Effect of Catchment Characteristics on the Biodegradability of Dissolved Natural Organic Matter

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Abstract

Dissolved natural organic matter (DNOM) is omnipresent in natural freshwaters. It is the main source of food for the aquatic heterotrophic respiration, though little is known of the bioavailability of the DNOM when flowing through the aquatic system. Most of the DNOM in surface waters is allochthonous (i.e., derived from the terrestrial environment). Therefore, the lake-water chemistry acts as a sentinel of the biogeochemical processes in the catchment. The increase in concentrations of DNOM (browning) is boosting aquatic respiration and suppressing the primary production, causing the lakes to become net sources of the greenhouse gases. The biodegradability of DNOM has been measured in a set of lake water samples from southeast Norway using a simple analytical method based on monitoring the rate of oxygen consumption during incubation. The spatial variation in biodegradability of the DNOM was not clearly explained by water chemistry. But there were significant ($p < 0.05$) empirical correlation between the biodegradability of DNOM and site and catchment characteristics. Forest cover is a key explanatory factor for the differences in biodegradability of the DNOM. Both high cover of forest and low cover (natural non-forest) had relatively high correlations, at -0.26 and 0.25. The variable that had the highest impact on the biodegradability of DNOM was the lake altitude. Altitude reflects multiple catchment characteristics that affect the DNOM, that again affects the biodegradability of DNOM. Lakes at high altitudes have foremost more low molecular weight (LMW) DNOM, which have an important effect on the biodegradability of DNOM.

Preface

I would like to thank my supervisor, Rolf D. Vogt, for guidance, suggestions and for always providing lots of feedback. And my co-supervisor, Camille Craprat, for helping me with the lab, statistical analysis and always having a solution when I was stuck.

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My office buddies, Camilla and Hanne, thank you for all the fun we had before lockdown.

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List of abbreviations

DNOM	Dissolved natural organic matter
HMW	High molecular weight
LMW	Low molecular weight
POPs	Persistent organic pollutions
BDOM	Biodegradable dissolved organic matter
UiO	University of Oslo
SOM	Soil organic matter
BP	Bacterial production
BR	Bacterial respiration
GHG	Greenhouse gasses
NIVA	Norwegian Institute for Water Research
CBA	Center of Biogeochemistry in the Anthropocene
TOC	Total organic carbon
DOC	Dissolved organic carbon
JOVA	The Norwegian Agricultural Environmental Monitoring Program
TN/DN	Total nitrogen / dissolved nitrogen
TP/DP	Total phosphor / dissolved phosphor
NPOC	Non-Purgeable Organic Carbon
UV-ViS	Ultraviolet and visible light

sUVa	Specific ultraviolet absorbance
sViSa	Specific visible absorbance
SAR	Specific absorbance ratio
PCA	Principal component analysis
NMBU	Norwegian University of Life Science

1 Introduction

Dissolved natural organic matter (DNOM) is omnipresent in natural freshwaters. The levels of DNOM are especially high in the boreal biome where the total mass of DNOM is typically greater than the content of inorganic ions. In these waters, the biogeochemical processes, governing the elemental cycling of carbon as well as macro-nutrients, are mainly controlled by the amount and quality of DNOM. Moreover, the DNOM is the main source of food for the aquatic heterotrophic respiration, though little is known of the bioavailability of the DNOM when flowing through the aquatic system. Most of the DNOM in these surface waters is allochthonous (i.e., derived from the terrestrial environment). Therefore, the lake-water chemistry acts as a sentinel of the biogeochemical processes in the catchment. The increase in concentrations of DNOM (browning) is boosting aquatic respiration and suppressing the primary production, causing the lakes to become net sources of the greenhouse gasses CO_2 and CH_4.

1.1 Dissolved natural organic matter

Dissolved natural organic matter (DNOM) is a term describing a heterogeneous mixture of high molecular weight (HMW) and low molecular weight (LMW) organic molecules naturally formed in soils, sediments, and waters by biochemical and chemical reactions during the decay and oxidation of plant and microbial remains. The main fraction of the DNOM is comprised of humic macro molecule substances, which are organic compounds found in the environment that cannot be classified as any other chemical class of compounds, such as, e.g., proteins. Carbon, hydrogen, oxygen, nitrogen, and sulfur are the main elements of DNOM. On average, carbon constitutes approximately 50% of the mass of DNOM. The compounds contain both aromatic and aliphatic structures, and functional groups such as hydroxyl groups, phenols, carboxylic acids, ketones, amides, and sulfhydryl groups (Gaffney et al., 1996, Leenheer and Croué, 2003). The large amount of weak organic acids allow the DNOM to regulate the pH and form strong metal-complexation with Fe, Al, and type B (heavy) metals, such as Hg and Pb (Rahman et al., 2010). Moreover, persistent organic pollutants (POPs) are lipophilically adsorbed to the DNOM (Grannas et al., 2012). DNOM thus plays an important role as a transport medium of micro-pollutants, thereby increasing their transport from their pool in the soil to surface waters. In the surface waters, these contaminants may become bioavailable and taken up into the food chain. The assimilation of heavy metals, especially mercury, is enhanced by methylation. DNOM is used as an energy source through heterotrophic respiration, and it contains nutrients. It is thus an important source of food for heterotrophic aquatic organisms, fuelling heterotrophic microbial-based food webs (Azam et al., 1983). DNOM therefore plays a key role in heterotrophic bacterial activities in freshwater, especially in dystrophic lakes. In addition, the DNOM absorbs radiation in the UV and visual spectra, attenuating potentially damaging UV radiation (Findlay et al., 1986) and reducing the depth of photosynthetically active radiation (PAR).

DNOMs multiple effects on pollution, nutrient, and energy transport as well as adsorption of radiation implies that it generally plays a vital role in governing the biogeochemical processes in aquatic ecosystems in the boreal domain, characterized by conifer forests with organic rich soils.

1.2 Environmental changes

The biology and chemistry of Norwegian surface waters are changing due to changes in environmental pressures, such as acid rain, climate, and biomass. In particular, the concentration of DNOM has increased, and the concentration of calcium has decreased, especially in southern Norway (Garmo and Skancke, 2020). These changes have in the past been related to the decrease in acid atmospheric sulfur deposition (decreased by up to 90% in southernmost Norway). The effect of decreased acid rain has subsided and will likely not contribute significantly to any further changes. Instead, climate change, causing increased intensity and amount of precipitation and increased temperature, is having the same effect on the water chemistry with regards to the DNOM. Moreover, indirect climate change along with changes in land-use and other factors, have caused an up to 30% increase in biomass in the boreal regions of Norway (de Wit et al., 2015). This has also had a profound impact on the water chemistry, especially the amount and quality of DNOM.

1.3 Biodegradability of dissolved natural organic matter

Allochthonous DNOM is an important food source for heterotrophic organisms. Aquatic systems are dynamic, with the water containing the DNOM flowing through the watershed and watercourse system. The bioavailability of this material thus depends on the rate at which the DNOM can be degraded (i.e., the biodegradability), and on the residence time of the water in soil, rivers, and lakes. The biodegradability is mainly governed by the characteristics of the DNOM. Usually, the role of DNOM as a source of energy for the aquatic ecosystem is assessed by measuring the biodegradable amount of dissolved organic matter (BDOM), i.e. the proportion of DNOM that is biodegraded during an operationally defined given time period. Since the aquatic systems are dynamic the focus in this study is on the biodegradability of the DNOM, measured as the rate of decrease in oxygen (O_2) concentration during an incubation of water samples with DNOM.

1.4 Aim of the study

In this study empirical correlations between spatial differences in biodegradability of DNOM and its characteristics, the chemistry of the water matrix, and the site and catchment characteristics are assessed. The aim is to induce the main explanatory parameters for the spatial variation in biodegradability and deduce the main chemical processes governing the biodegradability of DNOM based on our conceptual understanding of the biogeochemistry of the environment.

The hypothesis is that catchment characteristics such as vegetation type, and the ratio of peats in the watershed, are key explanatory factors for the differences in biodegradability of the DNOM. This is based on Wickland et al. (2007) and several previous MSc studies (Håland, 2017, Færgestad, 2019, Rajakumar, 2018, Stevenson, 2018, Francés, 2017) at the Section of Environmental Sciences at the Department of Chemistry, University of Oslo (UiO).

2 Theory

The theory starts with a short introduction to DNOM and its characteristics in Chapter 2.1 focusing on its natural role, mobility, and chemical structure of the DNOM. In Chapter 2.2 the increase in DNOM is discussed. It starts with why there is an increase, and how an increase in precipitation, because of global warming, is affecting the concentration of DNOM. Then it addresses how the increase in DNOM is affecting the environment. Chapter 2.3 provides a short explanation of biodegradability of DNOM. Chapter 2.4 focuses on the boreal domain and describes its common catchment types and characteristics. Finally, in Chapter 2.5 an introduction is given to The Center of Biogeochemistry in the Anthropocene's (CBA) survey named "CBA *100-lake survey*" which this thesis is a part of.

2.1 Dissolved natural organic matter characteristics

DNOM characteristics vary in space and time. DNOM may be divided into two different groups based on its origin. Allochthonous material is material that is made within the terrestrial environment and washed into the surface waters, while autochthonous material is generated within the lake itself, primarily by microorganisms. DNOM in non-eutrophic boreal watercourses is generally more derived from terrestrial soil and plant material and less from aquatic phytoplankton, i.e. more allochthonous than autochthonous (Tranvik et al., 2009). Therefore, it mainly reflects the properties of soil organic matter (SOM), which are strongly influenced by the catchment vegetation and climate (Ittekkot, 1988, Meybeck, 1982). Spatial dissimilarities in DNOM characteristics are thus mainly due to differences in biotic, edaphic and topographic factors. Temporal changes in allochthonous DNOM characteristics are mainly governed by fluctuations in hydrological flow-paths through the watershed, governed by weather, as well as changes in anthropogenic influences (Vogt et al., 2004).

DNOM lowers the pH in poorly buffered (i.e., soft) waters. The acidic properties of DNOM are mainly due to their weak carboxylic- and phenolic acid functional groups. The first usually have a pKa-range from 2.5 to 5 depending on the molecular structure, while phenolic groups usually have a pKa-range of 9 to 10. Due to the large range of different weak organic acid functional groups in the heterogeneous DNOM material no distinct set of pKa values may be defined for the DNOM. The pH in various ground- and surface waters will vary depending on soil composition. The presence of carbonate minerals in the soil renders a soil with a more circumneutral pH due to that the carbonates are readily weathered consuming the H^+ released from the carbonic acids. On the other hand, in poorly weatherable soils the weak carbonic- and organic acids may over time deplete the base saturation of the soils. An example of the latter are soils containing a high level of exchangeable acid cations such as Fe^{3+} and Al^{3+}, cations with a high ionic index.

