic possibly due to the extended summer growing season. The understanding of how diatom communities respond to environmental changes is extensive but would require excessive research to gain further insight in micro-organismal studies (Blanco et al., 2020).

Air temperature alone is not enough to describe the diatom-climate interaction and thus other abiotic pond and soil variables should be considered (Anderson, 2000). Previous literature has mainly focused on water temperature (Weckström et al., 1997) and air temperature (Sorvari et al., 2002), studying how they shape the diatom communities. There are many different focus points for diatom research with elevation such as water pH as it has shown to affect diatom assemblages (Antoniades et al., 2005). Water pH varies with water and air temperature and are thus likely to alter diatom assemblages with increasing global temperatures. Fránková et al. (2009) found that pH was the main environmental variable explaining diatom species richness. Nutrient availability appears to be a driver for diatom diversity, but it often has a secondary role along elevational gradients as it is shaped by several other catchment variables (Mogna et al., 2015). For subarctic ponds, the nutrient concentrations are generally small, but these are expected to rise with increasing global temperatures (Roiha et al., 2012). This further enhances the assessment of small-scale fluctuations in nutrient inputs and their sources along elevational gradients.

2.1. The elevational gradient

Elevation shapes several abiotic and biotic pond variables (Figure 5). Elevation itself does not directly influence species richness or community composition, but it influences many biotic and abiotic factors such as precipitation and temperature. These will then control other factors such as pH, nutrient availability, and biomass when shaping local diatom communities. When studying the relationship between organisms and elevation, the gradient for elevation should be as extensive as possible as otherwise there might be a dependency ratio between species richness and elevation (Rahbek, 2005). There is no universally accepted trend in the pattern between diatoms and any explanatory variables along elevation. Elevational richness patterns tend to show a variety of response shapes, such as monotonic, unimodal or other maximum richness points (Wang et al.,

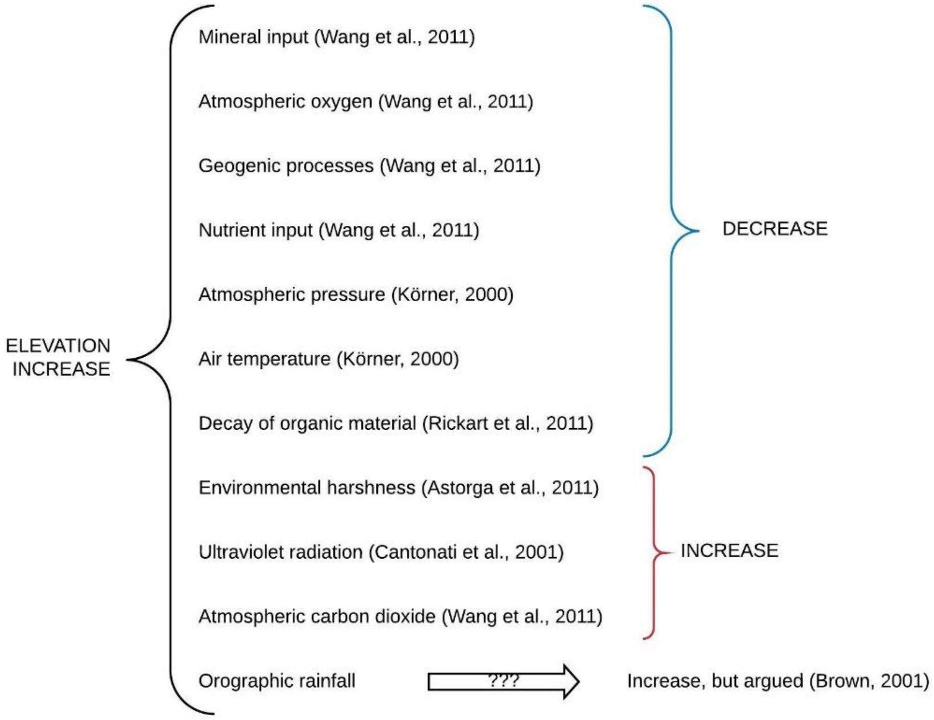


Figure 5. Variables changing in streams with increasing elevation based on previous literature. The change in orographic rainfall is argued (Brown, 2001), but generally it increases with increasing elevation.

2017). It can be illustrated that diatom diversity could be more affected by local environmental factors rather than by climatic variables associated with elevation (Blanco et al., 2020). For example, the variation in nutrient supply and an increase in bacterial richness, shape the diatom communities best along an elevational gradient (Wang et al., 2011). Unimodal trends have also been common (Weckström and Korhola, 2001), which indicates highest diatom species richness at mid-elevation also known as the mid-domain effect (McCain and Grytnes, 2010).

The features of the catchment leading to the pond will vary substantially between the ponds and their local communities. The catchment properties must be considered when conducting research in the field to understand better the role of different environmental factors explaining aquatic species composition and richness. There are some difficulties when assessing elevational trends and subarctic ponds. For example, in the lowland areas of the subarctic, some ponds tend to be fed by groundwater instead of surface water from the catchment (Yoshikawa and Hinzman, 2003). As demonstrated by the article, groundwater fed subarctic ponds may begin to shrink in size, which can be another issue when assessing subarctic ponds along elevational gradients. A similar trend was noted in Alaska where the subarctic ponds have shrunk by 54% in the past 50 years (Riordan et al., 2006). It is believed to be caused by increased evaporation due to increased global temperatures or increased drainage due to permafrost thaw.

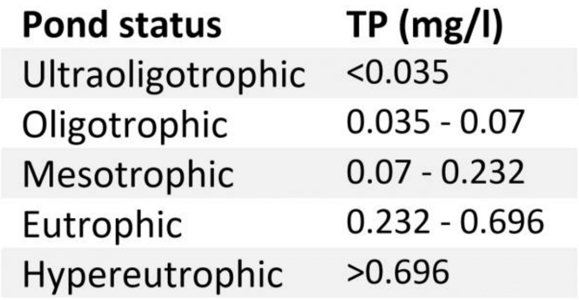
2.2. The subarctic pond environment

Subarctic ponds are a significant feature of the subarctic landscape with little or no vegetation as they appear generally above the treeline (Catalan et al., 1993). Not only are they a visual admiration to viewers and a biogeographical hotspot to researchers, they are also a key component in aquatic biodiversity. The most prominent feature of these ponds is the extreme seasonality with long, dark winters followed by a short summer with continuous sunlight (Forsström, 2006). Circumpolar environments can have some significant similarities in terms of climate and biology despite their spatial differences (Nauwerck, 1994). Circumpolar and alpine environments may share some similar characteristics. A subarctic pond at roughly 1000 meters above sea level (masl) would correspond to an alpine pond at 3000 masl based. The main environmental distinction between the two areas is light availability and strength of incoming radiation (Forsström, 2006). On top of environmental differences, alpine regions have a longer history of human occupation and thus are more influenced by anthropogenic impacts. Arctic-alpine regions therefore play an important role for the assessment of the impact of environmental changes at local levels (Blanco et al., 2020). Same conclusions can be made for the Himalayas (Nautiyal and Mishra, 2013). However, for the circumpolar more research is required to understand the pond environments and the habitats occupying them. Furthermore, there have been some studies in the bacterioplankton stock in relation to elevation (Karlsson et al., 2001), but very little focus has aimed on diatom communities.

Subarctic ponds provide the local floral and faunal communities with water and nutrients and serve as an important setting for many microbial communities. The productivity of the entire catchment may be a useful predictor of taxa in lake plankton communities (Soininen and Luoto, 2012). However, the smaller the aquatic ecosystem, the more often the ecosystem is influenced by small, yet harsh disturbances (Soininen and Meier, 2014). Thus, these influential disturbances will have a great impact on the pond ecosystem. Microbial communities have an important role contributing towards local production and must therefore not be excluded from the so-called food-chain. It is important to understand the interactions between cyanobacteria, green algae and diatoms as they contribute mostly towards the primary production of subarctic ponds. Macrophytes are rare and their biomass and productivity is generally low, which elevates the importance of microbial primary producers, such as benthic algae on ecosystem functioning in small subarctic ponds (Forsström, 2006).

Phytoplankton such as diatoms are subjected to many limitations from the subarctic pond environment. Due to their small surface areas and cold water temperatures, they are extensive periods covered by ice. The open water period can be only a couple of weeks (Welch, 1991), which slows down many biological processes and limits the growing season of phytoplankton as they are being grazed by zooplankton. At this point the amount of available nutrients becomes crucial. Nitrogen and phosphorus are both important nutrients for production, hence the reference to limiting nutrients. Subarctic ponds can be classified into five groups based on the concentration of total phosphorus (Table 1). Nutrient levels in subarctic pond and lake environments tend to vary quite a

Table 1. Trophic statuses of aquatic mediums based on total phosphorus levels (modified from Catalan et al. 1993).



Pond status	TP (mg/l)
Ultraoligotrophic	<0.035
Oligotrophic	0.035 - 0.07
Mesotrophic	0.07 - 0.232
Eutrophic	0.232 - 0.696
Hypereutrophic	>0.696

lot, but it can be assumed that in high altitude ponds, the nutrient levels would correspond to either oligotrophic or mesotrophic (Shortreed and Stockner, 1986). However, there can be distinctive regional differences (Forsström, 2006). Eutrophication tends to be a more common issue at lower elevations where anthropogenic influences are elevated (McCain, 2007), but within the circumpolar oligotrophication may be a greater issue as vital nutrients are washed out from the source pond due to increased runoff (Huser et al., 2018). As altitude decreases, phosphorus becomes more available, increasing species richness leading to a linear trend (Sorvari et al., 2002; Wang et al., 2011). The reason why phosphorus tends to be more limited compared to nitrogen is because nitrogen appears naturally in the atmosphere and becomes bound abiotically into the system (Moss, 1998, Ch.6). Nitrogen enters the subarctic habitat in four different forms: ammonium, nitrate, nitrite and molecular N_2 (Moss, 1998, Ch.7). The first three will be absorbed and used by phytoplankton. Phosphorus amounts are mainly contributed from other species for example from bacterial decomposition, reindeer, birds and other larger animals. Sundqvist et al. (2013) pointed out that both aboveground and belowground consumers should be included in the interpretation of community response to elevation. This method has been adapted in remote sensing studies which aim to bridge the gap between catchment productivity, including both animals and plants, and species richness (Soininen and Luoto, 2012).

Nutrient and carbon cycling are key components in subarctic ponds as they provide energy for benthic biofilms, thus affecting the productivity of the pond. Dissolved organic carbon varies spatially in subarctic ponds based on catchment type, runoff amount and other allochthonous variables (Roiha et al., 2012). Since the nutrient load is generally poor, even a small variation in pond temperature might alter the algal biomass in aquatic communities (Gudmundsdottir et al., 2011). This is likely to increase decomposition rates which may lead to higher nutrient turnover for primary producers. Linked with seasonal temperature change, the abundance of algal groups may vary significantly. Generally during winter and spring, diatoms dominate as they are among the first epilithic algae to grow while most nutrients are in the epilimnion (i.e. the upper most layer of the surface water). As temperatures increase, cyanobacteria and green algae outcompete diatoms due to intensified nutrient and carbon cycling. In the autumn, the profundal (i.e. the bottom layer of the pond) is reoxygenated causing a large supply of nutrients due to the mixing of the water column, which promotes diatom growth. In shallow lakes, the organic material arriving to the profundal is rich in energy and nutrients, but the quality of it can vary greatly with water temperature (Cross et al., 2015). However, some studies have found no significant differences among the three algal groups with temperature in subarctic streams (Gudmundsdottir et al., 2011). Overall the chance for nutrient cycling is smaller and the loading of nutrients is poorer for shallow ponds (Moss, 1998, Ch.6), which does allow researchers to make broad conclusions on the portions of benthic algae in subarctic ponds in relation to seasonal changes.

The water chemistry in the subarctic ponds usually corresponds quite well to the chemical composition of rainwater. The ratio between sulphides and chlorine in rainwater is 8:3 and it should match in small, high altitude subarctic ponds (Camarero and Catalan, 1993). The higher sulphide concentrations are from weathered rock from the catchment, whereas chlorine appears in the atmosphere. However, there is an increase in chlorine with melting snow. Snow melt also releases other anions and cations such as ammonium and nitrate, which will decrease the water pH. Rainfall tends to be slightly acidic and thus small and shallow subarctic ponds tend to be slightly acidic in terms of water pH (< 5.5). Acidic water of pH 5 or below will cause dissolution of harmful metals such as aluminium for pond ecosystems. At the same time, the ecosystem’s ability to neutralize the acidic water takes place. Cations like calcium, potassium, magnesium and sodium will enhance denitrification of ponds which will decrease acidity (Schnoor and Stumm, 1986). Subarctic ponds tend to have a low conductivity (< 30 µS/cm), which is reflected to the mineral content of the water (Cantonati et al., 2001). Many of the aforementioned causes are driven by temperature. Patterns of lower benthic macroinvertebrate diversity have been detected in cold areas, both high altitude and latitude (Lento et al., 2019). This diversity is expected to increase with increasing global temperatures (Heino et al., 2009) potentially increasing invasive and rare species numbers. However, regional diatom data sets usually have relatively high rare diatom species counts (Teittinen et al., 2016). Increased precipitation and temperature will increase mineral weathering, decomposition of soil organic matter, erosion and sedimentation in the circumpolar leading to increased dissolved organic matter, nutrient, and mineral concentrations (Nadelhoffer et al., 1997). Even though these causes are not severe around the circumpolar, the potential shading of primary producers through nutrient enrichment and cyanobacteria enrichment may have drastic consequences on the aquatic community compositions (Scheffer, 1989).

2.3. Biotic and abiotic factors driving diatom species richness and community composition along elevational gradients

Research based on the relationship between elevational gradients and species richness as well as community composition has gained publicity during the 1990’s, but many have focused on streams (Potapova, 1996; Teittinen et al., 2016) or springs (Cantonati and Spitale, 2009; Angeli et al., 2010). Community composition describes the composition of a specific community within a restricted area, whereas species richness describes the range of different species found within the restricted area. Biodiversity is not only about the number of species in an area, but also about the spatial changes in species composition (Taxböck et al., 2020). The variation in the assembly can be stochastic or deterministic, which are influenced by a variety of factors such as sampling constrains, habitat area and evolutionary history. However, the most important variables are the abiotic and biotic variables. Giving the scale of factors, it is difficult to draw a large-scale spatial conclusion on species richness and distribution because of the large variability between local communities. Local scales must be broadly reviewed first to get a clear view of some of the factors controlling the diatom habitats of the area as aquatic microbial communities have some biogeographical regional variation due to these local variables (Jyrkänkallio-Mikkola et al., 2017). Diatoms will provide us with the best understanding of both regional climatic factors controlling the richness and diversity, but also pond-specific water chemical components (Lotter et al., 2010).