Heterotrophic bacteria digest labile DNOM directly, as well as transform recalcitrant DNOM to more labile forms (Hessen and Tranvik, 1998). DNOM thereby becomes a part of a large aquatic food-chain which sustains organisms on all trophic levels. In doing so allochthonous organic carbon enhances both bacterial production (BP) providing the bacteria building blocks in form of carbon (Jansson et al., 2000), and bacterial respiration (BR) by providing bacteria a source of energy (Jones, 1992). It is the BP that provides a linkage between allochthonous organic carbon and higher trophic levels as bacteria are grazed upon (Hessen, 1998, Jones, 1992). Energy pathways fuelled by terrestrial-derived carbon are of large importance for the function of these relatively unproductive freshwater ecosystems (Jansson et al., 2007). In dystrophic lakes, up to 90% of bacterial carbon (Hessen, 1992) and up to 50% of zooplankton carbon (Pace et al., 2004) have been reported to be of allochthonous origin. BR returns organic carbon to the atmosphere (Cole et al., 1994) by production of CO_2 and CH_4 and accounts thereby for an important source of greenhouse gases (GHG) and an important process in the carbon cycle (Algesten et al., 2004).

As mentioned in the introduction (Chapter 1.1), DNOM forms complexes with toxic `soft` or `type B` metals. This is because metals such as Pb, Hg and Cd have high covalent index (i.e., $\text{Electronegativity}^2 \cdot \text{radius} > 2.63$). Long-range transported atmospheric deposition of heavy metals in the environment can be linked back to the start of the industrial revolution in the 1850s (Fitzgerald et al., 1998). In regions far from emission sources, such as Norway, this long-range transported air pollution has led to the accumulation of toxic metals in the organic forest floor horizon. Strongly bound to SOM, they remained largely inert causing minor impact on the environment. Increasing DNOM concentration in the soil water increases the formation of chelate bonds with type B metals. The type B metals thereby become dissolved in the water column and are subjected to transport to surface water where the DNOM is exposed to physical and chemical degradation reactions enhancing their bioavailability (Haitzer et al., 2002, Jordan et al., 1997).

Depending on the biodegradability of DNOM binding the heavy metals and methylating bacteria, the increased load of dissolved heavy metals may be taken up into the food web, and could therefore potentially affect the entire food chain, including humans (Fitzgerald et al., 1998, Zheng et al., 2012). The aqueous system is inherently dynamic with water constantly flowing through the soil and watercourse at various rates. The rate in which the DNOM is biodegraded in the limited time in which it is passing through the systems is thus essential in understanding the role of this process in space and time. This biodegradability is fundamentally different from the absolute or relative amount of BDOM, which gives an indication of the relative amount of DNOM that can be degraded over an operationally defined time. Previous studies highlighted this difference. For example, the correlation between BDOM and biodegradability of DNOM was assessed in a project covering several waterworks in the Nordic countries (Eikebrokk et al., 2018). This study shows a poor correlation between BDOM and the biodegradability of DNOM. Biodegradability on the other hand tells how fast DNOM is degraded, which is more relevant in dynamic systems where DNOM moves and is not in stagnant water.

The physicochemical characteristics of DNOM dictate its mobility, transport, biodegradability, and impact. By determining DNOMs physicochemical properties in different watercourses, we can set empirical spatial differences in these properties in context with the characteristics of their different water sources. This enables us to assess the conceptual factors governing the mobility, transport and biodegradability of DNOM. Using a “space-for–time” approach the knowledge of these governing processes is a prerequisite to predict how the DNOM characteristics will be influenced by changes in the environment, such as direct and indirect effects of global warming and changes in land-use and management practices.

2.2 Increase in dissolved natural organic matter and environmental effect

DNOM has in recent years had an increase in concentration. What this increase does to the watercourse and how global warming is continuing this increasing trend is explained in Chapter 2.2.1. DNOM is also influencing the environment with methyl accumulation and browning of surface waters, all explained in Chapter 2.2.2.

2.2.1 Increase in dissolved natural organic matter

Terrestrial export of DNOM to surface waters has increased during the past decades over the vast boreal and other biomes, causing a pronounced “browning” of surface waters (De Wit and Skjelkvåle, 2007). The initial increase in DNOM has been linked to the decrease in acid rain deposition. However, climate change, with increased intensity and amount of precipitation, as well as increased temperature, is also impacting the DNOM concentration in many watercourses. Rivers fed with water from the surrounding area may serve as a “chemical fingerprint” for that catchment. It is thus possible to get an impression of the biogeochemical processes taking place in the watershed by sampling and analysing soil and nearby water. However, the various concurrent hydrological and geochemical processes in a catchment influence the water quality differently. At low flow the compositional changes in a stream occur slowly. This is due to that the runoff is generated by a single large source of groundwater that is slowly seeping out. Because of its long residence time and good contact with the mineral soil the low flowing groundwater is typically relatively richer in weathering products like silica and base cations, low in DNOM and has a relatively high pH. When the water flow increases, more water is entering the stream. This water has had a shorter residence time and may only have been in contact with shallower soil. Hence, it will be richer in DNOM and have a lower pH and content of base cations. This causes the concentrations of DNOM and H^+ to increase while the levels of base cations decrease with temporal increase in flow.

As rain precipitates on to the land surface, most of it will percolate down into the soil. As the water moves through the soil it will eventually start to saturate the soils in topographic depressions. Increased global warming, leads to an increase in the amount and intensity of precipitation in the boreal biome. This causes more frequent saturation of the soil in the valley bottom and our common shallow till soil on the slopes. This will thereby give increasing water flow through the organic rich forest floor directly out into the streams. By bypassing the adsorptive capacity of the deeper mineral soil layers, this provides an increase in DNOM flux to the watercourse.

In Norway there has been an 18% increase in annual precipitation for the whole country between 1900 and 2015 (Hanssen-Bauer et al., 2015). Although, due to large differences in topography there are significant geographic differences in the trends in annual precipitation (Groisman et al., 1999). The increase in precipitation is believed to be caused by change in atmosphere circulation patterns due to increased greenhouse effect (Hanssen-Bauer and Førland, 1998). An 1.5 °C increase in annual average temperature in Norway has been measured in the last 25 years (Hanssen-Bauer et al., 2006). The increasing global temperature leads to longer growing seasons, which in turn leads to increased vegetation and biomass production, known as “greening”. Increased greening leads to the production of more DNOM in the degradation process. In southeast Norway there has been an almost 30% increase in forest biomass between 1971 and 2000 (de Wit et al., 2006). In addition to climate change the increase in biomass is caused by changes in land-use management. The increase in atmospheric CO_2 and accumulation of reactive nitrogen, are also contributing to the increase of biomass. Increased DNOM in surface waters has potential impacts on the carbon cycle and emissions of GHG (Tranvik et al., 2009), as well as ecological functioning of freshwaters (Finstad et al., 2014) as mentioned in Chapter 2.1 and described further in Chapter 2.2.2.

2.2.2 Environmental effects of dissolved natural organic matter

DNOM causes the water to become brown allowing less light to penetrate down to the deeper layers of the lake. This limits the extent of photosynthesis and causes the epilimnion to become warmer. A warmer epilimnion leads to a more oxythermal habitat for aquatic biota (Couture et al., 2015), and will give stronger and longer summer stratification. The allochthonous DNOM is also an important source of nutrition for heterotrophic organisms. Increased inputs of biodegradable DNOM boost the activity of heterotrophic bacteria, which may negatively affect autotrophs because of their high consumption of nutrients. Increased photo-oxidation, reduced photosynthesis and increased heterotrophic activity promote a net release of GHG (Yang et al., 2015), causing lakes to be hot-spots for GHG emissions (Tranvik et al., 2009). Streams are governing the transport of DNOM, with water residence time being a key explanatory factor. DNOM also impacts type B metal, especially mercury, accumulation in aquatic food webs (French et al., 2014), as well as ecosystem services providing safe and sound raw water for drinking water supply (Anderson et al., 2017).

2.3 Biodegradability of dissolved natural organic matter

Bioavailability of DNOM describes the potential for heterotrophic microorganisms to utilize the DNOM as a substrate for energy and nutrients. Biodegradability of DNOM is dependent on numerous factors. The most important are the DNOM characteristics, such as the molecular structure, aromaticity, functional group content, and the molecular size. As well as being lighter LMW DNOM will generally have less conjugated double bonds and aromatic rings, this makes them more liable, or easier to degrade for heterotrophic bacteria then HMW DNOM. This often means that relatively more biodegradable DNOM has more LMW DNOM then HMW DNOM. The water properties also influence the biodegradability, such as nutrient availability, and microbial community structure. Biomass growth in boreal conifer forest is often nitrogen limited, as opposed to lakes that are generally phosphor limited. In addition, there are important external factors such as the temperature and rainfall regime and the associated vegetational cycles. Increased amount and intensity of precipitation increase the transport of DNOM with HMW and greater aromaticity to surface waters (Håland, 2017). This is discussed further in Chapter 2.4.5. All these governing factors cause spatial and seasonal variability in DNOM biodegradability.

2.4 Boreal domain and catchments characteristics

The boreal forest is located in the north Eurasia and North America and is easily recognisable for its abundance of conifer trees, with a large proportion of inland waters and wetlands such as bogs and fens. The soil in the boreal forest is relatively rich in organic carbon which partly leaches into the surface waters. At high latitudes, including the boreal domain, ecosystem carbon balance depends largely on aquatic processes (Kling et al., 1992, Vonk and Gustafsson, 2013).

Boreal and arctic ecosystems comprise about half of the total global terrestrial organic carbon store (Tarnocai et al., 2009), part of which will be vulnerable to microbial processing and release to the atmosphere as GHG by the end of this century. Levels of DNOM in surface waters are especially high in the boreal and sub-arctic areas due to the high soil organic content and general lack of carbonates in the soil. This explains the significant physical and chemical impact of DNOM on the surface waters in these regions.

Since most of the DNOM in a boreal watercourse is allochthonous its biodegradability is mainly governed by catchment characteristics such as land-use, vegetation type, climate, and soil type. Most of the boreal domain have rather similar catchment characteristics, such as type of forest, type and proportion of peatlands, soils with similar pH and the flux of water percolating through the soil. The boreal domain in Norway comprises on the contrary large gradients in these characteristics.

2.4.1 Forest type

Forests are divided into two main categories dependent on tree type. Deciduous forests are composed of trees with leaves, while coniferous forests consist of needle trees. The boreal coniferous forests are generally nitrogen limited and have lower biodiversity than deciduous forests. One third of Norway is covered by both deciduous and coniferous forests. A mild climate, such as in southernmost Norway, allows deciduous forests to grow, while harsher climate, especially harsh winters, gives way to coniferous forests. Forest industry prefers to use the Norway spruce (Picea abies), which is why the Norwegian forest consists of a relatively high proportion of coniferous forests.

Studying the Alaskan boreal forest Wickland et al. (2007) found that DNOM leached from spruce forest soils, was dominated by hydrophobic compounds and was generally slow to degrade, whereas fresh DNOM leached directly from vegetation litterfall contain potentially large amounts of hydrophilic compounds and ranged from slow to fast biodegradability. Forest soils characterized by poor drainage contained the least labile DNOM, meaning that the large SOM pools in these systems have undergone significant microbial processing, and that the remaining SOM is relatively recalcitrant (Wickland et al., 2007). The biodegradability of DNOM is thus dependent on where the DNOM originates and travels through the watershed.

2.4.2 Natural non-forests

Natural non-forests are areas where few trees grow, generally at altitudes above the tree-line. This gives room for heather meadows, grass bogs, and lichen covered areas. It is common to have snow coverage all year except for a few months in summer, resulting in a short growing season. In eastern Norway the tree-line is at around 1000 meters above sea-level, being higher inland and down to sea level at the coastline.

2.4.3 Marine limits

The highest former sea level in a given area after disappearance of ice from the last ice age is called the marine limit. The land mass was weighed down by thick ice sheets, allowing sea water to flow over the land as the glaciers withdrew. The level varies throughout Norway. The highest marine limit can be found in the Oslo region, where it reaches 220 meters above today's main sea level. Below the marine limit one will find marine deposits. These are fine-grained sediments, most often well sorted sand, silt, and clay, containing carbonates and phosphates. Above the marine limits one mainly finds ground moraines that consist of loose sediment and rock debris deposited by glacier ice, known as till.

2.4.4 Peatlands

The characteristic that distinguishes peatlands is its capacity to accumulate organic matter. In peatlands the annual rate of biomass production is greater than the rate of decomposition. This is because of the generally cold and wet climate, giving rise to frequently anoxic conditions, a low nutrient content, and a small microbial population to decompose the plant tissues and peat. Peatland naturally releases CO_2 and CH_4 into the atmosphere, that are both GHG, and DNOM into the water because of decomposition of organic material. Peatlands are an efficient carbon sink, since they release less CO_2 than the CO_2 captured by its plants. Although they only cover 3% of the earth's surface, they store up to 30% of the earths carbon in the form of organic matter (Gorham, 1991). Peatlands are thus important for the regulation of GHG.

Peatlands are an important source of DNOM to aquatic ecosystems. Aquatic DNOM concentrations have been shown to be significantly correlated with peatland coverage, area, and type (Aitkenhead et al., 1999, Gorham et al., 1998, Xenopoulos et al., 2003). Peatland inputs of DNOM to streams can have a profound impact on the chemistry of aquatic ecosystems (Billett et al., 2006). Despite the recognition that peatlands are a substantial source of DNOM to surface waters, the chemical quality of DNOM from different peatland types and how it varies seasonally is not fully understood.

There are two main types of peatlands in the boreal zone, which are distinguished by their water source. The first one is fens (minerogenic peatlands) whose water source is mainly groundwater that is rich in minerals and has a pH that is neutral or alkaline. The surrounding bedrock and soil chemistry determine the chemistry of the water flowing into the fen. Bogs (ombrogenic peatlands) are peatlands whose water source is mainly directly from precipitation. These are thus often acidic and have a lower concentration of minerals. Both bogs and fens are strongly influenced by local climate, soil type and geomorphology, vegetation, as well as water chemistry. As a result, they occur on the landscape in several different ways. Streams draining peatland-rich catchments generally have high DNOM concentrations with high aromaticity (Ågren et al., 2008), therefore being more hydrophobic. Peatland-derived DNOM will often also have HMW (Kalbitz et al., 2005). All these factors give more recalcitrant DNOM.

2.4.5 Soil material and soil chemistry

The types of soil profiles found in an area are governed mainly by the type of unconsolidated material which is given by the area's geology, morphology, glacial and fluvial processes as well as whether the site is located above or below the marine limit (Chapter 2.4.3). In addition, climate, and the biomass production of DNOM has a strong influence on the soil profile development. Furthermore, the type of soils is often influenced by human activity such as agriculture, industry, and urban development. Soil is the binder for input from the atmosphere and output to the hydrosphere and alters water chemistry as it passes through the watershed. The physicochemical characteristics of soil govern how mineral ions, nutrients and water are distributed in the environment. Soil thus holds a central role in global nutrient hydro-biogeochemical cycling (C, P, N, S). The sorption of organic matter onto mineral surfaces alter the physicochemical properties of minerals (Angove et al., 2002, Wang and Xing, 2005) and influence the transport and availability of nutrients and contaminants in soils and waters (Xing, 2001).

In mineral soils, the sub-lateral surface runoff flowpaths become activated during conditions of elevated runoff. This water flowpath transports the DNOM from the organic rich forest floor directly into watersheds (Raymond and Saiers, 2010, Wilson et al., 2013) (Chapter 2.2). These flows flush out DNOM from upper soil layers where potentially available DNOM may have accumulated and become concentrated over time during prolonged dry periods (Boyer et al., 1997). Vertical percolation down through soil is the dominant flow path during minor rainfall episodes or light rain when the soils are not saturated. Most of the DNOM released from the forest floor is absorbed to mineral surfaces in these deeper percolating waters (McDowell and Likens, 1988). This results in relatively low DNOM concentrations during baseflow and deep-soil seepage in comparison to runoff following shallower paths across a watershed (Sebestyen et al., 2009).

Podzols is a soil type that mainly occurs in poorly weatherable material (e.g. unconsolidated material of igneous gneiss and granite) in cold to temperate zones where precipitation is much greater than evapotranspiration. The acidity in the forest floor is high with a pH down to between 3.5 and 4. Podzols are characterized by a spodic B-horizon made up of accumulated humus and iron- and aluminium oxides. Above the spodic horizon there is often a bleached eluvial (E) layer where clay and iron oxides have been leached.

Weathering consumes H^+ so the weathering rate affects the buffer capacity. Depending on the pH in the soil there are different buffering systems. In water with pH

> 5.5 to neutral, the HCO_3^- system is important for buffering, while Fe & Al-hydroxides are buffers in waters with pH < 5.5. DNOM, as it contains a heterogeneous mix of weak organic acids, constitutes an important buffering system over the whole pH range (from 3 to 8), commonly encountered in nature.

Natural soil acidification is caused by biological uptake and harvest, elution with anions that can either be natural such as DNOM, HCO_3^- and Cl^-, or mainly anthropogenic such as SO_4^{2-} and NO_3^-. Soil acidification can be a reduction in base saturation or a reduction of the soil pH. The production of H^+ in soils originates either from the protolyzation of weak organic acids and carbonic acids from the hydration of CO_2 from organic respiration, or from vegetation which release H^+ as they take up base cations. The resistance of a soil towards acidification is controlled by the presence of easily weatherable buffering components such as carbonates, and bicarbonates, by neutralizing the protons. Carbonate minerals in the soil therefore strongly influence the soil- and water chemistry, by rendering soil with high base saturation and soil solution with high pH and alkalinity.

Acidic soils have higher leaching of DNOM. This is because an increase in pH will increase the amount of dissolved carbonates giving a high concentration of Ca^{2+} (hard water), and this reduces the solubility of DNOM. Therefore, acidic soil will have higher solubility and higher leaching of DNOM.

2.4.6 Agriculture

Modern agriculture uses less pastures then in the past, this gives either way to more crop fields or regrowth of previous forest. In Norway there has been an increase in biomass in the outfields. During the past two to three decades, the standing forest biomass in the boreal regions of Norway has increased up to 30% (de Wit et al., 2015) which is partly linked to less use of pastures and grazing.

From agriculture there is often runoff to near watercourses. This runoff generally consists of phosphor, nitrogen, and organic matter. Generally, an increase in yearly precipitation will increase the runoff. This is documented in the Norwegian Agricultural Environmental Monitoring Program (JOVA), where 8 out of 11 catchments had an increase in phosphorus runoff, and 7 out of 11 catchments had an increase in nitrogen runoff (Hauken et al., 2020).

2.4.7 Lake environments and bacteria

The trophic state of the lake environment dictates the relationship between DNOM from the lake (autochthonous) and DNOM that has been transported to the catchment (allochthonous). Oligotrophic lakes have a low primary productivity, because of a low nutrient content. An oligotrophic lake will thus show low concentrations of DNOM. Eutrophic lakes have a high biological productivity because of high nutrient concentration, particularly nitrogen and phosphorus. A eutrophic lake will therefore have high concentrations of anions and cations, nitrate, and phosphate, which gives good growing conditions for algae that release autochthonous DNOM. Algae blooms give a similar effect as browning, hindering light to penetrate to lower levels of the lake. Mesotrophic lakes have a medium level of primary production and are classified between oligotrophic and eutrophic. Dystrophic lakes have high concentrations of organic carbon, meaning they have high concentrations of allochthonous humic substances and organic acids. A dystrophic lake has low primary production because of low pH and the browning effect.

Heterotrophic bacteria use preexisting organic matter from their environment to grow and reproduce. Autotrophic bacteria produce organic matter themselves from CO_2, water, and sunlight. Dystrophic lakes have more heterotrophic bacteria which gives higher respiration. Whereas meso/eutrophic lakes have more autotroph organisms.

2.5 CBA 100 lake survey

One thousand lakes all over Norway were sampled and analysed in 1986 and in 1995. A new synoptic *“1000-lake survey”* of the same lakes was conducted by Norwegian Institute for Water Research (NIVA) in the fall of 2019. The Center of Biogeochemistry in the Anthropocene (CBA) at UiO did a concurrent survey, named “*100-lake survey*”, by sampling manually 73 of these lakes, located in southern and eastern Norway, and analysing them in more details: including concentration of major cations and anions, DNOM characteristics, with focus on the biodegradability, as well as parameters such as absorbance, dissolved gases etc. Catchment characteristics of the watersheds of these 73 lakes are thoroughly documented and compiled (OSF, 2021). This subset of lakes will be used in this master thesis to study the effect of water chemistry and catchment characteristics on the biodegradability of DNOM.

3 Materials and method

The sample taking of the lakes (Chapter 3.1), and chemical analysis (Chapter 3.3) was executed in a joint effort by the CBA, mainly from the UiO. While the statistical analysis (Chapter 3.4) was done specifically for this thesis.

3.1 Sampling

The lakes chosen for *The 100-lakes survey* were a subset of the 1000 lakes sampled by NIVA. Multiple teams sampled over the course of 5 weeks, from 1st. October to 8th. November 2019, comprising 18 people that participated in the sampling. 81 lakes were selected and divided into 10 groups, for sampling trips of 1 or 2 days. Nine of these lakes were discarded due to closed roads or lack of time, ending up with the 73 lakes shown in Figure 1.