The relationship between elevation and species richness can be categorised into four categories: decreasing, low plateau, low plateau with mid-elevational peak or unimodal, and mid-elevational peak (McCain and Grytnes, 2010). In fact, some analyses have found the most diverse diatom communities appearing in high elevation ponds (Blanco et al., 2020). Environmental factors can only partially explain the response shape of the relationship between diatom species richness and elevation. For example, the number of microhabitats is one of the most important determinants of diatom diversity (Taxböck et al., 2020). From the environmental variables, water conductivity and light availability have been explained the diatom richness and community composition variation (Potapova and Charles, 2005). Abiotic variables such as nutrients, pH and metals further shape diatom diversity in subarctic ponds (Davis et al., 1983). Diatom taxa are not limited in the circumpolar since samples from similar latitudes have shown identical responses (Wrona and Reist, 2013). Diatom diversities seem to be mainly controlled by elevation (Blanco et al., 2020). On top of this significant distance-decay similarity trends were detected in the analysis of floristic composition among the samples. This stresses the importance of spatial factors which may override the effect of other abiotic factors highlighting the role of isolation and dispersal limitation in the configuration of the biogeographical patterns of benthic diatoms (Blanco et al., 2020). As well as influenced by spatial distance, communities and richness are influenced by elevational distance. Studies with linear trends with species richness and elevational gradient tend to have a short elevational gradient (Rahbek, 2005). The most prominent variables varying with elevation are water pH, total phosphorus, conductivity of water and nitrate concentration (Mogna et al., 2015).

3. Diatoms

Diatoms (*Bacillariophyceae*) are microscopic single-celled algae consisting of mostly benthic communities. Diatoms belong to a smaller group of eukaryotic algae with cryptophytes and green algae that have the capability of forming small colonies in the benthos (Cantonati and Lowe, 2014). The benthic algae consist of other microalgae such as cyanobacteria as well as macroscopic filamentous algae including green algae. However, diatoms tend to be one of the most dominant algal group with cyanobacteria in benthic samples due to their quick adaptation and growth. Diatoms, along with other algae rely heavily on nutrient supply especially phosphorus and nitrogen. Diatoms appear globally and in a variety of ecosystems comprising of different abiotic and biotic conditions (Pajunen et al., 2017). In the arctic regions, *Tabellaria flocculosa* (Kützig) appears to be one of the most common diatom taxa as it has a large pH tolerance and typically appears in small colonies (DeColibus, 2013). *Tabellaria flocculosa* is a typical example of a cosmopolitan diatom species as they are controlled only by environmental variables. Thus, the same species would thrive all over the globe as long as the environmental conditions would be the same. However, the definition has become controversial since more endemic species have been found within the diatom communities. Many previous studies that focus on the impacts of biotic and abiotic variables on pond algal communities have solely focused on diatom assemblages because they are the easiest to sample and analyse in terms of sample preparation methodology, identification and sampling techniques in the field. Due to their fast life cycle and great species turnover in space and time, they respond to the surrounding habitat even within days (Eloranta et al., 2007). Even though there are still believed to be thousands of species still to be identified, the taxonomy of diatoms is the most resolved among benthic algae.

3.1. Ecology and distribution

There is not only a single variable that affects the diatom ecology, as diatoms are affected by multiple abiotic and biotic variables (Pajunen et al., 2017). At larger scales, dispersal history, geology, bedrock and climate including ice cover duration play important roles in shaping the diatom communities. On the contrary at local scales, water chemistry, shaped by the local environment, seems to play a predominant role in determining algal species composition (Forsström, 2006). The basic requirements for shaping the diatom communities are moisture, nutrients, light and space (Vadeboncoeur and Steinman, 2002). Diatoms compete with one another against these resources, but they also are in a constant competition with other organisms, especially green algae (Huisman et al., 2004). Even though space is a limiting factor, it appears to be a secondary resource after nutrient and light availability, which are controlled by the size of the habitat. Shallow, subarctic ponds with clear water offer potentially the most suitable habitat for diatoms as the euphotic zone (i.e. the zone where photosynthesis occurs) is wide often including even the profundal (Hill, 1996).

Temperature has a direct and an indirect role on diatom communities especially through an interplay with light (Anderson, 2000). The direct effect of temperature is related to cell growth as metabolism speeds up at higher temperatures (Montagnes and Franklin, 2001). In addition, particularly for shallow subarctic ponds, sunlight can quickly heat up the water and have drastic changes on diatom communities. In contrast, subarctic ponds fed from groundwater are cold and can thus have very different species composition. The source of water can have varying effects on water chemistry and geochemical properties (Cantonati and Spitale, 2009; Mogna et al., 2015). This may have an impact on the acidity of waters which affects diatom community composition, metabolism and mortality. Equally, extremely high water pH can have similar effects on diatoms (Eloranta et al., 2007). Based on the water pH, diatoms can be categorized into three main groups: acidophiles (pH <7), neutrophiles (pH ~7), and alkaliphiles (pH >7) and can be used to explore freshwater acidification (Hirst et al., 2002). Most diatoms are specialised in a certain pH, but there are a few rare species which are classified as indifferent having no clear pH optimum.

Nutrient (especially phosphorus and nitrogen) availability is one of the most significant variables in shaping aquatic diatom communities. There can be significant variation in nutrient supply between nearby sites and thus diatom community composition and species richness can vary drastically (Soininen and Meier, 2014). In harsh, subarctic environments, the increase in nutrient availability seems more likely to increase species richness rather than decreasing it, which might be the case in already eutrophicated waters (Alahuhta et al., 2019). Based on four general substrate types, total phosphorus tends to be the limiting nutrient in the circumpolar (Figure 6). Epipsammic, and epipelic substrates often accumulate or remove relatively rapidly meaning that the substrate provides and unstable habitat for diatoms. In subarctic ponds the type of substrate is not as important as in streams where turbidity and constant disturbance have an impact on the microhabitats (Eloranta et al., 2007). Due to the lack of macrophytes in the subarctic, most common diatoms are epilithic and epipsammic, which are usually limited by nutrients and light (Vadeboncoeur et al., 2014). Generally speaking, the rapid seasonal change in diatom communities is driven by light availability in the subarctic (Rantala et al., 2017). The rapid ongoing climate change will bring direct effects on the aquatic habitats and thus indirect effects on the diatom communities (Rühland et al., 2015).

Research has shown that population divergence can take place over several hundreds of kilometres, which cannot be explained by dispersal related factors. In this case environmental filtering is more important compared to spatial factors (Teittinen and Soininen, 2015). Diatom distribution takes place mostly in aquatic ecosystems and they are spread through the water column either within the lake or in streams, but can be distributed through fish, birds, mammals, air and insects on larger spatial scales. However, some of these transport methods are rare among diatoms as their cells become dry when in transport. The various methods of distribution could explain the appearance of rare species. Competition and grazing by benthic invertebrates may cause a surge in

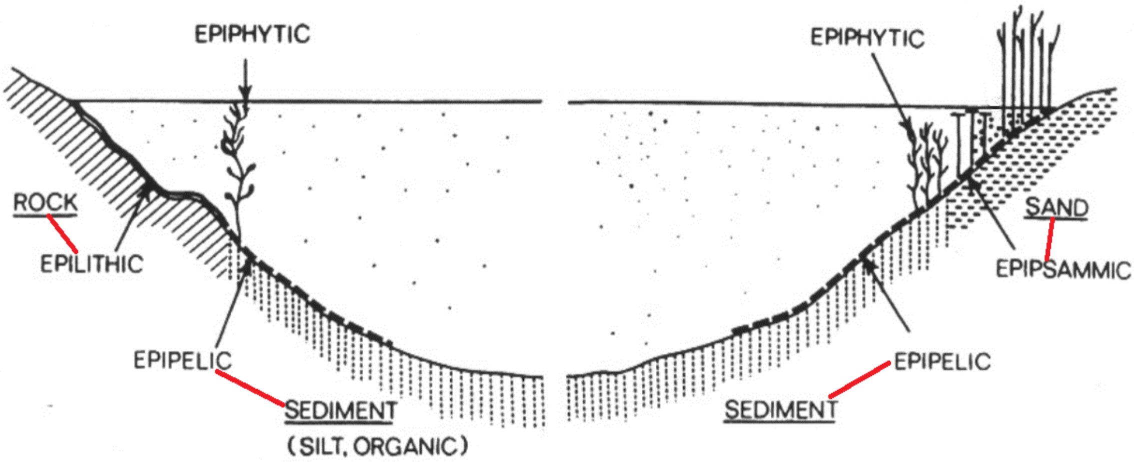


Figure 6. Substrate types for benthic algae (modified from Wetzel, 2001).

rare/uncommon species in the environment. For example, Lowe (1996) demonstrated that pennate diatoms tend to be more sustainable to disturbance, when attached to a substrate. Their unique shape allows them to be more firmly attached, while maintaining enough flexibility for the cell not get damaged. Partly due to this, epilithic diatom sampling offers the best substrate in terms of methodology.

3.2. Morphology & Identification

Diatoms are characterized by an outer frustule made of silica, which allows long term preservation in aquatic environment or deeper in sediments. The cell consists of several parts allowing good preservation and protection for the diatom (Figure 7). The size and shape of the hypotheca is predetermined by the parent-cell. Typically, the cell size decreases after cell-splitting, but in some cases due to the flexible girdle bands, the cell can retain its size and shape through cell-splitting. The morphology of diatoms varies in size, shape and the texture of the outer cell.

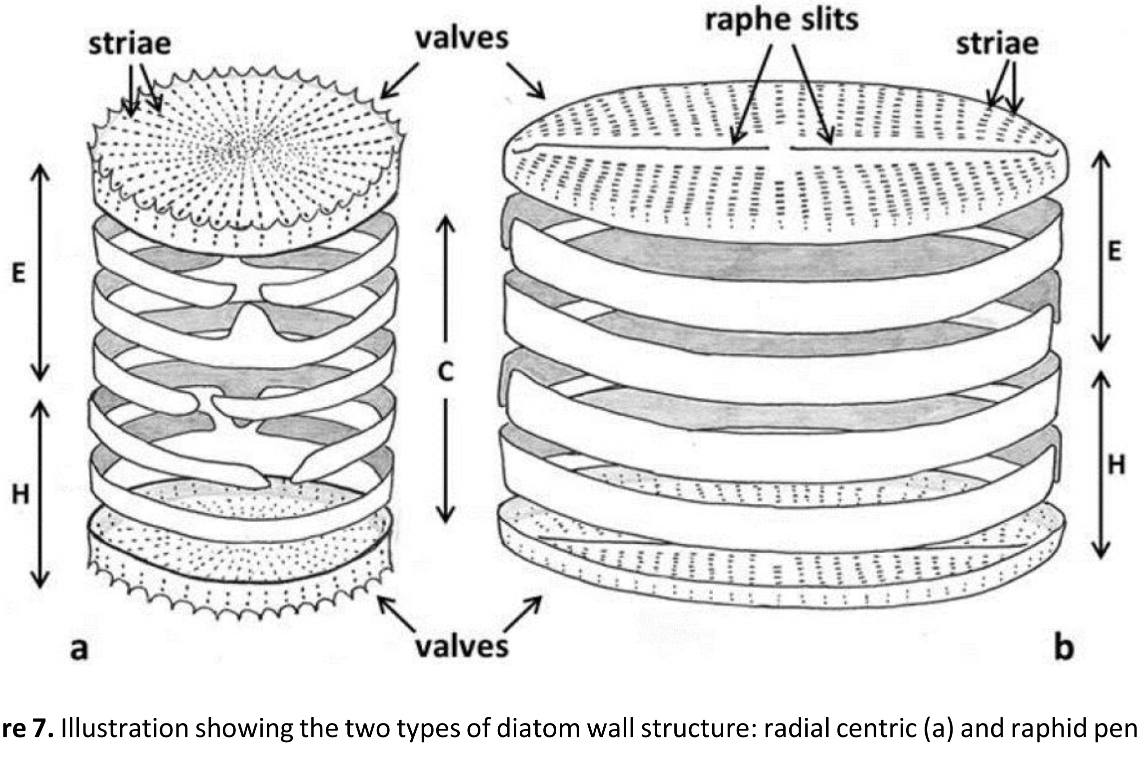


Figure 7. Illustration showing the two types of diatom wall structure: radial centric (a) and raphid pennate (b). Both types consist of an epitheca (E) and hypotheca (H), which are linked with a series of girdle bands (C; modified from Cox, 2014).

Taxonomists have divided the diatoms into several groups based on a combination of valve characteristics and phylogenological data traditionally according to the books by Krammer and Lange-Bertalot (1986-1991). However, with increasing research being done on the subject, new species are being found and researchers have begun to reorganize and rename diatom taxa, which increases the fact that shape and symmetry are not reliable guides for identification (Alverson et al., 2006). Instead of academic literature, many are now found on the internet (Guiry & Guiry, 2020) and as research goes on, the number of diatom species is expected to increase (Soininen, 2007). Better organisation of diatom taxa is required to allow faster, universally recognised and easier identification. Basic identification key includes outlining the location, density and number of striae, amount, shape and length of raphe, and overall shape and size of the frustule. The frustule, spines, girdle bands, striae, and raphe are very frequently formed in the same way meaning that species within a genus share similar basic morphologies (Cox, 2010).

4. Study area

The study area of slightly over 100 km^2 consisted of 100 subarctic ponds around Ailakkavaara, Malla-fjeld in Kilpisjärvi and Skibotn (68°56’58.9” - 69°22’37.9” N; 20°02’42.5” - 21°03’55” E) within the Finnish and Norwegian subarctic (Figures 8 and 9). The area consists of a mixture of marshland, dwarf birch (*Betula nana*)- dominated woodland and steep, rocky fjelds with a mixture of heathland. These areas with annotations to Kauhanen (2013), were used for comparison between biotopes. There were 13 grass heath and rocky, 32 dwarf shrub heathland, seven tree and shrub heathland, 43 mountain birch woodland and five Norwegian areas categorised. The categorisation was mainly done from photographs taken by researchers in the field. For Norwegian sites, there is a higher possibility of anthropogenic influence from traffic, whereas in Kilpisjärvi the potential disturbances are from hikers as some ponds were located near popular hiking paths such as the Malla and Halti trails. Reindeer may have some impact on pond chemistry as a few were spotted at pond sites. The chosen subarctic pond locations were relatively remote and were chosen as they represent a large variability in terms of explanatory variables and topography. The total elevational range used was 879 meters with a minimum of 8 masl and a maximum of 887 masl. However, when excluding the five sample sites from Norway, the elevational range would be 398 meters.