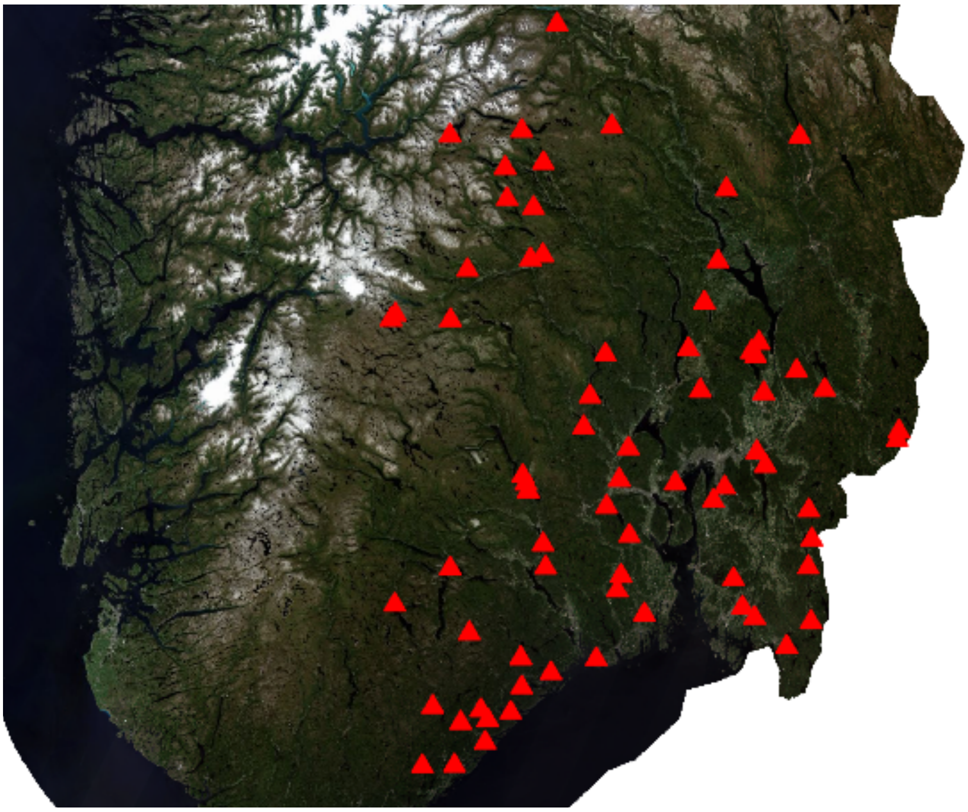


Figure 1. Satellite image of south Norway showing location of sampling sites, marked with red triangles. Source: Modified from (©Kartverket, 2021)

The water samples was collected from the lake's surface into a bucket using a sampling rod with a sampling beaker, with minimal disturbance possible. Whenever possible, the outlet of the lake was selected as a sampling point.

3.2 Sample preparation

The samples were filtered through a 0.2 µm filter at the sampling site in order to practically sterilize the sample. A nutrient solution was made with 5 mM dipotassium phosphate and ammonium nitrate. An inoculum was prepared with the nutrient solution and 2.0 µm filtered water from lake Langtjern. Prior to incubation 250 µL of inoculum and nutrient solution was added to a 25 mL sample, producing four replicates for each sample. This was performed as described in the method for analysis of the biodegradability (Crapart et al., 2021)

3.3 Analytical methods

3.3.1 Biodegradability

Instrumentation

The biodegradability of DNOM during incubation was monitored by measuring the oxygen content using a PreSens SensorVial (SV-PSt5-4mL) with a SensorDish® Reader (SDR) Basic Set. The SensorVials used in the experiment are borosilicate glass vials, and screw caps with a rubberized septum to seal the vials. To further ensure the seal, Parafilm was wrapped around the screw caps as well. A spot type sensor (PreSens Oxygen Sensor Spot SP-PSt5) is located at the bottom of each SensorVial. The sensors are made of hydrophobic silicon doped with rubidium and have a luminescent dye. The vials are placed in a 24-well plate, which is placed on top of the SDR. Every minute, the SDR emits a flick of light that excites the dye. After excitation, the sensor phosphorescent light is detected by the SDR. Oxygen in the solution acts as a quencher for the phosphorescence and reduces the luminescent lifetime. The partial pressure of oxygen in the sample thus determines the luminescent lifetime in the sensor. This luminescent lifetime is translated back to oxygen concentration by the software (PreSens SDR_v4.0.0), giving the concentration of oxygen in the sample. The sensor plates are mounted on the Sensor Reader in an incubator (Digital incubator Incu-line 23L), maintaining a constant temperature at 25 ^{o}C.

Reactions in the incubator

The PreSens software calculates the oxygen concentration using the Stern-Volmer relationship (Equation 1).

Equation 1. Stern-Volmer relationship

$$\frac{I0}{I} = 1 + K_{sv} \times [O_2]$$

Where I_0 is the luminescent lifetime in absence of oxygen, I is the actual luminescent lifetime of the sample, K_{sv} is the Stern-Volmer constant that denotes quenching rate, and $[O_2]$ is the oxygen (quencher) concentration. K_{sv} is temperature dependent. After reaching constant temperature the O_2 concentration stabilizes. During the initial phase, or “lag phase”, the inoculated bacteria adapt to its new environment and substrate. During this phase oxygen consumption is low. Some activity is nevertheless taking place since the bacteria are synthetizing new enzymes and multiplies, though no significant biodegradation occurs. Then the decomposition phase starts causing the O_2 concentration to decline. During the initial linear decomposition, one may assume that the amount of DNOM and O_2 are not limiting factors. The maximum rate of which the O_2 is consumed, i.e., the steepest slope of the declining O_2 concentration, is then reflecting the biodegradability of the DNOM. The bacteria will often consume the most labile parts of the substrate first before they start to consume other more recalcitrant parts. At the end of the incubation, the O_2 concentration decreases less. This may be due to that there is no more DNOM to decompose, but it may also be due to lack of O_2, nutrients, or accumulation of toxic wastes from the bacterial community.

Finding biodegradability

The biodegradability is the respiration rate, which is the maximum slope of the decrease in O_2 concentration during incubation. Typically, the curve of oxygen concentration with time has a negative sigmoid shape. An R-script was developed to extract descriptive parameters for the biodegradability of DNOM from each curve (https://github.com/CamilMC/100_lakes_2019). Data were removed from the initial hours of incubation, due to instable temperature at the start, the lag phase, and measurements after 30 hours.

First the oxygen concentration values, $[O_2]_{\text{initial}}$, were normalized, $[O_2]_{\text{corrected}}$, by the ratio of oxygen concentration in the corresponding blanks, $[O_2]_{\text{blank}}$, divided by the mean of all the blanks, using Equation 2:

Equation 2. Normalizing O_2 concentration

$$[O_s]_{corrected} = \frac{[O_2]_{initial}}{\left(\frac{[O_2]_{blanks}}{mean_{([O_2]_{blanks})}}\right)}$$

Then the decline in oxygen concentration in each vial was fitted as a constrained spline and the derivative of the equation was calculated. The derivative curve displays a peak, corresponding to the maximum rate of oxygen consumption. From the peak the absolute biodegradability can be extracted. Then biodegradability was determined for each of the 73 lakes as a median of 4 replicate samples. To make sure the biodegradability is not dependent entirely on the DNOM content the relative biodegradability can be calculated. The relative biodegradability is a quality factor describing the relative speed of biodegradability and is thus independent of the amount of DNOM. This is found by dividing the absolute biodegradability by the dissolved organic carbon (DOC) concentrations of the sample.

3.3.2 Chemical analysis

Chemical analysis was done at the Department of Chemistry, the Department of Biosciences at the UiO and at the Faculty of Environmental Sciences and Natural Resource Management at the Norwegian University of Life Sciences (NMBU). The following parameters were determined: pH, conductivity, alkalinity, TOC/DOC, UV-Vis absorbance, major anions and cations, gases, TN/DN – total and dissolved nitrogen, and TP/DP – total and dissolved phosphorus (OSF, 2021).

pH

The pH is measured according to ISO 10523. A Thermo Scientific Orion^{TM} DualStar^{TM} pH/ISE Dual Channel Benchtop Meter with an Orion^{TM} ROSS Ultra^{TM} pH electrode were used. This electrode is a combination electrode that combines the glass electrode and the reference electrode. pH is measured by measuring the H^+ as a potential between the internal electrode and the reference electrode in millivolts. The pH meter is calibrated using two buffer solutions.

Conductivity

Conductivity was measured using a Mettler-Toledo FiveGo^{TM} conductivity meter and an LE703 conductivity probe, according to ISO 7888 (1985). Prior to measurement, the instrument was calibrated using an 84 μS*cm^{-1} calibration solution.

Alkalinity

Alkalinity was measured by titrating the amount of 0.1 M HCl needed to bring the sample to a stable pH = 4.5 using a Titrino 702 SM autotritration. Alkalinity is a measure of the buffering capacity.

Dissolved organic carbon analysis

Total and dissolved organic carbon (TOC, DOC) was measured at the Department of Biosciences, UiO, in accordance with the ISO 8245 method (1999) using the Shimadzu instrument TOC-VCPH with an ASI-V automatic sampler. The instrument setting used in this analysis was Non-Purgeable Organic Carbon (NPOC). With this setting the inorganic carbon in form of bicarbonate in the samples were first protonated to carbonic acid by adding HCl. The CO_2 is then purged out with N_2 gas. The organic carbon in the DNOM in the aqueous sample is then combusted to CO_2 at 680°C using a titanium oxide catalyst. This CO_2 is measured by a non-dispersive infrared (NDIR) detector.

Potassium hydrogen phthalate standards were prepared and used to make a calibration curve.

UV-ViS absorbance

Absorbance spectra were measured to detect structural changes in the DNOM. This was conducted at wavelengths 200 - 800 nm using a Shimadzu UV – ViS - 1800 spectrophotometer with 1 cm quartz cuvettes. Two cuvettes containing Type I water were used to perform background correction, and one of the cuvettes was kept as a reference during the analysis for the samples.

Based on the absorbance at 254 and 400 nm, as well as DOC concentrations, specific UV absorbance (sUVa), specific visible absorbance (sVISa) and specific absorption ratio (SAR) were calculated. sUVa is the UV absorbance at 254 nm relative to the amount of DOC, measured in mg L^{-1}. The proxy is a useful way to describe the relative amount of aromatic components in DNOM. sVISa is a similar proxy based on visible light instead of ultraviolet radiation, and it is defined as the absorbance at 400 nm relative to the concentration of DOC. Adsorption of radiation at higher wavelengths requires longer chains of conjugated double bonds. sVISa thus generally reflects the relative amount of larger molecular size aromatic components in the DNOM. SAR is defined as the UV absorbency at 254 nm relative to the visible absorbance at 400 nm. Reflecting the difference between the sUVa and sVISa, a higher SAR value corresponds to a smaller size, i.e. the absorbance is relatively greater at lower wavelengths. Equation 3, 4, and 5 show how these values are calculated.