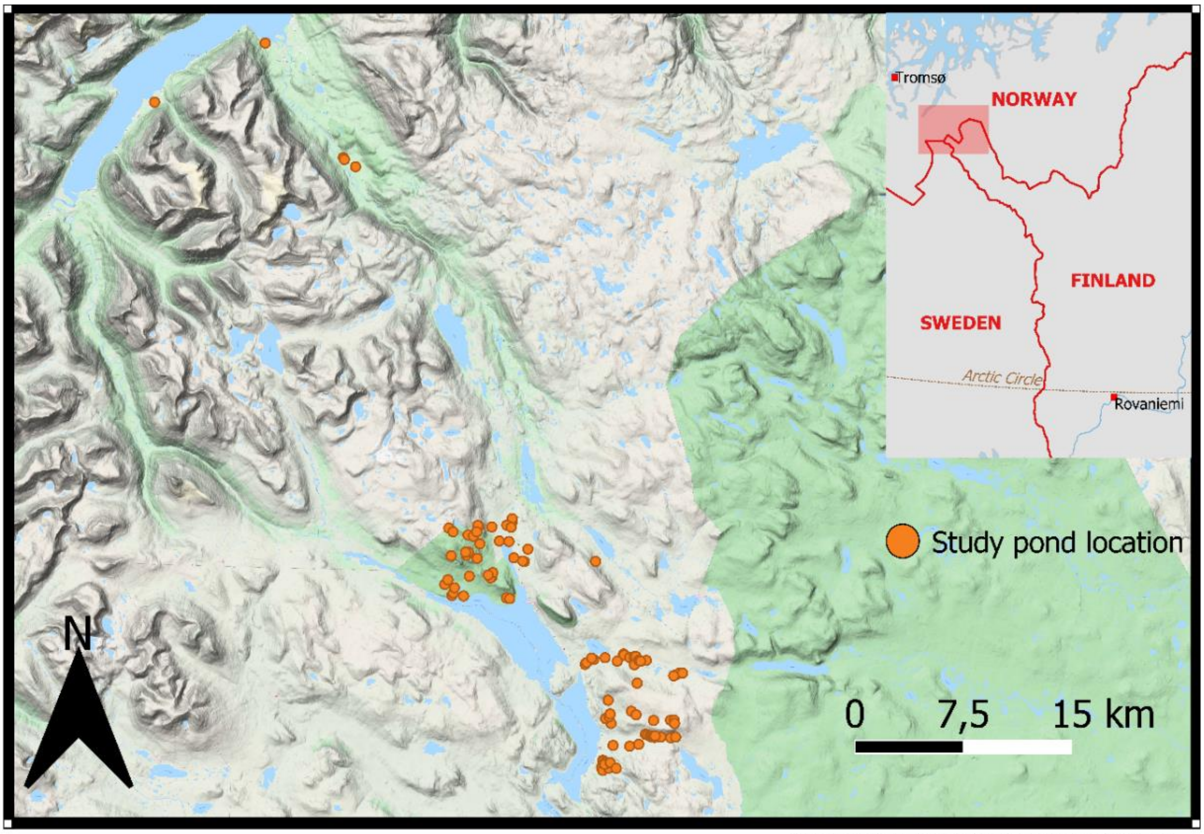


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Figure 8. An overview of the study area showing the 100 subarctic ponds sampled.

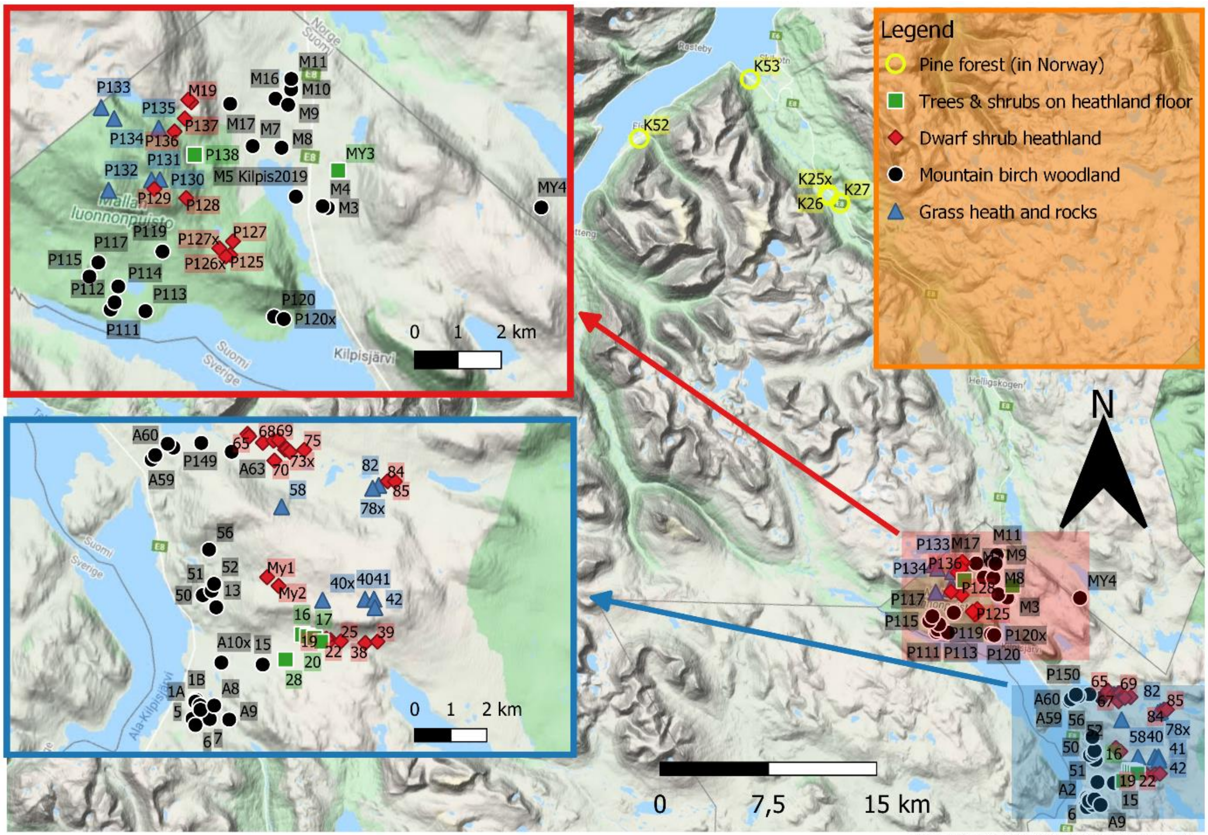

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Figure 9. A detailed map of studied subarctic ponds categorised according to local biotope. The pond ID matches to the ones presented in Appendix 1.

There was some distinctive variation in surface areas of the subarctic ponds (Figure 10). The surface areas of the studied ponds varied between 203m² to 5.43ha. The surface areas and elevations were obtained from satellite images using Google Earth. Air temperature measurements were obtained from the *in-situ* loggers located in the region. The loggers measure air temperature at a two hour interval at a height of 1.5 m from the ground (Aalto, n.d.). The distance to the loggers from the studied ponds varied from 15 m to 3 km, averaging to 795 m over the loggers. The distance to the loggers from the studied ponds varied from 15 meters to over 3 kilometres, giving an average range of 795 meters from the loggers to the subarctic ponds. For ponds located in Norway, the air temperature data was collected from Meteoblue (2020) and an average of the data sampling month (August) was used.

Figure 10. The variation in pond size from 5.43ha (bottom left panel, 50) to 203m^2 (top, My4). Bottom right panel shows the extent of which some subarctic ponds had dried out (A9). The ID numbers are outlined in Appendix 1.

5. Field and laboratory methodology

The pond data were collected in two separate campaigns in 2018 (48 ponds) and 2019 (52 ponds). As all the site locations are nameless, they were and are in this research referred by their given ID-name (see Appendix 1). At each site, *in-situ* pond measurements were taken (pH, water temperature [°C], conductivity [µS/cm]) with an YSI measurement tool. Water chemistry samples for nutrients, anions and cations were collected into a 250 ml bottle and metals were sampled into a 10 ml tube consisting of 0.05 µl of nitric acid [HNO_3] through a 0.45 µm filter. At each site the diatom sampling comprised of collecting ten stones under water, but near the shore. In some locations only a few stones were able to be collected. In these cases, the larger stones were sampled twice or even three times. Diatom samples were scrubbed on a 25 cm^2 surface with a sponge from stones and drained into allocated biofilms for further preservation. The stones were then measured and transformed according to the Wentworth scale (1922). Finally, an overall description of the study site was made with annotations to current weather conditions, macrophytes, shoreline vegetation, coordinates, shore direction, shading, and any additional information. Macrophyte communities were converted into binominal values (1=present, 0=absent) at each site. All water and diatom samples were stored in a fridge (4°C) until analysed at the Department of Geosciences and Geography laboratories at the Kumpula campus, Helsinki University. The overall sampling method used was an adaptation from a globally recognised standard (CEN, 2003). During data collection in 2019, after the diatom samples were collected the *in-vivo* BenthoTorch measurements were taken from each stone. The values at each site were then summed together for cyanobacteria, green algae and diatoms and converted into a percentage value to represent the benthic algal community at each site (see Appendix 2).

For diatom samples a universally standardized method, SFS-EN 13946 was used (Eloranta et al., 2007). The aim was to count and identify at least 500 valves per sample using a light microscope. As in some samples more than 500 valves were counted, relative abundances of species were used. Due to difficulties in species identification, some species were left to genus level and were marked in the data as sp. (species plural). Taxa were identified first according to literature by Krammer and Lange-Bertalot (1986-1991) and later the taxonomic names were changed according to Guiry & Guiry (2020) to match their currently accepted taxonomic names and given an abbreviation before statistical analysis.

The nutrient concentration analysis was conducted according to standardized methods, which are EN1189 (1996) for total phosphorus (TP) and EN ISO11905-1 (1998) for total nitrogen (TN). For the analysed nutrients, the TN values are based on the ratio of the two measurements done in wavelengths 275nm and 220nm. Normally this ratio should not exceed 5%, but as it was expected from these near pristine subarctic ponds, the TN values are very small which enhances the risk of disturbance, and thus all values were included in the statistical analyses despite their disturbance. For TP measurements, the limit of determination is 0.005 mg/l. A total of 25 samples fell under this value and were thus marked as 0.004 to indicate very small values of TP. An ion chromatography – machine was used to analyse the amounts of cations (Na [mg/l], K [mg/l], Ca [mg/l], Mg [mg/l]) and anions (Cl [mg/l], NO_3 [mg/l], SO_4 [mg/l], Alkalinity [mmol/l]). The information was then used to calculate the ion balances for each subarctic pond as ratios indicate phosphorus rich waters (Williams and Wacker, 1967). Mineral composition (Al [ppb], Si [ppm], P [ppb], V [ppb], Cr [ppb], Mn [ppb], Fe [ppb], Co [ppb], Ni [ppb], Cu [ppb], Zn [ppb], As [ppb], Se [ppb], Mo [ppb], Cd [ppb], Pb [ppb], U [ppb]) was conducted with an inductively coupled plasma-mass-spectrometer. All nutrient and mineral concentrations were converted into mg/l before statistical analyses. Not all nutrients, heavy metals, anions and cations were used to model diatom species richness and community composition as they were either not detected due to small amounts or their role is insignificant for diatoms (see Appendix 1).

6. Statistical analyses

Computer based numerical analysis was performed in R-software version 4.0.1, which enables multivariate statistical testing between species richness, species composition, and local physio-chemical variables (Pajunen et al., 2017). For the taxonomic diatom data, total valve count was marked next to each sampled pond (see Appendix 1). For individual elevational range of the taxa, the data were arranged from highest to lowest according to mean elevation. A simple error bar diagram was performed in R through the errbar-function in hmisc-package to visualise community composition along elevational gradient (Teittinen et al., 2016). Before analysis, the data (excluding altitude and pH) was logarithmically converted ($\log_{10}(x+1)$), so the data would resemble better a normally distributed data (Figure 11). Some chemical variables were directly incorporated in the analysis as they have been found to have a significant impact on diatom communities such as

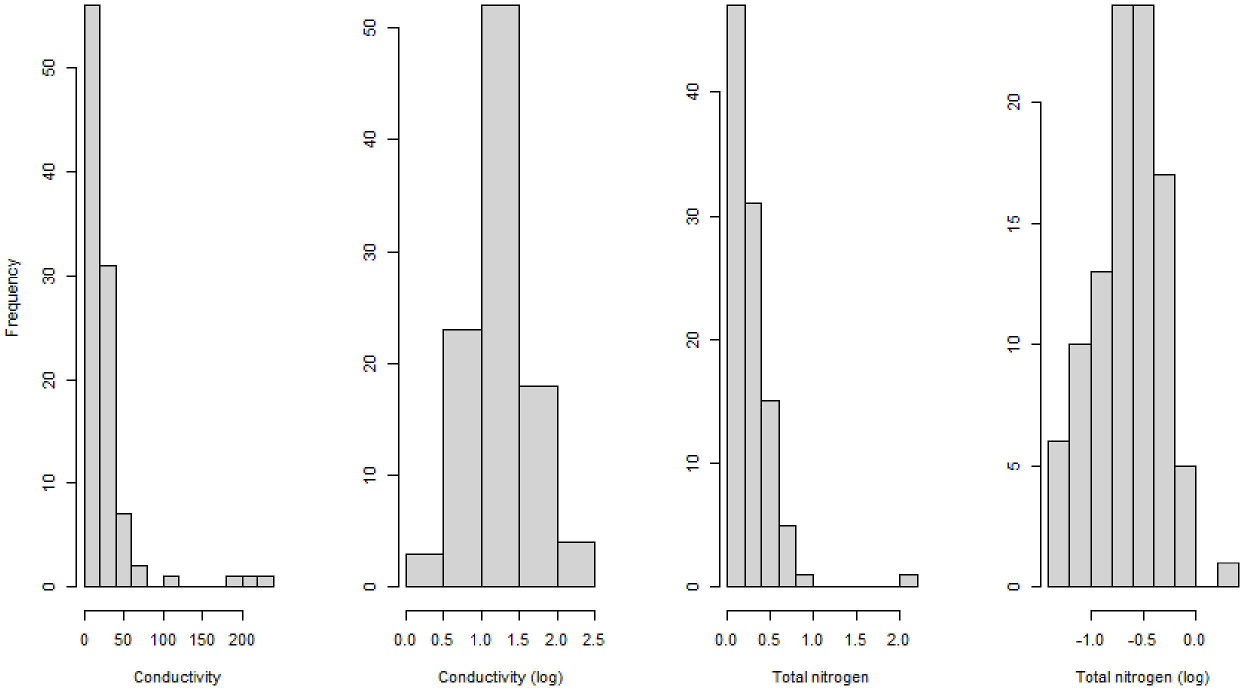


Figure 11. For explanatory variables a logarithmic conversion was made. Here conductivity and total nitrogen show how the conversion affects the distribution. In some cases, the resulting figure did not perfectly resemble normally distributed data.

manganese, silica and iron (Hutchins and Bruland, 1998; Passy, 2010). Other minerals were selected as they showed high variation between the studied ponds (e.g. aluminium). Once these variables were selected, they were checked for multicollinearity. Multicollinearity means that two or more explanatory variables are strongly correlated between one another, meaning that they contribute equally to the same outcome, which may lead to inaccurate and unreliable results. The char.correlation-function within the PerformanceAnalytics-package, provides a table of correlations and statistical significance of all explanatory variables. A simple Spearman's correlation method was used. If the relationship between two variables exceeded ±0.8, they were referred as strongly multicollinear. Other correlation coefficients above ±0.5 would be considered as moderate and values under ±0.5 were treated as low or very low. In this research, multicollinearity was taken into account by using a variance inflation factor (VIF). Explanatory variables are not independent enough to explain the factor if the VIF-value would exceed four. The vif-function is a tool within the car-package in R.

Spatial autocorrelation analysis shows how identical nearby sites are and how similarity decreases with increasing scale. A positive spatial autocorrelation indicates similar values in ponds near each other, which is typical in ecological research (Legendre and Fortin, 1989). Due to spatial autocorrelation, statistical analyses such as correlation and regression would otherwise show significant values despite being not significant. The raw data for explanatory variables and species richness was autocorrelated before any multivariate or correlation analyses. Spatial autocorrelation is done through null hypothesis i.e. there is no autocorrelation between sites. The analysis is performed with a Moran's I coefficient, which performs correlation analysis for pairwise sites (Nurhayati and Pasaribu, 2006). The result is a correlogram, showing the autocorrelation values in relation to the distance between the sites. In R software, a correlog-function in the ncf-package was used to calculate the autocorrelation between sites. A Bonferroni criterion was made ($\alpha'=\alpha/k$), where α = statistical significance (0.05) and k = number of distance classes (10). If all values are statistically significant the correlogram can be accepted.

Generalized linear modelling is a multivariate method for non-normally distributed data, which in this thesis is used to explain the variation in species richness between subarctic ponds. As all of the data collected was positive and had no maximum values, the richness distribution was analysed through Poisson distribution. In the initial model all variables with their quadratic terms were used and analysed to see whether there were any single order values. In the final model, a stepAIC-function from the MASS-package was used to determine the final terms for each variable. This method chooses a model with the smallest Akaike's information criterion (AIC) value, creating the most simplified model yet a one that has good explanatory power. Based on this method and from individual determination of p-values, a final model was built. The coefficient of determination was calculated for each model to see which one explains the variation in species richness the best. The final model was then run through a variety of model tests to quantify the model. The residualPlots-function from the car-package in R generates residual plots, which can be used to detect non-linearity. Confidence regions provide a simultaneous measure of the precision of parameter estimates (Weld et al., 2019). Another method for model validation was done through influenceIndexPlot-function in the car-package. This function provides Cook's distance values, which measure the influence of each observation in the given model, i.e. what would happen if each single observation was excluded from the model (Banerjee, 1998). As a general rule, if a single observation is more than three times the mean, it can be detected as an outlier (Glen, 2016). Finally, the role of each individual variable used in the final model was interpreted through hierarchical partitioning. The hier.part-function and -package provides percentage values on how strongly a single explanatory variable affects diatom species richness.

The relationship between diatom species composition and environmental variables was analysed using redundancy analysis (RDA) within the vegan-package. A principal component analysis ordination method was done for explaining study pond differences. In terms of species, relative amounts with a Hellinger transformation were used. A Hellinger transformation for species data provides more accurate degree of determination as it decreases the length of the ordination axes (Ruokolainen and Blanchet, 2014). The ordination biplot illustrates the location of the object in relation to a series of response arrows. When discussing the significance of the biplot, it is important to note the eigenvalues of the arrows and the coefficient of determination. Even though the ordination plot would explain a small portion of the change in species richness, the diagram still might be very informative. The ordination plot shows the taxa or sites as point values and their distance from one another can be determined qualitatively by the arrows. Therefore, sites or taxa which appear close to each other in the plot favour similar biotic and abiotic conditions. The longer the explanatory arrows shown in the ordination are, the stronger its influence is on community composition or on individual diatom taxa.

Non-metric multidimensional scaling is an analytical method to compare environmental factors and diatom taxa. As there was no clear spatial structure in the data, the study sites were split here according to biotope type following Kauhanen (2013), leading to two-dimensional scaling. The ordihull-function provides visual analysis between the biotopes, whereas the orditorp-function performs biplots between explanatory variables and individual diatom taxa. Numerical analysis for RDA was constructed and possible differences between the groups of sites in different biotopes were tested using decorana-, decostand-, anova.cca- and rda-functions. The decorana- and decostand-functions were used to check the length of the axes. If the value would be above 2, the gradient is unimodal. The anova.cca-function was used to verify whether the final RDA model was statistically significant. Analysis of similarities (anosim) was used to quantify the similarities or dissimilarities of diatom species richness and community composition between the different biotopes. The result will provide a numerical value between -1 and 1 according to the null hypothesis, which assumes no variation between sites. When the values are near 0, the null hypothesis can be accepted so that there appears to be no significant similarities between the sites.

Negative values indicate that the average of the rank dissimilarities is greater within groups compared to between them (Anderson and Walsh, 2013). Between and within group rank dissimilarities were observed from the distributional summaries' tables from minimum to maximum. A Mantel test was performed for community composition to see whether it changes with spatial and elevational distance. The vegdist-function in the vegan-package provides with point data on how communities vary with spatial and elevational distances, provided with either coordinates or altitude. In both cases pairwise Euclidean distance and 9999 permutations was used. The resulting plot will show community similarities decrease with increasing spatial distance and elevational distance.

7. Results

In-situ water pH ranged from very strongly acidic (4.69) to moderately alkaline (8.1) between the subarctic ponds (Table 2). Based on total phosphorus concentrations 91% of the subarctic ponds studied are classified as ultraoligotrophic, 6% as oligotrophic and 4% as mesotrophic (see Table 1). The highest levels of total phosphorus and total nitrogen were found at low elevations. The ratio between total nitrogen and total phosphorus (i.e. N:P ratio) varied between 1 and 152 with an average of 35. From the explanatory variables, air temperature, conductivity, calcium and sulphate showed the greatest variation with elevation (Table 3). All of the mentioned variables decrease strongly with increasing elevation. Out of all the explanatory variables used in the final model only ion balance was not spatially autocorrelative as it did not show significance in any of the allocated distance classes. The value for ion balance was not significant after the Bonferroni criterion was adjusted (p= 0.05/10 = 0.005). All other variables were spatially autocorrelative either in one distance class or in several (Figure 12). For example, pond temperature was spatially autocorrelative in three distance classes, which means the correlogram was found to be statistically significant.

Table 2. Explanatory variables used in this study to explain species richness and community composition.

Explanatory variables	Minimum	Maximum	Mean	Median	Standard deviation
Elevation (masl)	8	887	596	613	164
Surface area (m^2)	203	54 279	4 653	1 769	8 160
Conductivity (µS/cm)	0	222	27	19	36
Ion balance, IB (%)	-17	84	16	10	21
Water temperature (°C)	4.8	18.0	12.2	12.0	2.5
Air temperature (°C)	7.7	14.6	10.4	10.3	1.8
pH	4.69	8.18	6.54	6.51	0.85
Total phosphorus, TP (mg/l)	0.004	0.074	0.014	0.008	0.015
Total nitrogen, TN (mg/l)	0.050	2.010	0.300	0.230	0.250
Sodium, Na (mg/l)	0.001	9.357	1.021	0.845	1.199
Sulphate, SO_4 (mg/l)	0.001	27.436	1.272	0.393	3.148
Silicon, Si (mg/l)	0.001	4.031	1.448	1.255	1.228
Aluminium, Al (mg/l)	0.001	147.006	47.955	40.965	32.535
Chlorine, Cl (mg/l)	0.001	16.710	0.985	0.791	1.833
Calcium, Ca (mg/l)	0.001	35.457	2.418	0.506	5.692
Iron, Fe (mg/l)	0.001	291.736	28.142	13.987	44.354
Manganese, Mn (mg/l)	0.001	36.060	2.094	0.613	4.477

Table 3. Explanatory variables that had the most significant correlation with elevation. These were conductivity (r= -0.7), air temperature (r= -0.65), calcium (r= -0.68) and sulphate (r= -0.64).

Explanatory variable	0-100 masl	400-600 masl	600-900 masl
Conductivity (µS/cm) avg	159	25	16
Conductivity (µS/cm) min	73	5	0
Conductivity (µS/cm) max	222	69	59
Air temperature (°C) avg	14.55	11.15	9.40
Air temperature (°C) min	14.55	9.04	7.70
Air temperature (°C) max	14.55	12.63	12.22
Calcium, Ca (mg/l) avg	23.289	1.764	0.935
Calcium, Ca (mg/l) min	6.931	0.001	0.001
Calcium, Ca (mg/l) max	35.457	8.572	3.899
Sulphate, SO_4 (mg/l) avg	11.634	1.066	0.435
Sulphate, SO_4 (mg/l) min	4.708	0.001	0.001
Sulphate, SO_4 (mg/l) max	27.436	4.708	1.796

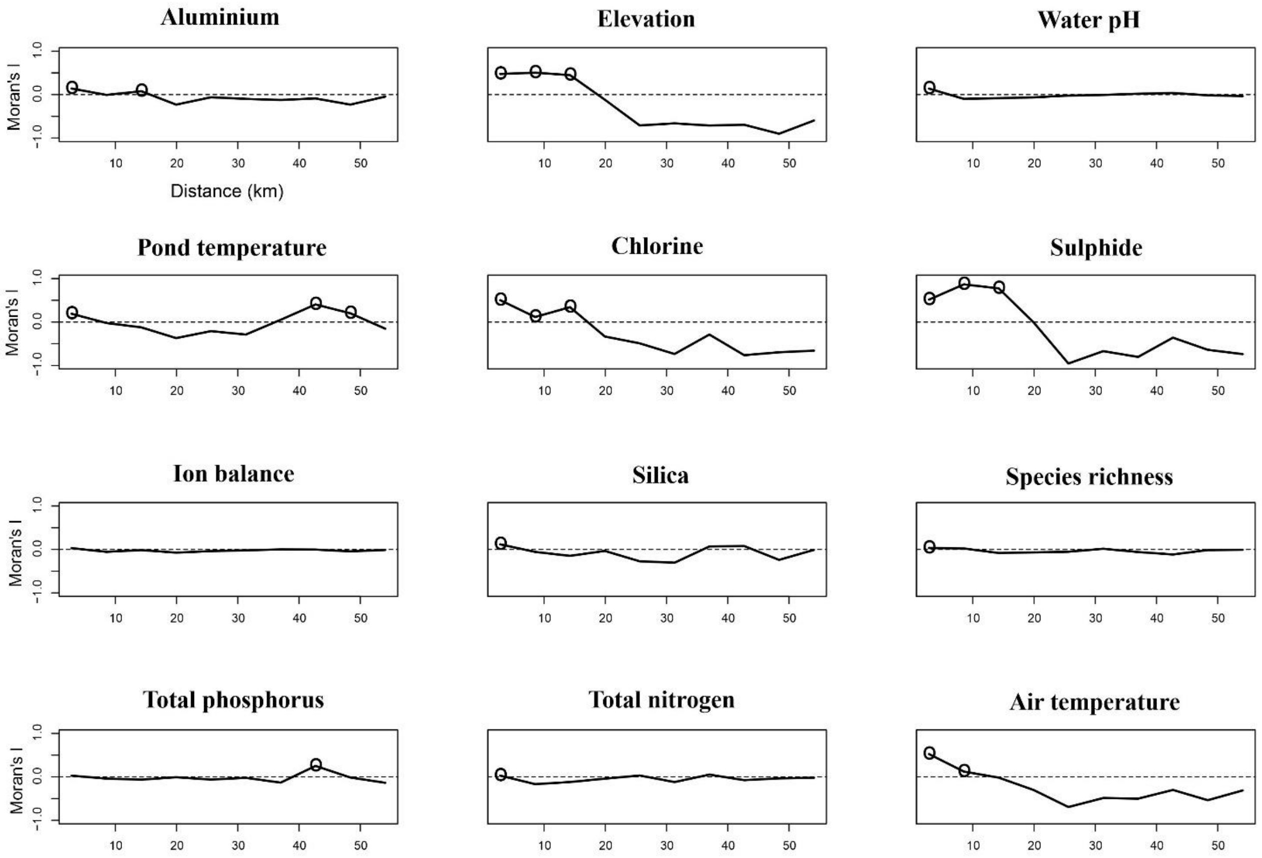


Figure 12. Species richness, explanatory variables and their spatial autocorrelations. Circles indicate statistically significant values after Bonferroni's criterion. The correlogram was significant if one or more distance class showed statistical significance.

Based on Spearman's correlation coefficient, the only strong intercorrelations were between sodium, calcium, conductivity and chlorine (Table 4). Sodium (r= 0.81) and calcium (r= 0.94) correlated strongly with conductivity but did not show statistical significance ($p > 0.05$). Based on this, sodium and calcium were excluded from the upcoming analysis. All strongly correlative variables had a positive correlation with each other. The same was true also between sodium and calcium (r= 0.8) and chlorine and sodium (r= 0.92). There were some very significant ($p < 0.001$) correlations between the explanatory variables with moderate correlations for instance between elevation and conductivity (r= -0.7), air temperature and elevation (r= -0.65), and most anions and cations with elevation. All these moderate correlations were negative. When examining the VIF-values of the model which included all explanatory variables, the maximum value was 198.7 for sodium. After the strongly multicollinear variables were removed and a final model was established, the maximum VIF-value was 3.6 for chlorine making it acceptable.

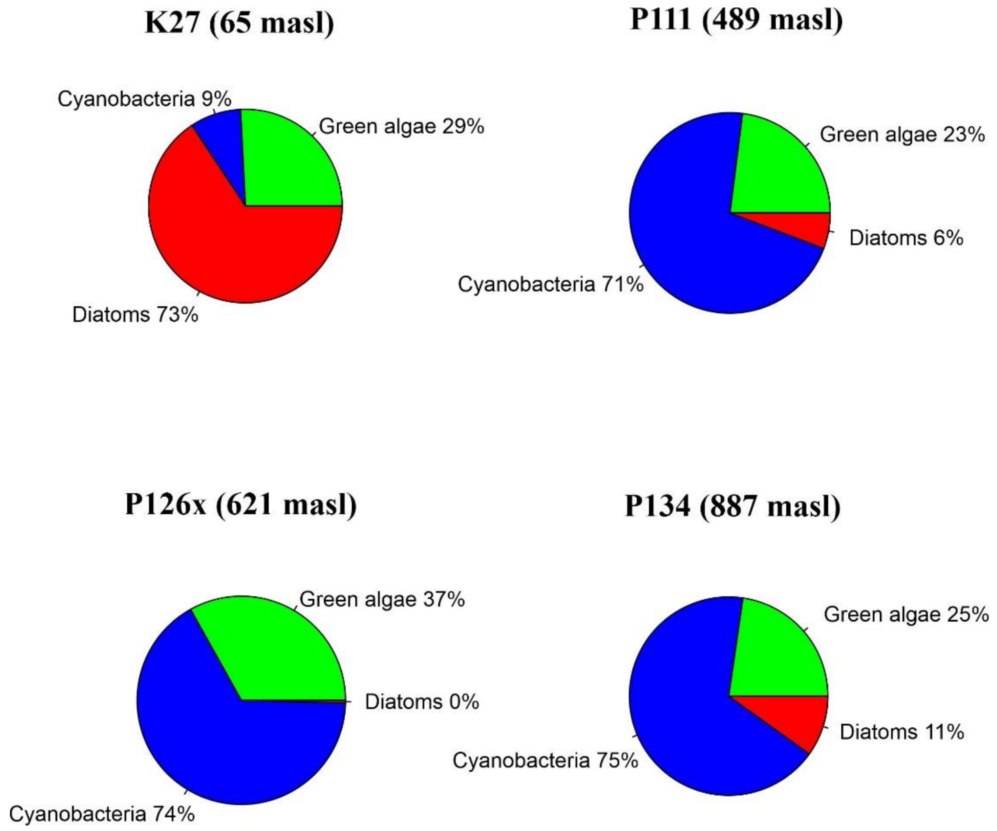
Table 4. Environmental variables and their correlations (r) and statistical significances based on p-values: not significant (-), $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). A strong correlation (±0.8) is indicated as bold numbers.