Equation 3. sUVa calculation

$$sUVa = \frac{Abs(254\ nm)}{DOC\ (\frac{mg}{L})} * 100$$

Equation 4. sViSa calculation

$$sViSa = \frac{Abs(400\ nm)}{DOC\ (\frac{mg}{L})} * 100$$

Equation 5. SAR calculation

$$SAR = \frac{Abs(254\ nm)}{Abs(400nm)} * 100$$

DNOM with high aromaticity indicates high molecular weight (HMW), which often correlates to high content of DNOM (thus dystrophic lakes have more HMW DNOM).

Major cations

Calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) and potassium (K^{+}) concentrations were analysed using an Agilent 410 microwave plasma atomic emission spectrometer (MP-AES). The MP-AES utilizes a microwave plasma and runs on air instead of combustible gases. This is a highly sensitive instrument which requires only acidification after filtration as sample preparation.

A nebulizer converts the liquid sample into mist and is introduced in plasma. The sample mist collides with the ions and electrons in the plasma, and the analyte is thereby converted to ions. Their electrons reach an excited state, and they emit photons at specific wavelengths when they return to their ground state. This emission is detected by the instrument and its wavelength identifies the element, while the intensity of the emission is proportional to the number of atoms of the element.

Major anions

The concentration of fluoride (F^-), chloride (Cl^-), phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}) and nitrate (NO_3^-) were determined by ion chromatography using a Thermo Fisher Scientific Dionex Integrion HPIC^{TM} instrument with a Dionex^{TM} AS-DU autosampler, a DionexTMAG18 guard column and AS18 separation column.

The autosampler injects the sample into the eluent stream and is pumped through the column, where the ions are separated based on their charge and radius due to their interaction with the stationary ion exchange sites. The stationary phase in the column is positively charged and interacts with the negatively charged anions. After moving through the column, the eluent and sample move through a suppressor. This is a high-capacity cation exchange membrane, which removes cations from the eluent and replaces them with H^+. The H^+ neutralizes highly conductive HCO_3^-, CO_3^{-2} and OH^-, to non-conductive H_2CO_3 and H_2O. This decreases background noise and enhances sample detection. Stronger interaction with the stationary phase results in a longer retention time in the column. Ions are identified based on their retention time, and the chromatogram peak areas and heights are compared to the peaks produced by the standard solutions. Peak areas are used to determine ion concentration.

Gases

The gases were measured using gas chromatography (GC) at the Norwegian University of Life Sciences (NMBU). The gasses Ar, N_2, CO_2, was measured using GC thermal conductivity detector (GC-TCD), N_2O was measured using GC electron capture detector (GC-ECD), CH_4 was measured using GC flame ionization detector (GC-FID), and O_2 was measured using GC XRX-620 conductivity, temperature, pressure (depth) sensors (CTD).

Total nitrogen (TN) & Total phosphor (TP)

Total amount of nitrogen (TN) and total amount of phosphor (TP) were measured at the Department of Biosciences, UiO. TN was measured using a total nitrogen measuring unit (TNM-1) attached to the Shimadzu TOC-VWP analyzer. The sample is delivered to the combustion furnace. It decomposes and is converted to nitrogen monoxide (NO) gas. The NO generated is cooled and dehumified, and then sent to the total nitrogen detector. The TP was measured on a SEAL 2-channel auto-analyzer 3, using a continuous segmented flow analysis principle, as phosphate after wet oxidation with peroxydisulfate.

3.4 Statistics

Correlation (r) explains the strength of the relationship between an independent and dependent variable, R^2 indicated to what extent the variance of one variable explains the variance of the second variable. The p-value is used as an alternative to rejection points to provide the smallest level of significance at which the null hypothesis would be rejected. A smaller p-value means that there is stronger evidence in favor of the alternative hypothesis. In Chapter 3.4.1 a short explanation of empirical correlations is provided. Then in Chapters 3.4.2 and 3.4.3 the principal component analysis and cluster analysis, respectively, are explained. And finally in Chapter 3.4.4 the Carlson trophic state index is described.

3.4.1 Empirical correlations

Empirical correlation between the variables were found using Pearson Correlation. It is a measure of linear correlation between two sets of data. The covariance of two variables is divided by their standard deviations, so that the resulting correlation coefficient (r) always has a value between −1 and 1. It only reflects a linear correlation of variables. A correlation coefficient of 1 means that for every positive increase in one variable, there is a positive increase of a fixed proportion in the other. A correlation coefficient of -1 means that for every positive increase in one variable, there is a negative decrease of a fixed proportion in the other. Zero denotes that the two parameters are not empirically related. A correlation matrix is a table showing correlation coefficients between variables. Each cell in the table shows the correlation between two variables. This can be used to summarize a large amount of data where the goal is to see patterns. Computations of the correlation matrix were done using the packages “hmisc”, and “corrplot” in R.

3.4.2 Principal component analysis

Multivariate data analysis was performed by principal component analysis (PCA), which is a method that allows summarization and visualization of the information in a data set containing multiple intercorrelated quantitative variables. Important information can be extracted from a multivariate data table and be expressed as a set of few variables called principal components (or dimensions). These new variables correspond to a linear combination of the originals. The number of dimensions is less than or equal to the number of original variables. The goal of PCA is to identify directions with the largest variances that describe most of the variance in the data with minimal loss of information. In multivariate analysis, where several variables are correlated, the number of principal components that are relevant for interpretation is low compared to the number of original variables. Variables located in one direction are positively correlated with samples located in the same direction. Computations of the PCA were performed with the “ggbiplot”, “devtool” and “ggrepel” packages in R.

3.4.3 Cluster analysis

Similarly, to a PCA, a cluster analysis organizes items into groups, or clusters, on the basis of how closely empirically correlated they are. The purpose of cluster analysis is to construct similar groups while ensuring that within a group the variance in observations is as similar as possible, while observations belonging to different groups are as different as possible. For each data set the number of optimal clusters differs. Therefore, the number of clusters must be calculated for each analysis (appendix

Figure E). Since there is no standard correct cluster, a few samples often end up in the wrong cluster. This can be either because they should be in a different cluster, or because they don't fit in with any of the clusters. To get an overview of the number of wrong samples a silhouette plot can be used. It is a bar plot that goes up to 1. If the line is above 0, the observation is correctly grouped. The closer the coefficient is to 1, the better the observation is grouped. If the line is under 0, the observation has been placed in the wrong cluster. If the line is equal to 0, the observation is between two clusters. The cluster analysis was made with the "cluster", "tidyverse" and "factoextra" packages in R.

3.4.4 Carlson trophic state index

The Carlson trophic state index for lakes is an index in a scale of 0 to 100. Each major division (10, 20, 30, etc.) represents a doubling in algal biomass. The index number can be calculated from any of several parameters, including total phosphorus. Comparing the total phosphorus concentration in µg/L with the ranges given in Table 1 one can determine the trophic state or class. Concentration of phosphorus in µg/L gives specific trophic class (Carlson, 1977). Figures were made with "ggplot2", and "ggrepel" packages in R.

Table 1. Dividing lakes into trophic classes using concentration of phosphorus (Bilgin, 2020).

Total phosphorus µg P/L	*Trophic class*
0 – 12	Oligotrophic
12 – 30	Mesotrophic
30 – 100	Eutrophic

4 Results and discussion

The results and discussion starts with describing the distribution of the biodegradability of DNOM in the lakes in Chapter 4.1. Then in Chapter 4.2 the correlation between biodegradability and the water chemistry in the lakes is discussed, and how the water chemistry can help classify the lakes. Chapter 4.3 discusses the catchment characteristics, how they correlate to each other, and how the catchment is distributed between the lakes. Then in Chapter 4.4 we explore how the biodegradability of DNOM is affected by the catchment characteristics. Also, the relative biodegradability of DNOM is discussed, including a comparison of how catchment characteristics effect the absolute and relative biodegradability of DNOM.

4.1 Biodegradability in lakes

The distribution of absolute and relative biodegradability of DNOM from highest to lowest absolute biodegradability in the lakes is shown in Figure 2.

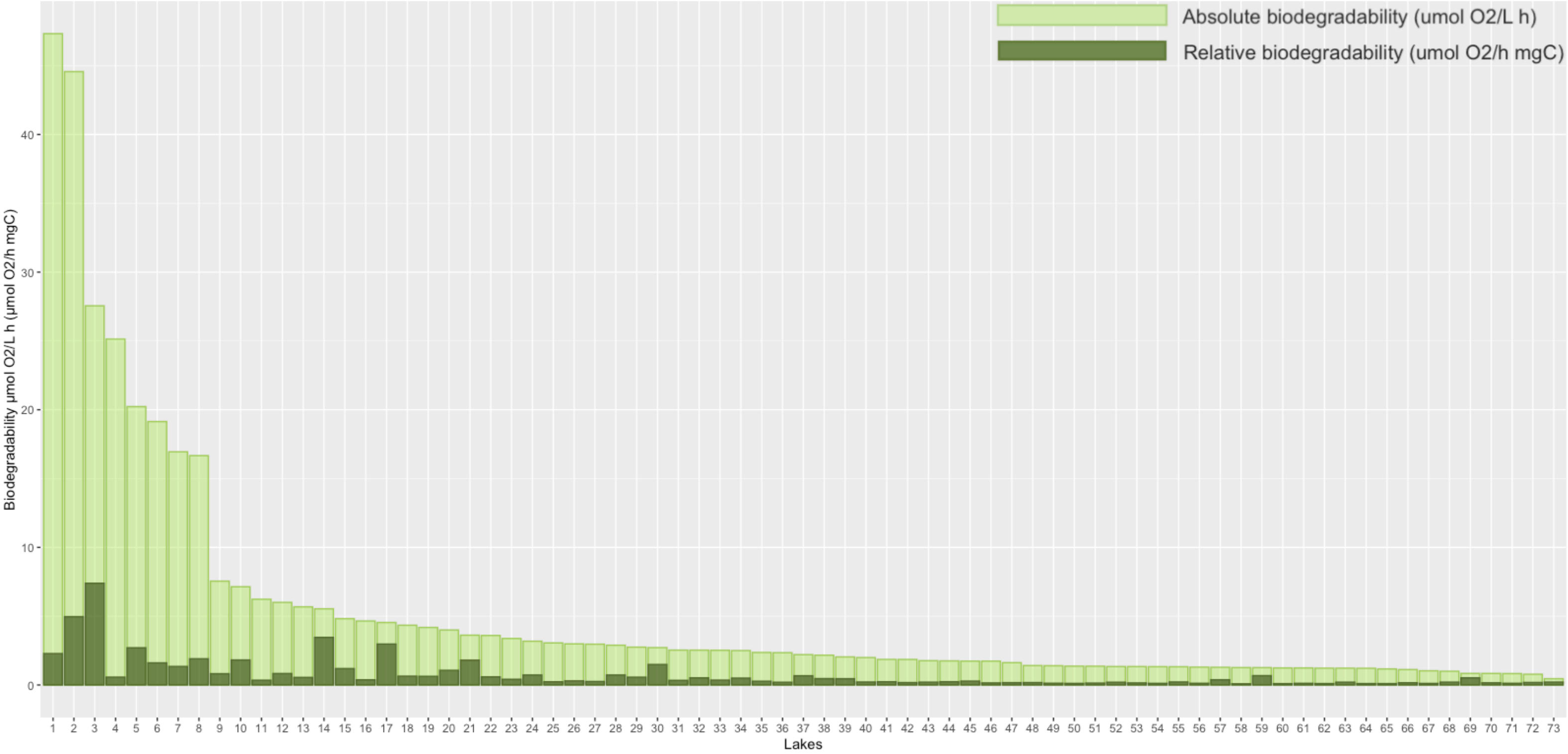


Figure 2. Biodegradability of DNOM for each lake, absolute biodegradability in a descending order in light green, with lake numbers representing absolute biodegradability from 1-73, and relative biodegradability in dark green.

Figure 2 shows the lakes from highest to lowest absolute biodegradability. Lakes 9 to 73 have relatively low biodegradability (under 10 μmol O_2 L^{-1} h^{-1}). On the contrary, lakes 1 to 8 have a relatively high biodegradability (from 47 μmol O_2 L^{-1} h^{-1} down to 16 μmol O_2 L^{-1} h^{-1}). Considering the distribution of the 73 values, these 8 lakes can be considered as statistical outliers according to Tukey's definition (see Appendix Figure A). With the exception of lakes 5 and 7 the lakes 1 to 8 are located close geographically, the longest being around 90 km apart, visualised in Figure 3. This proximity may possibly indicate that the catchment characteristics or water chemistry for each lake with high biodegradability are similar.

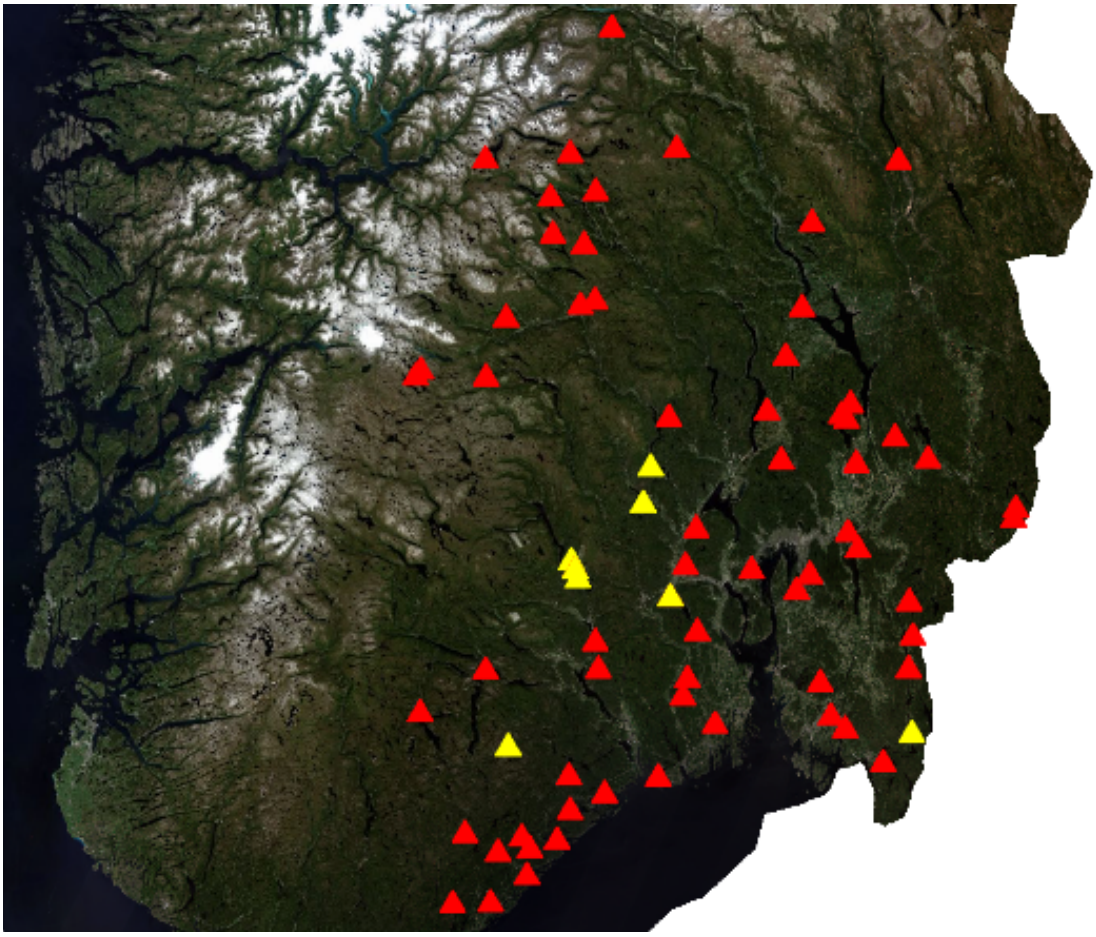


Figure 3. Map over sampling sites, with yellow triangles marking lakes with high biodegradability (above 10 µmol O_2 *L^{-1} h^{-1}), and red triangles marking the others. Source: Modified from (©Kartverket, 2021)*

As well as the absolute biodegradability, the relative biodegradability is also depicted in Figure 2. The relative biodegradability is slightly differently distributed, and the perhaps clearest example is lake 4, in which the absolute biodegradability has relatively high concentrations but is one of the lowest for the relative biodegradability. This means that the biodegradability in lake 4 is highly dependent on the amount of DNOM.

4.2 Correlations between biodegradability of dissolved natural organic matter and water chemistry

A simple linear fitting of all the water chemistry variables showed no statistically significant correlation between absolute biodegradability and water chemistry. But as this assumes a normal distribution this is not unexpected as not all the parameters are normally distributed.

The correlation matrix for water chemistry parameters with biodegradability is shown in Figure 4. Dark blue colour shows a high positive correlation, while dark red shows a high negative correlation. No colour denotes no significant ($p < 0.05$) correlation.

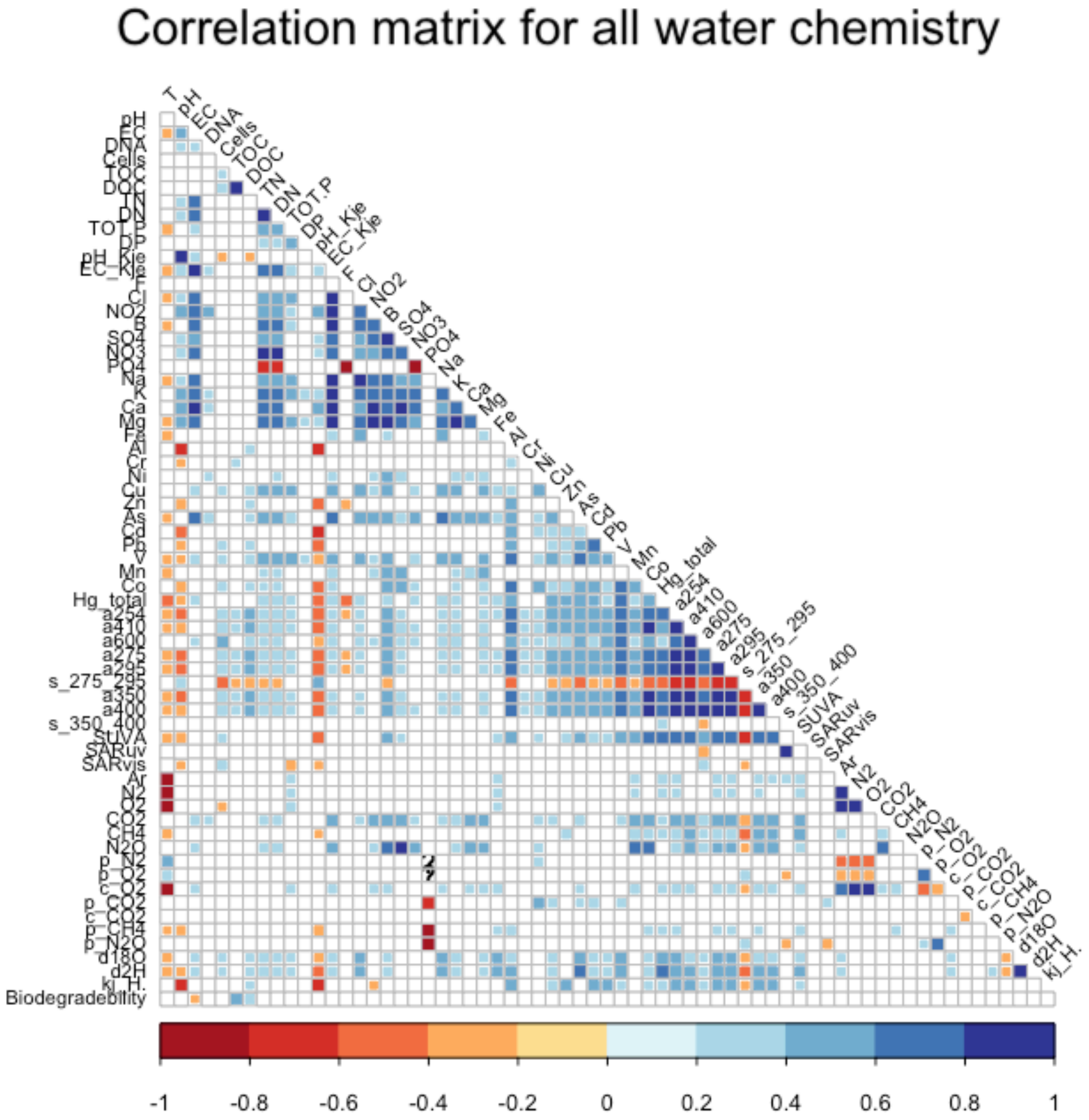
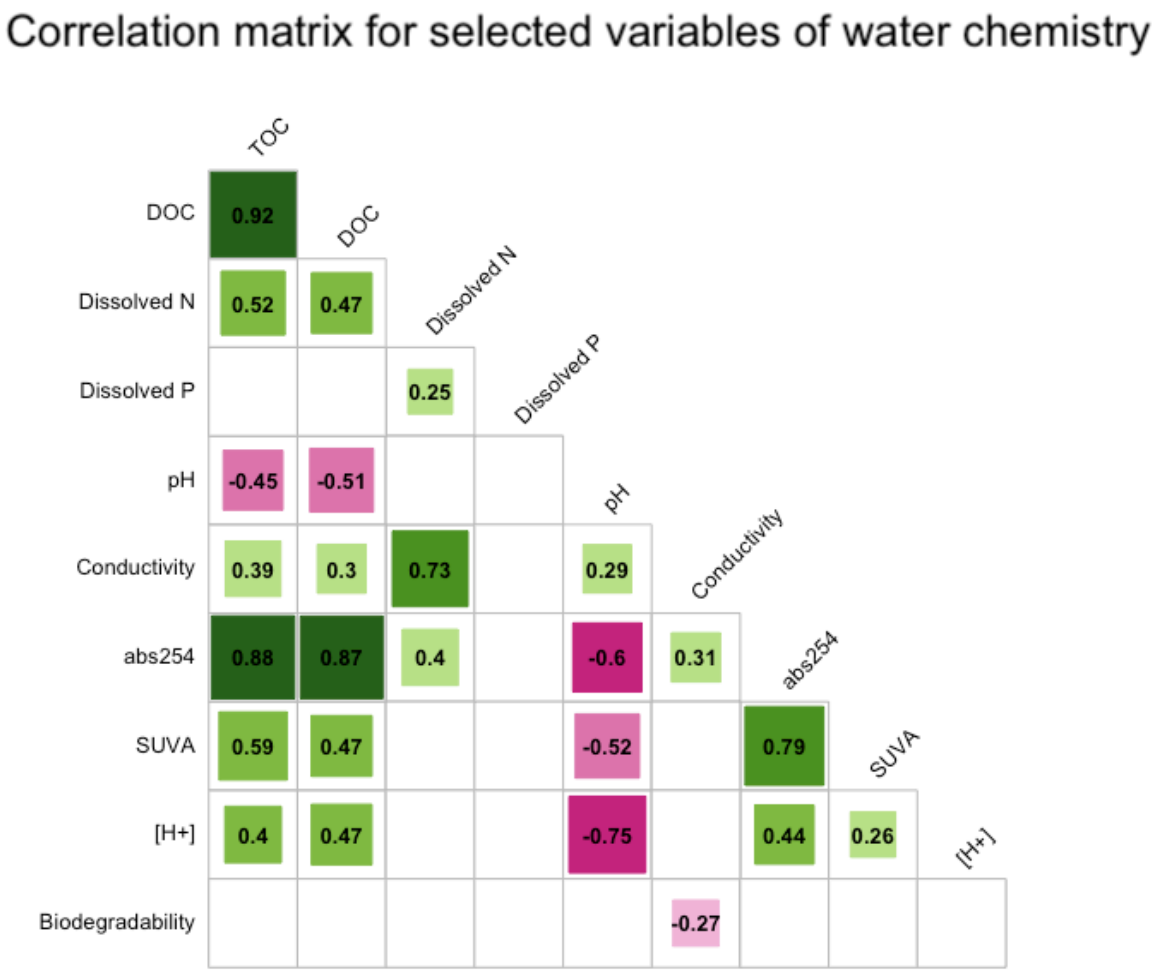