	Elevation	Surface area	pH	Conduct	Pond temp.	Cl	SO4	Na	Ca	IB	Al	Fe	Mn	Si	Richness	TP	TN	Air temp.
Elevation		-	-	***	-	***	***	***	***	-	-	*	***	-	-	*	**	***
Surface area	-0.06		*	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-
pH	0.13	0.25		-	***	-	-	-	-	-	-	**	***	***	-	-	**	***
Conduct	-0.70	0.00	0.13		*	-	***	-	-	**	**	*	***	*	-	-	-	-
Pond temp.	0.00	-0.17	-0.33	-0.21		-	-	-	*	-	-	-	-	***	**	-	**	***
Cl	-0.63	-0.11	-0.13	0.78	0.13		***	-	-	-	-	***	-	-	-	-	-	***
SO4	-0.64	-0.03	0.05	0.57	-0.08	0.36		***	***	-	*	-	-	-	-	-	-	***
Na	-0.66	-0.11	-0.03	**0.81**	0.06	**0.92**	0.54		-	-	*	***	***	-	-	-	-	***
Ca	-0.68	-0.08	0.05	**0.94**	-0.20	0.76	0.54	**0.80**		-	*	*	***	-	-	-	-	***
IB	0.08	-0.21	-0.51	-0.26	0.16	-0.03	-0.14	-0.05	-0.10		**	-	-	***	-	-	-	-
Al	0.02	-0.16	-0.09	-0.28	0.04	-0.18	-0.21	-0.20	-0.24	0.26		*	-	***	*	-	-	-
Fe	-0.23	-0.19	-0.28	0.24	0.14	0.54	-0.02	0.41	0.24	0.18	0.21		-	-	-	-	***	-
Mn	-0.42	-0.17	-0.34	0.48	0.06	0.77	0.08	0.62	0.48	0.15	0.15	0.76		-	-	-	**	***
Si	-0.01	0.17	0.48	0.28	-0.45	0.05	0.19	0.15	0.19	-0.43	-0.33	-0.10	-0.07		-	-	***	*
Richness	-0.03	0.03	-0.06	0.09	0.26	0.07	0.00	0.06	0.08	0.04	-0.23	-0.06	0.02	-0.05		-	-	-
TP	-0.22	-0.03	-0.04	0.09	0.13	0.18	0.08	0.18	0.06	0.00	0.00	0.04	0.08	-0.13	-0.11		-	**
TN	-0.27	-0.12	-0.26	0.07	0.31	0.17	-0.01	0.15	0.01	0.03	0.18	0.43	0.28	-0.34	0.16	0.13		**
Air temp.	-0.65	-0.19	-0.52	0.46	0.37	0.56	0.45	0.54	0.47	0.12	-0.10	0.16	0.37	-0.24	0.13	0.30	0.26	

Over the 52 subarctic ponds most showed very low values for diatom biomass (< 4 µg/cm²). Only one sample location showed a value of over 4 µg/cm². In general diatoms showed the smallest biomass with a mean value of nearly two times lower compared to green algae and five times smaller compared to cyanobacteria (Table 5). Cyanobacteria showed the largest range over the three groups with a range of 13.62 µg/cm². The other two groups only had a range of 4.08 µg/cm² for green algae and 4.57 µg/cm² for diatoms. There is great variation in the proportions of benthic algae as measured with the BenthoTorch between the subarctic ponds with elevation (Figure 13). The portion of diatom biomass dominated over cyanobacteria and green algae at four pond locations. On the rest of the locations, either cyanobacteria or green algae or both were more dominant. Correlation between chlorophyll-a values and diatom species richness was weak between all benthic algal groups. The strongest correlation was between green algae and diatom species richness (r= -0.09). Cyanobacteria and diatom chlorophyll-a values showed slight positive correlations (r= 0.06 for diatoms and r= 0.02 for cyanobacteria).

Table 5. Chlorophyll-a statistics obtained from the values measured by the BenthoTorch

Benthic algae (µg/cm^2)	Minimum	Maximum	Mean	Median	Standard deviation
Green algae	0.02	4.10	1.37	1.04	1.00
Cyanobacteria	0.32	13.94	3.46	2.22	3.06
Diatoms	0.00	4.57	0.69	0.33	0.93



K27 (65 masl)

P111 (489 masl)

P126x (621 masl)

P134 (887 masl)

Figure 13. Percentage values from the portions of chlorophyll a for each benthic algae (cyanobacteria, green algae and diatoms) measured by the BenthoTorch. The four sites were chosen to represent a large spatial and elevational variation over the study area in terms of the portions of benthic algae.

7.1. Patterns in diatom community composition

A total of 357 diatom species were identified with 10 taxa remained at genus level. At most there were 74 species identified at a single pond and 19 at the least. On average there were 41 species per pond. Many of the identified diatom taxa appeared as rare. Out of the 357 identified diatom taxa, 92 were detected in only one pond. From these rare taxa, 58 were singletons. A total of 23 species (6.4%) covered the entire elevational gradient (Figure 14). The average elevation of the gradient at Kilpisjärvi was located at 688 masl, whereas the mid-point average for all taxa was located at 615 masl. As established earlier, there are several locations, where only few diatom species were found. This is why the graph shows several single dots as the taxa only appears at a single elevational point.

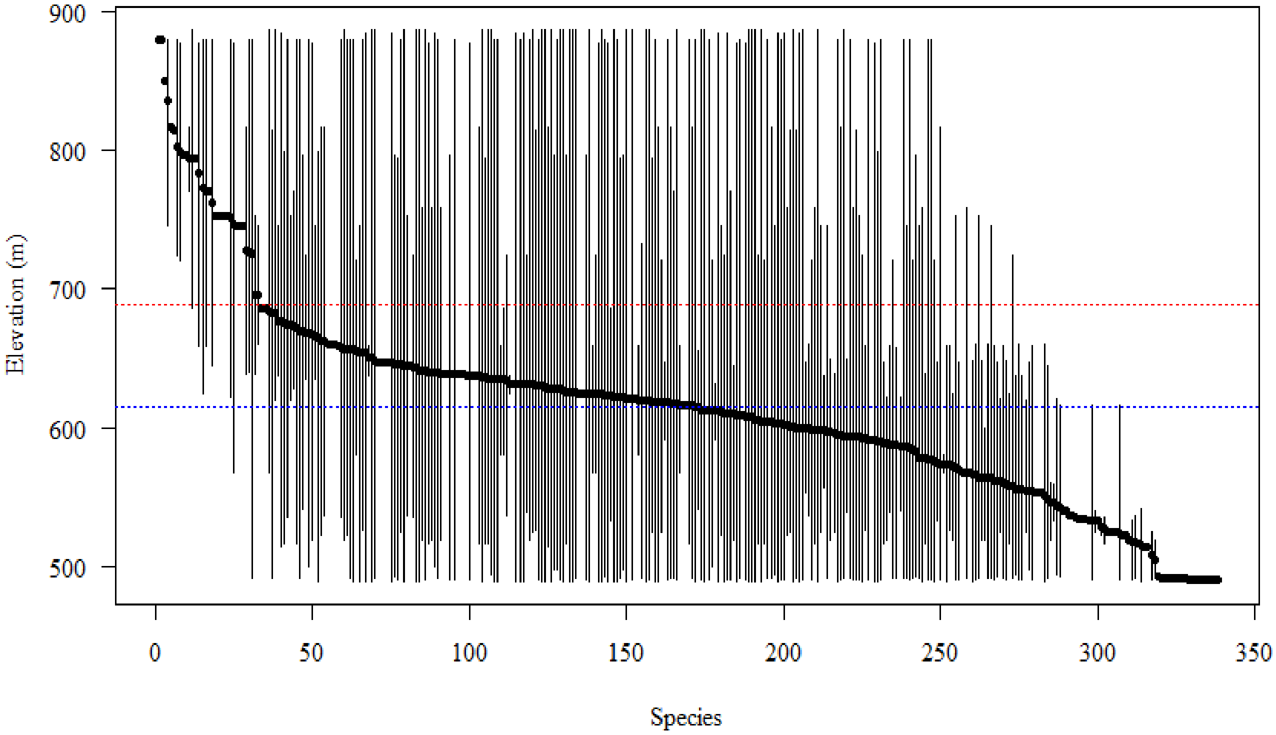
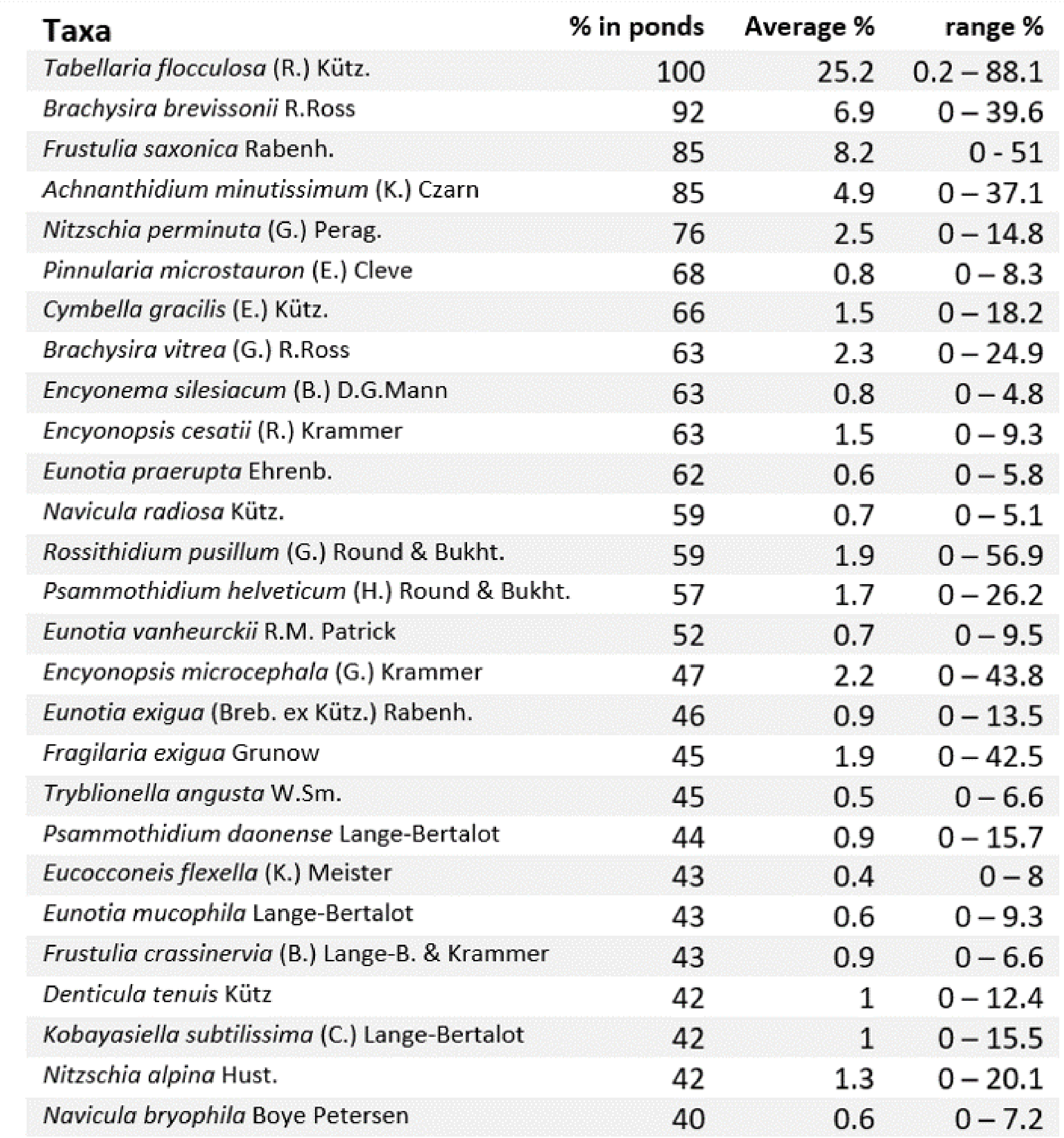


Figure 14. The range and mid-points of diatom taxa in Kilpisjärvi region. The red dotted line indicates the mid-elevational point at Kilpisjärvi region (688 masl) and the blue dotted line indicates the mid-point average of species.

Tabellaria-genus represented 26.1% of all the valves counted (51 046 total valves) combined from all study locations. The other relatively high abundant genera were *Brachysira* (11.0% of all cells), *Frustulia* (9.3%), *Nitzschia* (7.2%), *Eunotia* (6.0%), and *Achnanthidium* (5.5%). From the *Tabellaria*-genus, four taxa were identified with *Tabellaria flocculosa* (25.5% out of all identified valves) mainly explaining the high abundance of the *Tabellaria*. *Tabellaria flocculosa* was the only species that was present in every subarctic pond sample (Table 6). At most, *Tabellaria flocculosa* consisted of 88.1% of all identified species in a single pond. In 16 sample locations, *Tabellaria flocculosa* covered over 50% of the identified diatom taxa.

Table 6. The most common diatom taxa found at least in 40% of the study ponds. Average indicates the mean relative abundance over all counted taxa and the range shows the minimum and maximum of relative abundances at a site of a taxa.



Taxa	**% in ponds**	**Average %**	**range %**
Tabellaria flocculosa (R.) Kütz.	100	25.2	0.2 – 88.1
Brachysira brevissonii R.Ross	92	6.9	0 – 39.6
Frustulia saxonica Rabenh.	85	8.2	0 - 51
Achnanthidium minutissimum (K.) Czarn	85	4.9	0 – 37.1
Nitzschia perminuta (G.) Perag.	76	2.5	0 – 14.8
Pinnularia microstauron (E.) Cleve	68	0.8	0 – 8.3
Cymbella gracilis (E.) Kütz.	66	1.5	0 – 18.2
Brachysira vitrea (G.) R.Ross	63	2.3	0 – 24.9
Encyonema silesiacum (B.) D.G.Mann	63	0.8	0 – 4.8
Encyonopsis cesatii (R.) Krammer	63	1.5	0 – 9.3
Eunotia praerupta Ehrenb.	62	0.6	0 – 5.8
Navicula radiosa Kütz.	59	0.7	0 – 5.1
Rossithidium pusillum (G.) Round & Bukht.	59	1.9	0 – 56.9
Psammothidium helveticum (H.) Round & Bukht.	57	1.7	0 – 26.2
Eunotia vanheurckii R.M. Patrick	52	0.7	0 – 9.5
Encyonopsis microcephala (G.) Krammer	47	2.2	0 – 43.8
Eunotia exigua (Breb. ex Kütz.) Rabenh.	46	0.9	0 – 13.5
Fragilaria exigua Grunow	45	1.9	0 – 42.5
Tryblionella angusta W.Sm.	45	0.5	0 – 6.6
Psammothidium daonense Lange-Bertalot	44	0.9	0 – 15.7
Eucocconeis flexella (K.) Meister	43	0.4	0 – 8
Eunotia mucophila Lange-Bertalot	43	0.6	0 – 9.3
Frustulia crassinervia (B.) Lange-B. & Krammer	43	0.9	0 – 6.6
Denticula tenuis Kütz	42	1	0 – 12.4
Kobayasiella subtilissima (C.) Lange-Bertalot	42	1	0 – 15.5
Nitzschia alpina Hust.	42	1.3	0 – 20.1
Navicula bryophila Boye Petersen	40	0.6	0 – 7.2

7.2. Variables explaining diatom community variation

Aluminium and pond temperature had statistically significant correlation with species richness with pond temperature having the highest correlation (Figure 15). The correlation is positive, meaning that as the temperature in the subarctic ponds rises, the species richness increases. Elevation did not have a significant role in explaining species richness. The generalized linear model including all explanatory variables explained 42.43% of variation in species richness and its AIC-value was 795.47.

Once the multicollinear variables (Ca and Na) were removed, the model explained 42.2% of the variation and its AIC-value was 788.12. The final model with all significant explanatory variables apart from elevation explained 34.17% of diatom species richness variation and its AIC-value was the lowest, 775.06. Explanatory variables in this model included latitude, elevation, pH, pond temperature, macrophytes, ion balance, aluminium, silica, total phosphorus, air temperature, chlorine, sulphide and total nitrogen. In this model pH, aluminium (Figure 16), ion balance and sulphite also included second degree terms. Out of the included explanatory variables, no non-linearity was detected in the final model when run through influence index plots. Based on Cook's distances on influence index plots (Figure 17), there were 10 observations that exceeded the threshold of 0.088. According to hierarchical partitioning the highest independent effect from an explanatory variable on species richness was pond temperature (20.8%) with aluminium being the second most influential with 15.5%. All other variables had an independent effect of under 10% (Figure 18).

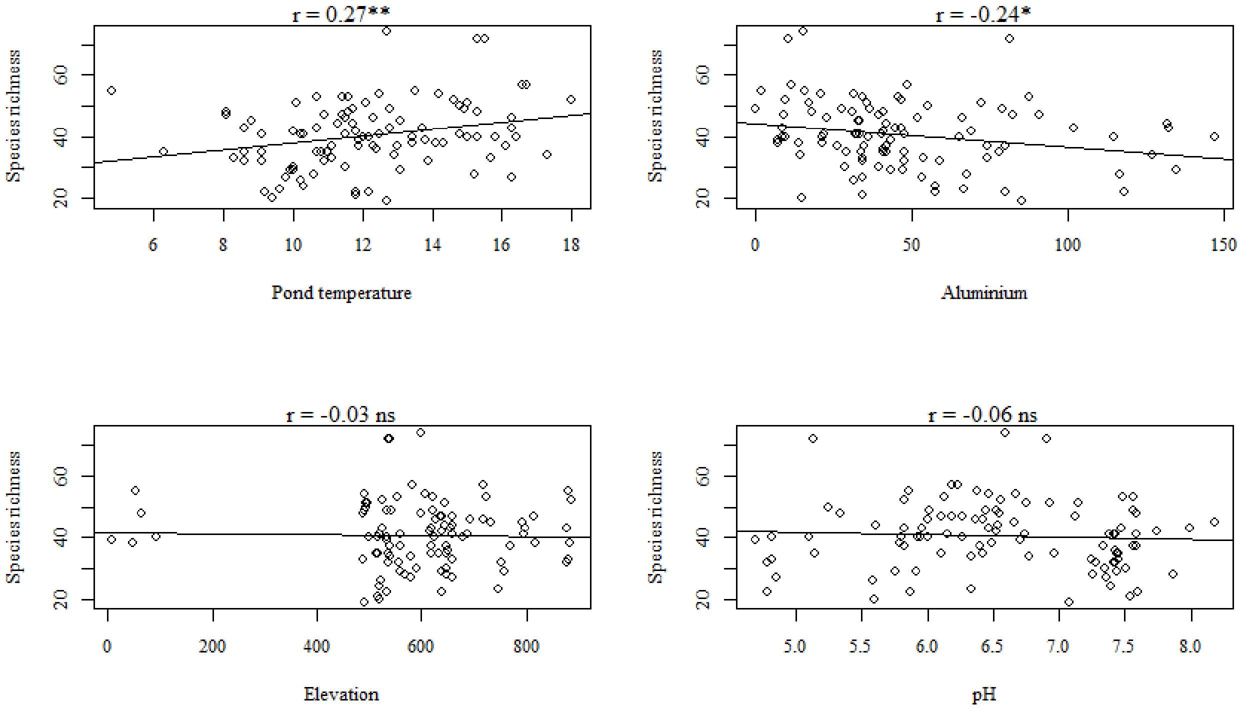


Figure 15. Scatterplots of the most significantly correlative variables with species richness (pond temperature [$p < 0.01$**] and aluminium [$p < 0.05$*]). Also showing elevation and water pH with both non-significant (ns) correlation with species richness.

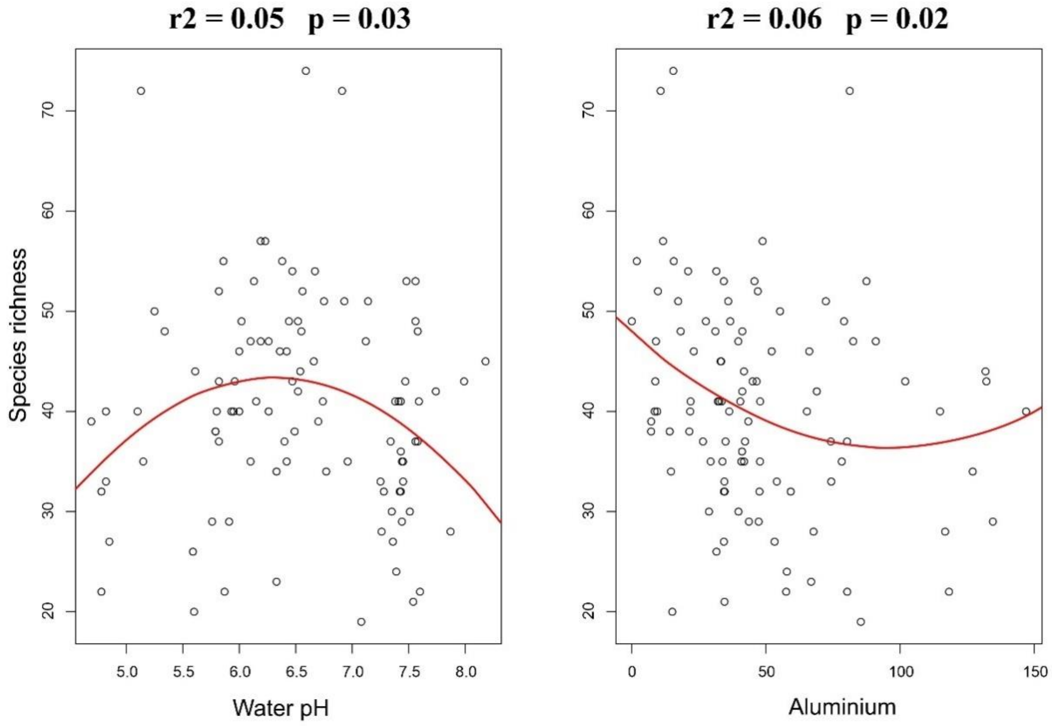


Figure 16. Species richness against pH and aluminium with second degree terms showing the response curves as a trend line.

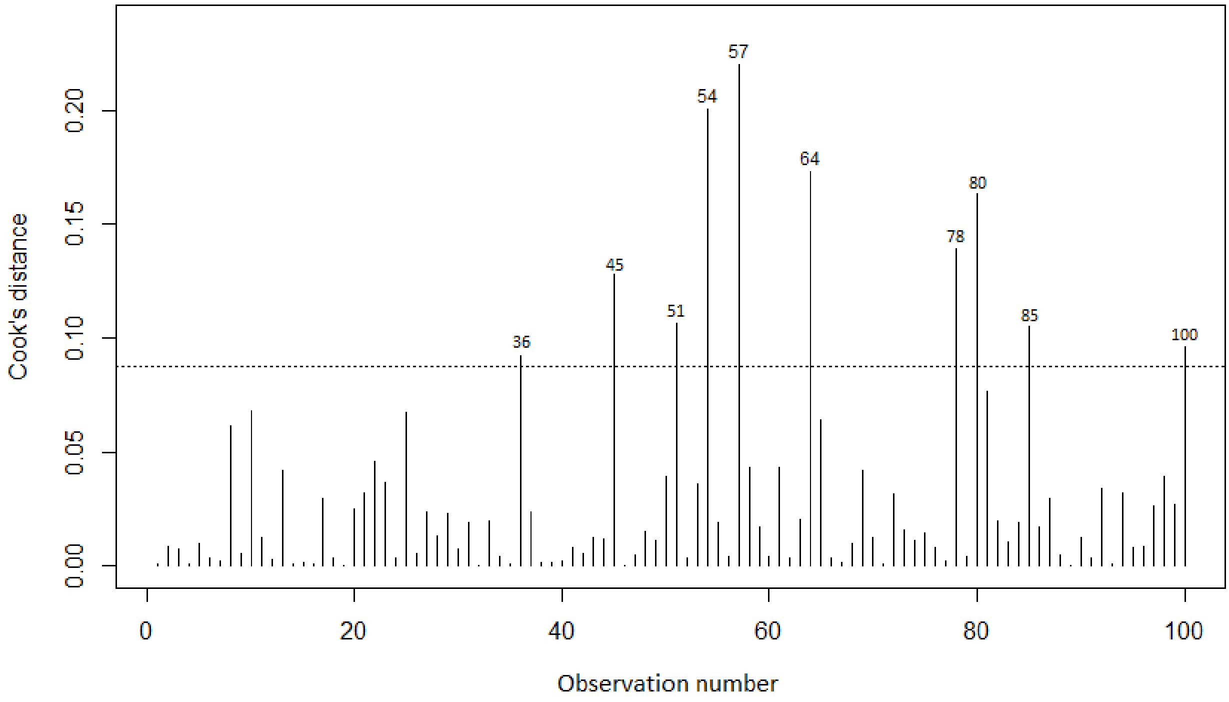


Figure 17. Model validation based on Cook's distances for each study location. The dotted line indicates the threshold of 0.088, which is three times the mean. Observation number refers to a subarctic pond, which corresponds to the numbers in Appendix 1.

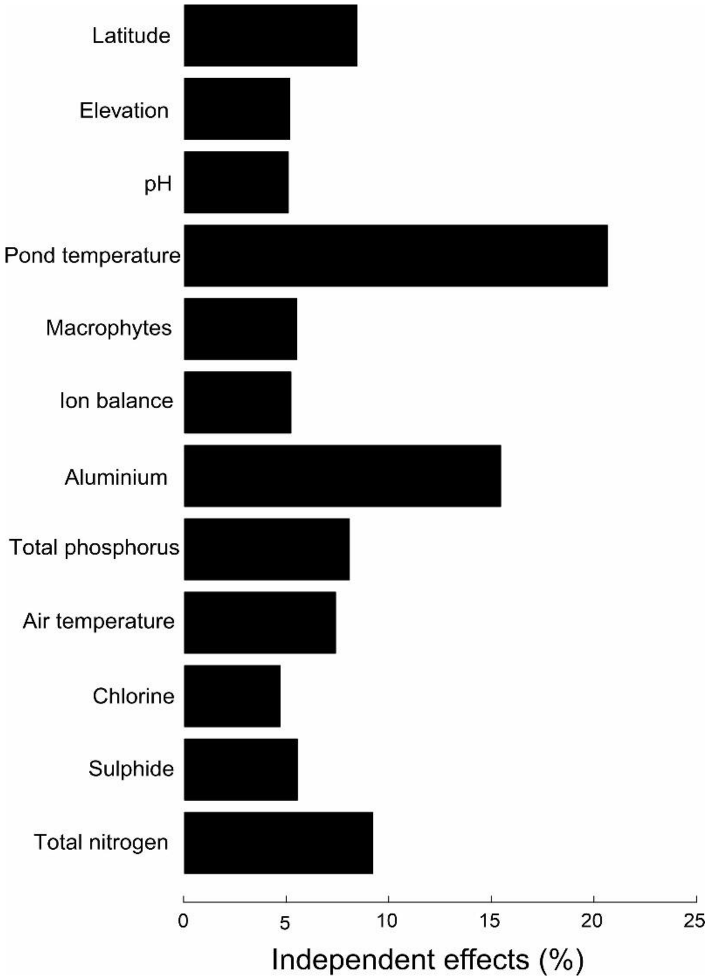


Figure 18. The independent effect of variables affecting species richness using hierarchical partitioning.