Figure 4. Correlation matrix for water chemistry for all the lakes, with only significant ($p < 0.05$) correlations colored.

In the bottom row in Figure 4 showing biodegradability, stronger colours indicate that biodegradability is more strongly correlated (i.e. high r) to the listed variable of water chemistry. This clearly shows a strong and significant positive correlation (r) of biodegradability with TOC at 0.57, and DOC at 0.40, and negative correlation with conductivity at -0.45. Non-significant ($p > 0.05$) variables that correlated to the biodegradability were dissolved nitrogen (DN) at -0.44 with a p-value = 0.08, dissolved phosphor (DP) at -0.47 and a p-value = 0.06, and sUVa at -0.31 and a p-value = 0.06. I.e., all showing a negative correlation to biodegradability (see Appendix Figure B).

TOC, DOC, Conductivity, DN, DP, and SUVA were selected for more detailed statistical studies based on their correlations to biodegradability as well as conceptual relevance. In addition, pH, concentration of H^+ and absorbance at 254 were included as they are also considered to be conceptually related to biodegradability. The correlation matrix of the selected parameters for lakes 9-73 is shown in Figure 5.


Correlation matrix for selected variables of water chemistry

	TOC	DOC	Dissolved N	Dissolved P	pH	Conductivity	abs254	SUVA	[H+]
DOC	0.92								
Dissolved N	0.52	0.47							
Dissolved P			0.25						
pH	-0.45	-0.51							
Conductivity	0.39	0.3	0.73		0.29				
abs254	0.88	0.87	0.4		-0.6	0.31			
SUVA	0.59	0.47			-0.52		0.79		
[H+]	0.4	0.47			-0.75		0.44	0.26	
Biodegradability						-0.27			

Figure 5. Correlation matrix (r) for selected variables that are expected to be correlated to the biodegradability for lakes 9 to 73. Only significant ($p < 0.05$) squares are shown.

Figure 5 shows no significant ($p < 0.05$) correlation to biodegradability, except conductivity at r = -0.27. This is not a surprising trend, as biodegradability of DNOM is not easily measured with simple chemical analysis.

For lakes 1-8, with a relatively high biodegradability, there are stronger correlations between water chemistry and biodegradability of DNOM but none are significant ($p < 0.05$) (Appendix Figure C).

Based on the correlation matrix shown in Figure 5 for conceptual relevant water chemistry parameters seven variables were studied further in a principal component analysis (Figure 6). The variables are concentration of H^+, conductivity (EC), TOC, dissolved nitrogen (DN), dissolved phosphorus (DP), sUVa and absolute biodegradability. pH, DOC and abs 254 were left out because they are highly correlated to H^+, TOC and sUVa respectively. Figure 6 is for lakes 9 to 73 only.

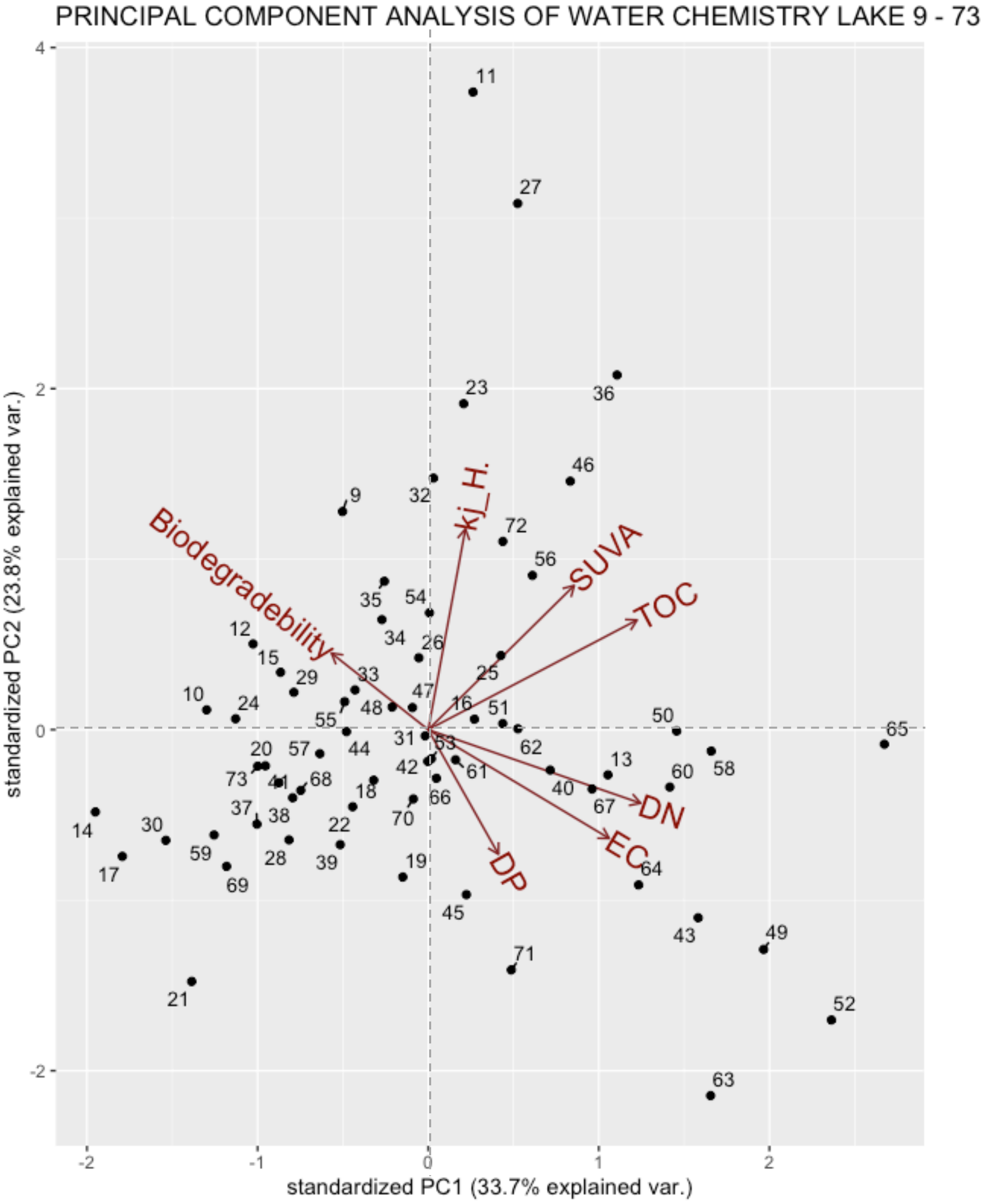


Figure 6. Biplot of principal component analysis for water chemistry on lakes 9 to 73. Variables included are absolute biodegradability, concentration of H+, SUVA, TOC, DN, EC, DP. With PC1 and PC2 explaining 57.5% of variance.

The principal component analysis in Figure 6 illustrates how the biodegradability of DNOM in the lakes depend on the selected variables, and how the different explanatory parameters relate to each other. Biodegradability has a negative correlation to dissolved nitrogen (DN) and phosphorous (DP), as well as conductivity (EC), also seen in the correlation matrix in Figure 5. There is no correlation to organic carbon (TOC) nor aromaticity (sUVa).