The explanatory variables describing community composition explained 20.18% of total variation in redundancy analysis. Of the total variation, 39.79% is explained by the first two RDA axes. Out of the variables, ion balance and total phosphorus were not significant ($p>0.05$). The length of the axis exceeded 2 in all cases despite performing decostand- and decorana-functions. The first axis length was 4.02 and the second axis length was 2.71, thus assuming a unimodal gradient. First four axes were statistically significant ($p>0.05$, RDA1 = 0.001, RDA2 = 0.005, RDA3 = 0.043, RDA4 = 0.017). The first RDA axis indicates mainly elevation and temperature gradient, whereas the second axis is water pH and ion balance (Figure 19). Elevation increases towards the left side of the graph, where for instance sulphide and chlorine show smallest values. Sulphide and chlorine increase towards the right side of the graph and have thus an opposite effect as elevation on species community. As several arrows have identical lengths, they have similar explaining power with diatom community composition between study sites. Most of the variables decrease as temperature decreases. The

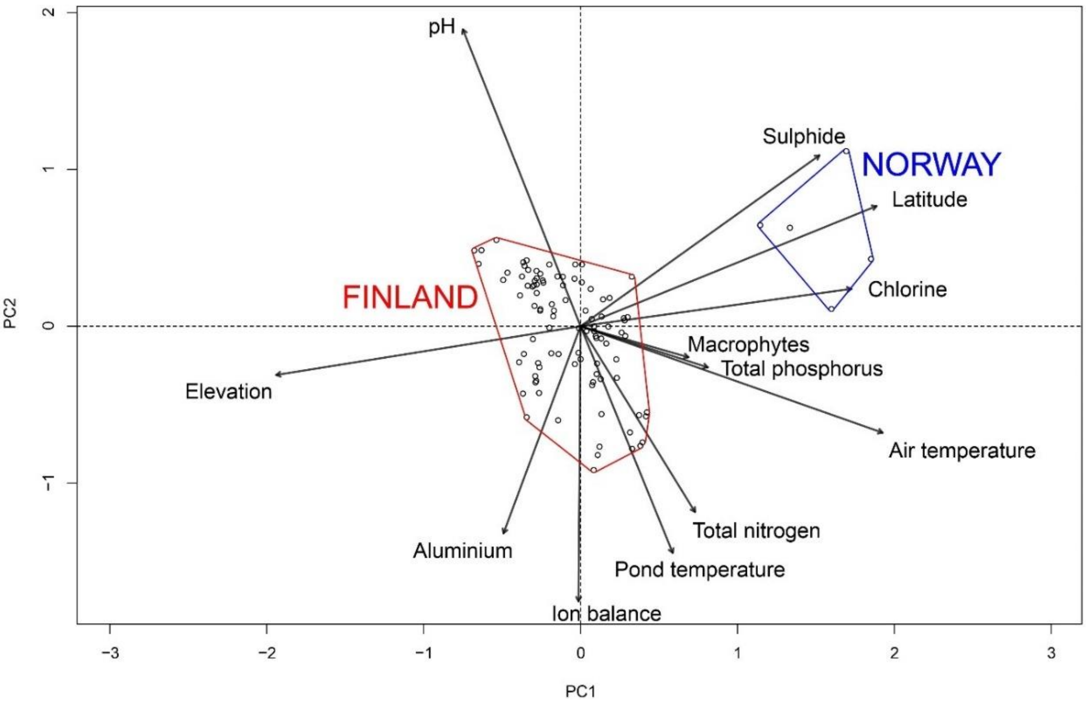


Figure 19. A redundancy plot showing the relationships between the explanatory variables and studied subarctic ponds.

ordination plot of subarctic pond locations clearly separates the sites in Norway at <100 masl, while all other locations are clustered in the centre. The analysed ponds in Kilpisjärvi are oriented more along with the second axis according to pH.

Species within the same genus preferred similar conditions with only slight variation within the genus. For example, *Cymbella*-genus appeared generally at high sulphide but low ion balance, aluminium and pond temperature values (Figure 20). *Frustulia*- genus appeared at varying elevations. *Frustulia amphipleuroides* (Grunow) appeared at the highest elevations, compared to *Frustulia rhomboides* (Ehrenberg) and *Frustulia saxonica* (Rabenhorst). Simultaneously, these taxa occur at lower chlorine and air temperatures. Species such as *Fragilariforma virescens* (Ralfs) and *Navicula cryptocephala* (Kützig) situated in between two explanatory variables seem to have another variable affecting them positively which is not included in the model. There does not seem to be a general orientation for diatom taxa, but rather all species are evenly distributed around the ordination plot. *Tabellaria flocculosa* is affected by a combination of all explanatory variables preferring mean environmental conditions due to its location at the centre of the plot. According to species composition similarity tests based on spatial and elevational distances, community similarities decrease steadier with elevation compared to spatial distance, whereas spatial distance shows clearer grouping of communities (Figure 21).

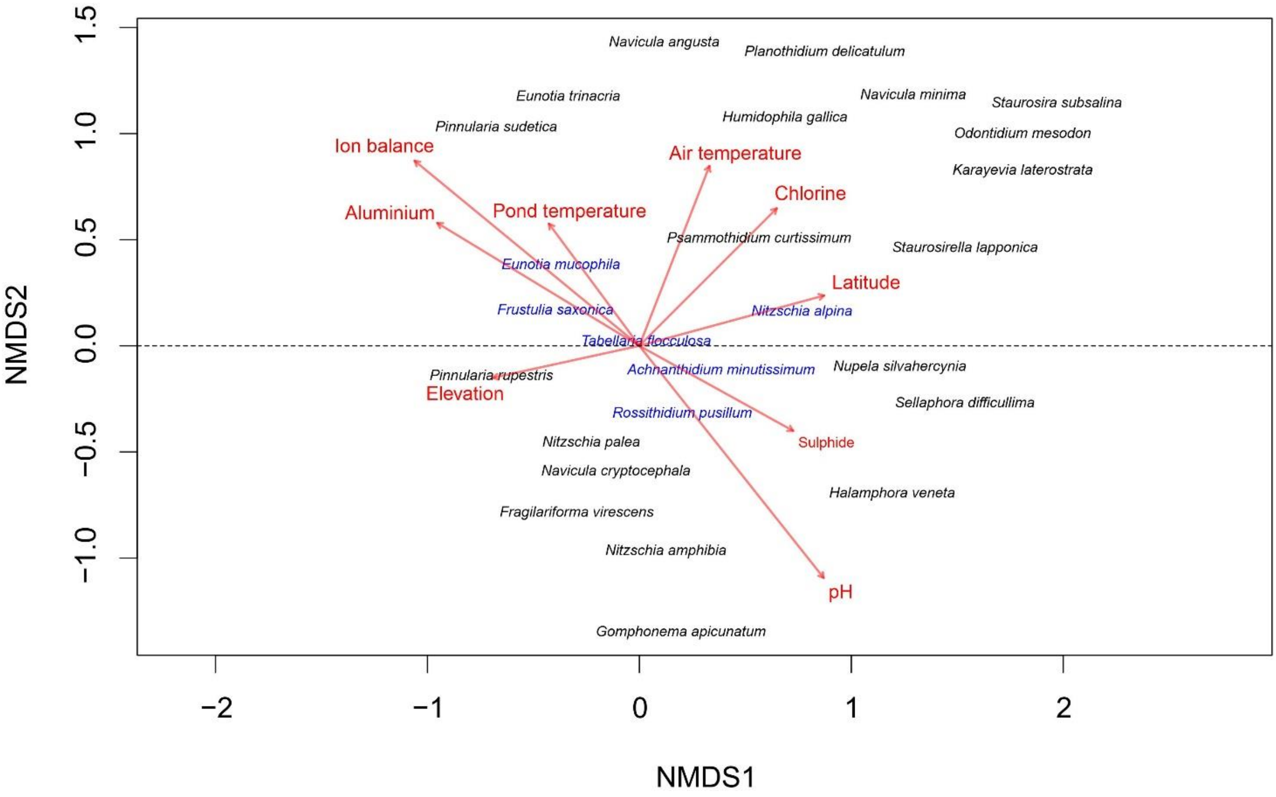


Figure 20. An ordination plot performed through non-metric multidimensional scaling. For better visual interpretation only a few taxa have been included at the perimeter of the final plot. Some of the most common taxa (see Table 6) were included as well and labelled in the graph in blue.

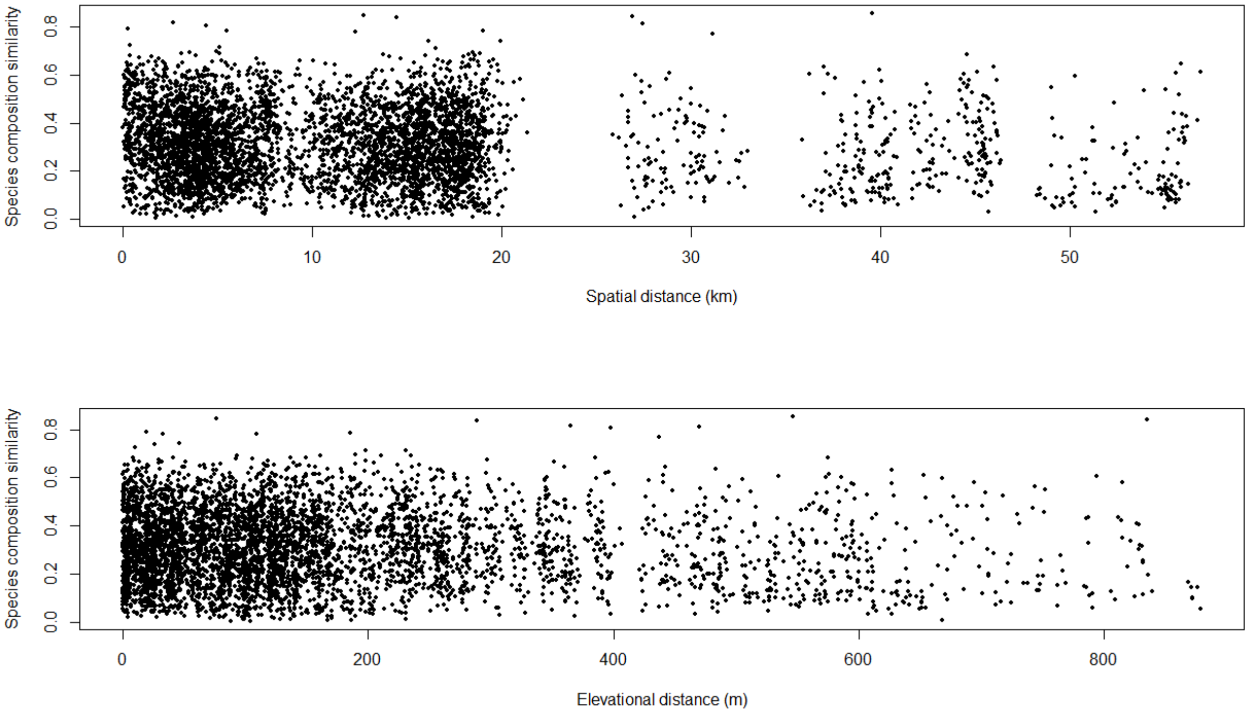


Figure 21. Showing the species composition similarities with spatial distance (above) and elevational distance (below). Both analyses were composed with Euclidean distance with 9999 permutations.

7.3. Diatom community composition and species richness at different local biotopes

Based on biotope descriptions half of the subarctic ponds studied in this research were located above the treeline with little or no vegetation. There is a moderate trend between elevation and the biotopes (Table 7). The areas were visualised with a non-metric multidimensional scaling plot (Figure 22). The coefficient of determination between the five areas was 6.7% and it was statistically

Table 7. The five biotope types arranged according to mean elevation

Biotope type	Elevational range (masl)	Mean elevation (masl)
Grass heath and rocks	659 - 887	815
Dwarf shrub heathlands	599 - 799	670
Tree & Shrub heathlands	560 - 640	621
Mountain birch woodlands	489 - 616	534
Pine forest (in Norway)	8 - 93	53

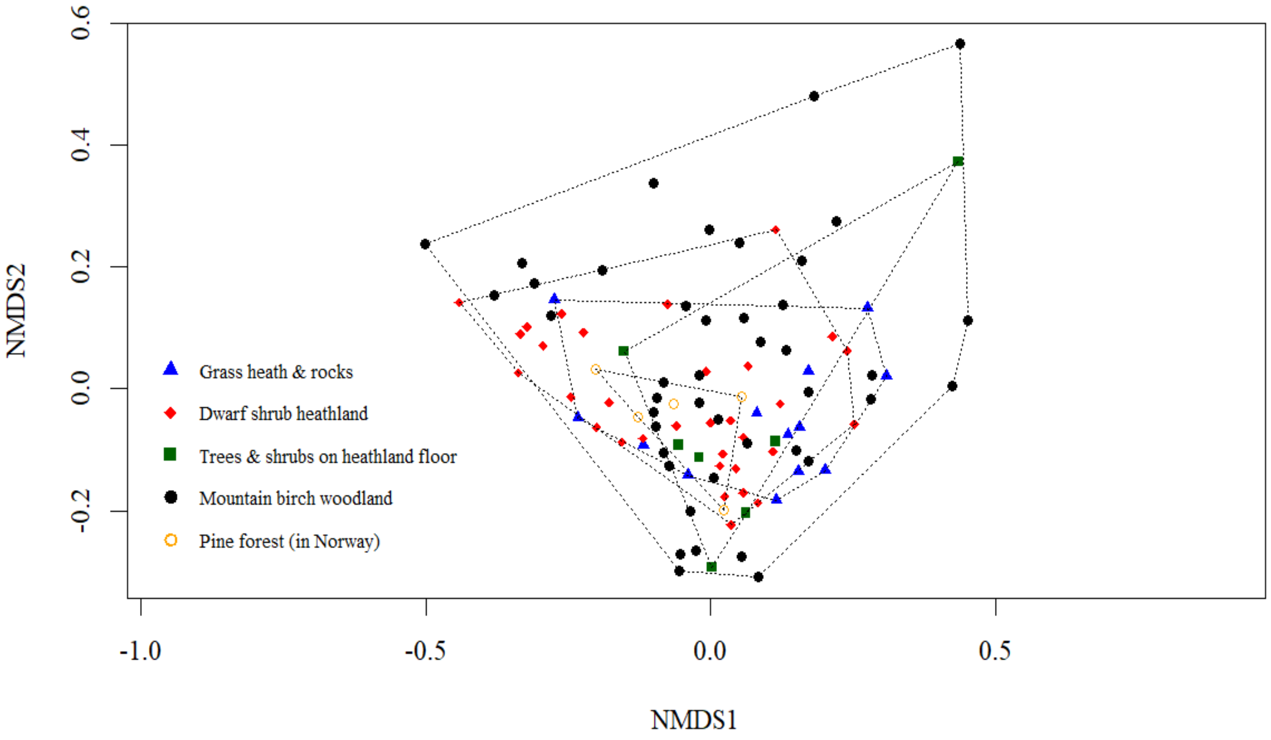


Figure 22. The differences and similarities between the five biotopes were visualised using non-metric multidimensional scaling. The range of the biotope is outlined with a black dotted line.

significant (p = 0.007). When investigating both species richness and relative abundances of community composition between the five areas with analysis of similarity, there was no significant difference between them and neither of them showed statistical significance. For community composition the test statistic between areas was -0.04 (p = 0.86) and for species richness it was -0.04 (p = 0.85). When assessing the rank dissimilarities between and within groups for community composition and diatom species richness, there appears to be some heterogeneity within biotopes rather than between them (Table 8). The table shows the distributional summaries on between and within group rank dissimilarities from the minimum (0%) to the maximum (100%). From the graph it can be seen that all areas are arranged together, with very little variation between them. The largest area consists of mountain birch woodland, which also consisted of most of the sample points. Mountain birch woodland thus had the largest range of species richness and community composition between all biotope sites. Even though the five biotopes categories used in the analysis were done unbiased from other variables, they do follow an elevational trend. Only very slight overlapping was detected, which means the biotope patterns are in this research to some extent correlative with elevation.

Table 8. Community composition (above) and species richness (below) ranks at different biotopes to distinguish heterogeneity between areas. Between refers to the heterogeneity between the different biotopes, whereas each individual biotope describes the heterogeneity within that biotope. N refers to the number of observations or replicates.

COMMUNITY COMPOSITION	0%	25%	50%	75%	100%	N
Between	1	1 223	2 406	3 654	4 950	3 442
Grass heath & rocks	8	469	1 367	2 888	4 526	78
Dwarf shrub heathland	15	876	2 196	3 296	4 875	496
Trees & shrubs on heathland floor	35	724	1 271	4 001	4 839	21
Mountain birch woodland	3	1 736	3 038	4 096	4 948	903
Pine forest (in Norway)	88	634	844	1 355	2 682	10

SPECIES RICHNESS	0%	25%	50%	75%	100%	N
Between	64	1 207	2 411	3 685	4 947	3 442
Grass heath & rocks	64	698	1 609	2 784	4 047	78
Dwarf shrub heathland	64	1 256	2 444	3 585	4 814	496
Trees & shrubs on heathland floor	162	1 704	2 684	4 061	4 887	21
Mountain birch woodland	64	1 375	2 780	3 945	4 950	903
Pine forest (in Norway)	64	1 850	3 207	3 429	4 632	10

8. Discussion and conclusions

Water temperature varied among the study ponds around the same range as measured in previous studies (Weckström et al., 1997). The water pH range was slightly larger here compared to previous literature, which could be explained by the large variation in local geology or some biotic factors, especially primary production. Another explanation could be the seasonal differences between the different studies. However, in general studied subarctic ponds showed relatively similar chemical and environmental characteristics indicating that pond physical and chemical conditions are primarily shaped by the same variables such as climate and bedrock type.

This study further found that macrophyte communities and pond surface area decreased with elevation. As a result, subarctic ponds at higher elevations are under greater threat from disturbance compared to those at lower elevations (Soininen and Meier, 2014). The small subarctic ponds that were analysed can be referred to as slightly acidic which could be connected with the water source of the subarctic ponds as many are believed to be rainwater-fed leading to slightly acidic water pH levels. Low pH values at these ponds were strongly correlated with elevated values of aluminium and low elevation as increased aluminium concentrations promote acidification (Helliweli et al., 1983). The exact ratio of 8:3 between sulphides and chlorine was found in only three subarctic ponds thus contradicting the argument set by Camarero and Catalan (1993) about the source of the water in the ponds being only from rainwater. If only based on the sulphide-chlorine ratio, the studied ponds were not only fed by rainwater. Thus, there might be an additional source of water from soils and groundwater input that could explain the misbalance of sulphides and chlorine.

On average, conductivity of the ponds matched with those found in the research by Cantonati et al. (2001). Low conductivity is typically correlated with low nutrient concentrations, which could explain the large number of laboratory measurements falling under detection limits. The levels of total nitrogen and total phosphorus were low at most study locations most likely due to their near pristine conditions due to low anthropogenic impact and overall low productivity and nutrient poor soils in the study region. The trophic statuses of the subarctic ponds correspond to the results found in previous literature (Shortreed and Stockner, 1986; Catalan et al., 1993; Forsström, 2006). Even if subarctic ponds seem all nutrient poor, some regional differences could be notified which could help in classifying subarctic ponds based on their trophic status.

The BenthoTorch measurements indicated that the chlorophyll-a concentrations were low and in general could be seen as reliable according to laboratory-based tests when the values are below 4µg/cm^2 (Harris and Graham, 2015). Therefore, it seems that based on these initial tests, measurements acquired from the BenthoTorch in this study are useful for (ultra)oligotrophic subarctic pond benthic algae chlorophyll-a estimations. Specially, this study found that diatom species richness was low when the chlorophyll-a values for green algae were high. Given the time of the season, this correlation would make sense as green algae outcompete diatoms when nutrient cycling towards the end of the growing season intensifies. However, as previous literature has not shown any real relationship between seasonal changes of benthic algal communities (Gudmundsdottir et al., 2011), the found correlation between chlorophyll-a measurements and diatom species richness is inconclusive. Total benthic biomass did not show any correlation with nutrient levels in studied ponds. However, increasing global temperatures will elevate the importance of the benthic algae and will provide useful information on seasonality changes in the future. There was no correlation between total benthic algal biomass and total nitrogen or total phosphorus thus providing no further information on nutrient levels in the studied ponds.

8.1. Explaining species richness and community composition

The relationship between species richness and elevation was found to be statistically non-significant. Despite elevation did not directly affect species richness or community composition, it was a strong underlying gradient as it did shape other explanatory variables such as water pH, pond temperature, ion balance and aluminium concentrations, which did explain diatom species richness and community composition along the elevational gradient (Figure 23). Ion balance and water pH have a clear optimum point approximately at mid-elevation as they show a unimodal trend along the elevational gradient. The relationship with aluminium appears U-shaped, meaning that the optimums are at both ends of the gradient and the lowest species richness is at the mid-point. The nutrients have similar independent effects between one another based on hierarchical partitioning. From the variation in the N:P ratios it can be said that phosphorus is a limiting nutrient in the studied environment. These findings also support from previous literature (Catalan et al., 1993; Sorvari et al., 2002; Wang et al., 2011). Nevertheless, total phosphorus was not a significant variable in explaining species richness or community composition. Perhaps one reason is that total phosphorus

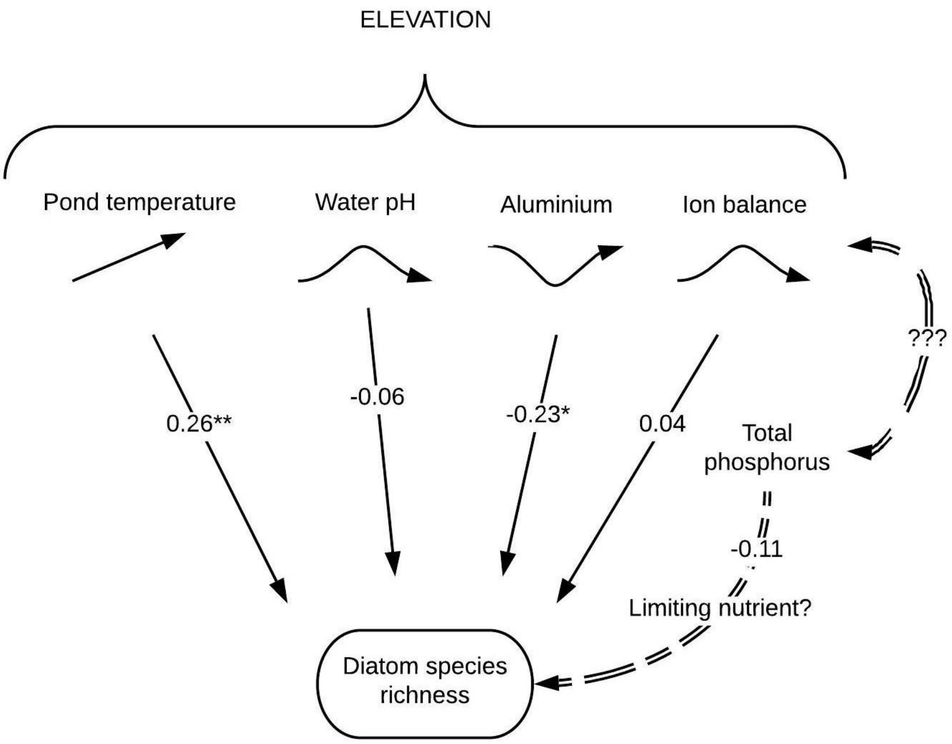


Figure 23. Variables that were shaped by elevation and correlated with diatom species richness as found in this study. The shape of the arrow below the variable indicates the relationship of species richness with the given variable. Pond temperature had a very significant ($p < 0.01$**) and aluminium a significant ($p < 0.05$*) correlation with richness. The role of ion balance is questioned in this research and should be investigated further through total phosphorus, which is considered to be a limiting nutrient in subarctic settings.

concentrations were typically very low and even below the detection limit in some ponds. Further studies about nutrient and ion balance may help to investigate the roles of limiting nutrients in such ponds in the future (Williams and Wacker, 1967). The other explanatory variables shaped by elevation enhance the role of mineral and nutrient inputs to the subarctic ponds. Total nitrogen and total phosphorus decrease with increasing elevation and thus low elevation serves as a better setting for diatom species growth (Sorvari et al., 2002; Wang et al., 2011). As established in this study, highest species abundance occurred below the mid-elevational point. Based on the findings of this research, elevation has an underlying role through several other environmental variables in explaining diatom species richness and community composition along an elevational gradient (Davies et al., 1983; Rahbek, 2005).

For community composition between sites, total nitrogen had a more significant role compared to total phosphorus in shaping the subarctic ponds. This elevates the importance of other explanatory variables in shaping diatom communities and explaining species richness along the elevational gradient. Community composition between sites was best explained by pH, air temperature and elevation, which again shows the importance of elevation as a secondary indicator for community composition. Both pond and air temperatures show opposite trends with pH, which further highlights the secondary role of elevation on diatom community composition through temperature.

Most taxa were distributed evenly around the ordination plot highlighting the importance of multiple variables affecting community composition. Elevation was not the most influential explanatory variable but was assisted by the importance of water pH and air temperature. There seems to be no clear categorisation between different diatom taxa. The resulting ordination plot can be interpreted in terms of which variables had the most significant effect from the length of the ordination arrows. Species within the same genus are likely to share similar responses to explanatory variable influence in subarctic ponds (e.g. *Frustulia* - and *Cymbella* – genera). The variation within the genus is relatively small and would require genus-specified analysis to get a better view on what variables affect individual taxa within a genus. The abundance of *Tabellaria flocculosa* is common in freshwater and in slightly acidic waters typically in the northern hemisphere, which explains the appearance of it in every subarctic pond analysed in this research. They colonize very quickly and usually appear in colonies of five or more (DeColibus, 2013). The amount of rare species found in this research was relatively significant as over a quarter of the identified species only appeared in a single study location. These rare taxa may appear by chance and could disappear within a relatively short time period (Taxböck et al., 2020). From the relationship between elevation and diatom community composition, it can be argued that the greatest elevational ranges appear at low plateau areas, i.e. below the mid-elevational point. Rare taxa were focused at maximum and minimum elevations, while most being at the minimum end of the gradient where species richness was also slightly higher. The range increases until the low plateau point, but does not increase further after that, but rather begins to decrease contradicting the findings of Blanco et al. (2020).

8.2. Potential errors

The greatest potential source of error considered for this research is human induced, such as inaccurate species identification, incomplete sampling and possible cross-contamination. Species found in a pond that were only counted once could cause errors in the results as they may have been misidentified by researchers. During identification, species appearing on their girdle band side (e.g. *Eunotia* and *Fragilaria* – species) were difficult to identify, which increases potential errors. As the valve size decreases, identification will become harder and potential identification errors will increase. Another challenge can arise during identification as different environmental conditions such as temperature may slightly alter the structure of the valve (Vanormelingen et al., 2009). Diatom identification was split between two researchers, which could explain the high abundance of rare species. Whether it would be necessary to increase the valve count per sample to help with the overestimation of rare taxa is questionable as it would be very tedious. Incomplete sampling can lead to underestimation of total species richness, which can also cause biases in the calculation of community similarity between sites. These errors can be corrected through statistical analyses (Chao et al., 2005), but could also be fixed somewhat using more extensive sampling. Diatom sampling in the subarctic should focus on several substrates instead of only one (Taxböck et al., 2020). Since this study only accounted for epilithic sampling, we cannot fully draw conclusions on the different diatom assemblages within the microhabitats of a subarctic pond even though epilithic sampling is recommended to be used for the highest statistical support for the relevant environmental determinants.

8.3. Future prospects and conclusions

This thesis explored the relationship between diatom species richness and community composition along an elevational gradient in the subarctic and aimed to indicate which variables are the most influential in driving diatom richness and composition. The results showed indications towards an indirect elevational influence through other explanatory variables on diatom species richness and community composition. Further knowledge could be gathered about limiting nutrients through N:P ratio with the help of ion balance calculations. This has further potential to broaden the knowledge of sulphide-chlorine ratios in subarctic ponds, which could give rise to better interpretations of water pH levels. Understanding water chemistry will also help to unveil the source of water on the subarctic ponds and thus it should be a one main focus in future research.

An *in-vivo* benthic algae chlorophyll-a measurement device; the BenthoTorch was used in this study to get a better understanding of its potential. The results indicated that chlorophyll-a values were typically low in the ponds but still more research on the subject is required for quantitative results on benthic algae portions in the circumpolar. Detailed performance analysis of the device should be done excessively to see how reliable the in-vivo chlorophyll-a measurements are in both subarctic ponds as well as in less oligotrophic waters. At the moment measurements of below 4 $\mu g/cm^2$ are reliable (Harris and Graham, 2015). Perhaps there should be more measurements from eutrophicated waters in which benthic algae have higher biomasses to compare with the results obtained in the subarctic. For example, high cyanobacteria abundances could offer potential setting for BenthoTorch performances to detect high-risk waterbodies (Quiblier et al., 2013). There is potential, that the seasonal change in the benthic algal community can be detected from the BenthoTorch measurements since at the moment literature is contradicting whether there are any links between seasonal changes and benthic algae portions (Gudmundsdottir et al., 2011). In general, the benthic algae chlorophyll-a appeared low in the subarctic ponds, but as temperatures increase the lower grazing macroinvertebrate diversity could potentially increase significantly with increasing global temperatures (Heino et al., 2019) making them an increasingly important study setting for future research.

Over the past years, aquatic research around the circumpolar has increased and further knowledge has been gained. That being said, more research in these remote regions is required for greater knowledge on these harsh ecosystems. Similar research on subarctic ponds needs to be done for regional comparison, which could help understand how and to what extent does elevation shape diatom community composition and species richness through other environmental variables. To bridge this gap a larger gradient, a greater variety of explanatory variables and further specialisation into diatom taxonomy will provide sufficient data in future research in aquatic ecosystems. For example, future species richness studies should include remote sensing data of the catchment as maximum normalized difference vegetation index has been found to be a significant variable in describing planktonic richness at lake level (Soininen & Luoto, 2012). This method could be adapted with a better categorisation of biotopes compared to this research, which relied only on photographic images. Currently the results from this research indicate that the difference between and within groups is primarily a difference in dispersion (Anderson and Walsh, 2013). Methodologically, future diatom biodiversity sampling should additionally focus on rare taxa to guarantee more complete community composition (Taxböck et al., 2020). Afterall, subarctic aquatic ecosystems provide valuable information on the rapid changing environment and should thus be studied extensively in the future.

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Supporting information

Supporting information can be requested from the author at janne.g.heikkinen@helsinki.fi

Appendix 1 Main explanatory variables and the most common diatom counts for studied ponds.

Appendix 2 Chlorophyll-a measurements of benthic algae from 2019 including the sum and percentage values of each group.