Summing up, the spatial variation in absolute biodegradability of the DNOM was poorly explained by the water chemistry. Including 8 samples with a biodegradability that were considered as outliers based on high values, we find that about 30% of the variance in biodegradability may be explained by the level of DNOM. Excluding these outliers, the trophic level assumes an explanatory value, with lower biodegradability at higher trophic level. High access to nutrients, particularly nitrogen, can give a relatively high carbon - nitrogen ratio. A high C:N ratio indicates recalcitrant, terrestrially (allochthonous) derived DNOM (Rantala et al., 2016), which will give low biodegradability of DNOM. While catchments that are mainly conifer forest it is likely that they are nitrogen limited. The addition of nutrients prior to the incubation is found to boost the respiration in samples where the DNOM was previously nitrogen limited (Crapart et al., 2021).

Based on phosphorus levels the lakes are classified into three trophic classes in Figure 7, also showing the distribution of TN and DOC concentration.

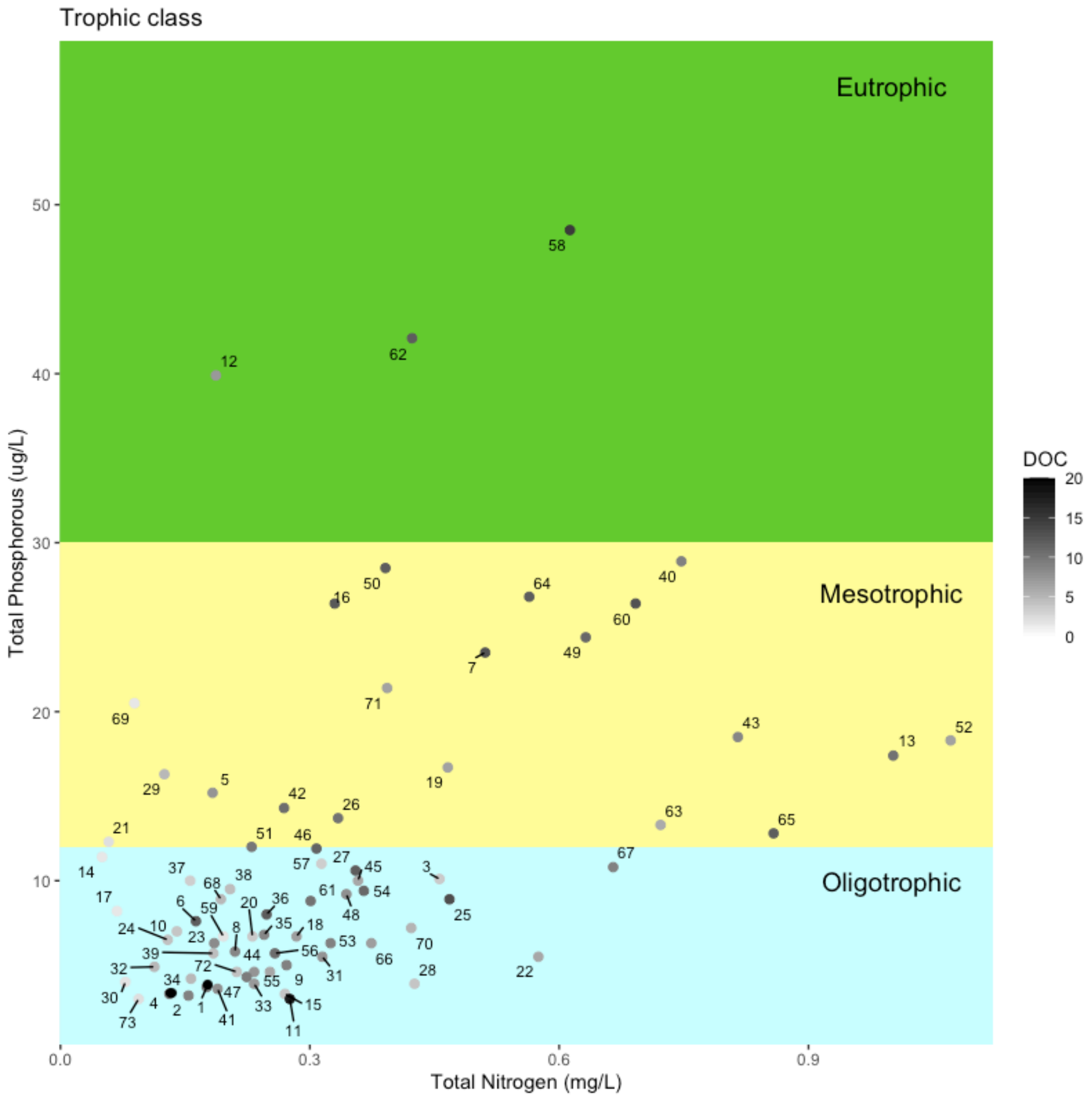


Figure 7. Trophic class based on Carlson's trophic state index

Figure 7 shows that the majority of the lakes are oligotrophic lakes, which are lakes that have low nutrient content. Oligotrophic lakes with high concentration of DOC are dystrophic or humic lakes, having high concentration of allochthonous DNOM. Three of the lakes are eutrophic with a high nutrient content, which often leads to high algae concentrations. While the rest of the lakes are mesotrophic which have a medium nutrient content.

4.3 Catchment characteristics

Catchment characteristics of all lakes are compiled by NIVA. The data include: average precipitation from the sampling day to six months back, average temperature changes from the sampling day and six months back in time (i.e 180 days); longitude, latitude and altitude of the lake; lake area, as well as the catchment types, i.e. developed land, agriculture, forests, natural non forests, peat, glaciers, or freshwaters.

A cluster analysis was done to assess which lakes can be grouped together based on their catchment similarities (Figure 8). The optimal number of clusters is five (Appendix Figure D).

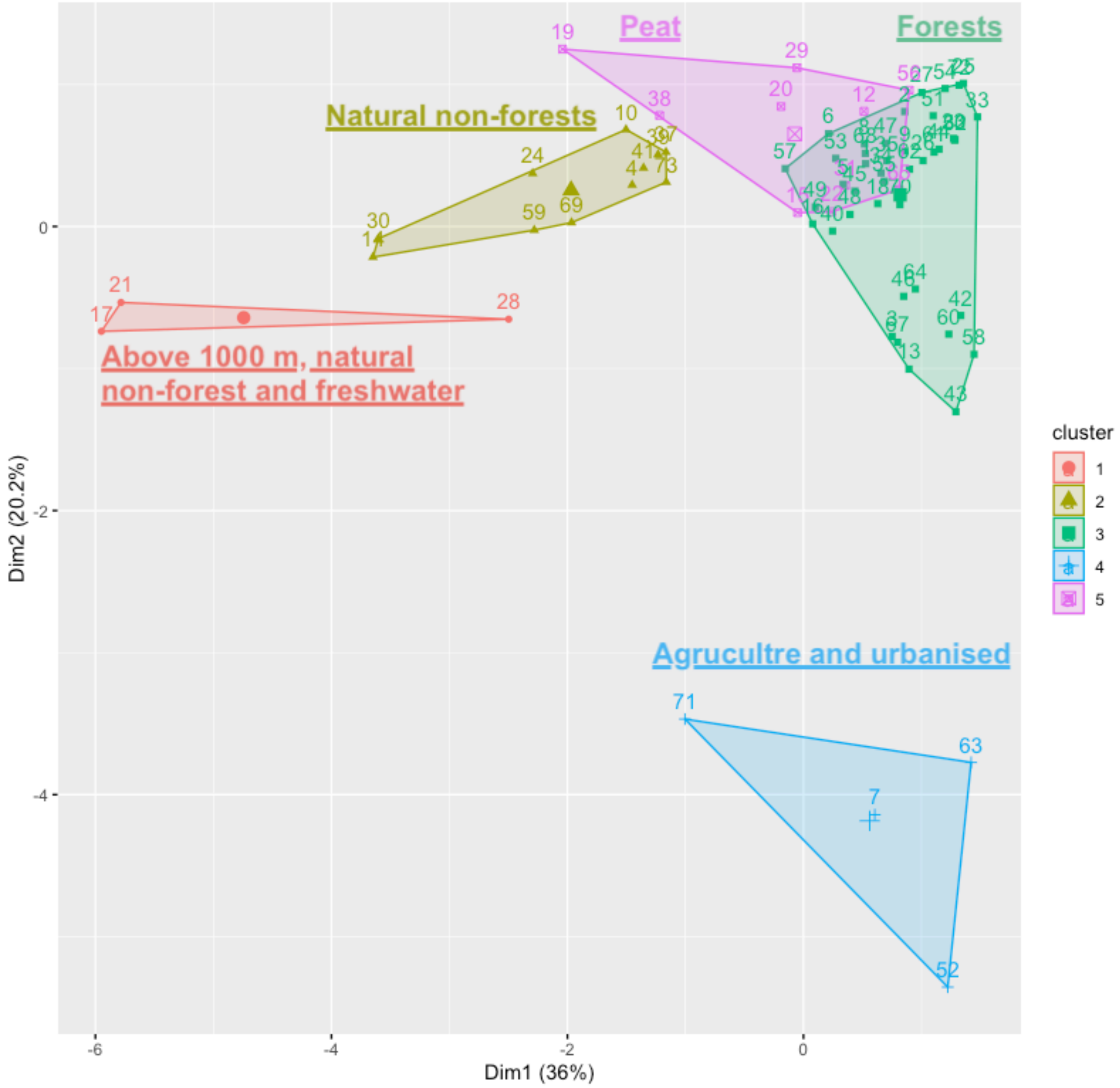


Figure 8.Cluster analysis of catchment types. Grouping the lakes with similar catchment together. K-mean quality partitioning at 63%, appendix Equation A.

Cluster 1 consists of 3 lakes, with similar amounts of natural non forests, peat, and freshwater, and non or very little forest. In this cluster, lakes 21 and 17 are lakes above the tree-line at over 1000 meter above mean sea level. Cluster 2 is composed of 11 lakes characterized by catchments with natural non forests. Cluster 3 is composed of the 42 lakes with a high proportion of forest in the catchment. Cluster 4 groups 4 lakes whose catchment are covered by agriculture or developed landscape. Finally, cluster 5 represents 10 lakes with a similar ratio of peat, higher than the other lakes. The forest cluster (3) and peat cluster (5) overlap a great deal. The catchments in both groups have a very low proportion of developed land and glaciers.

It is common in clustering analysis that some data does not fit perfectly with the model. Using a silhouette plot (Appendix Figure E), 3 observations are proved to be in the wrong cluster, lakes 28, 71, and 7. This cluster analysis shows it is likely that the main differences in catchment type is the coverage of forest and peatland.

The percentage coverage of the type of land-use coverage in the catchments for each lake, grouped using the clusters found in Figure 8, is depicted in Figure 9.

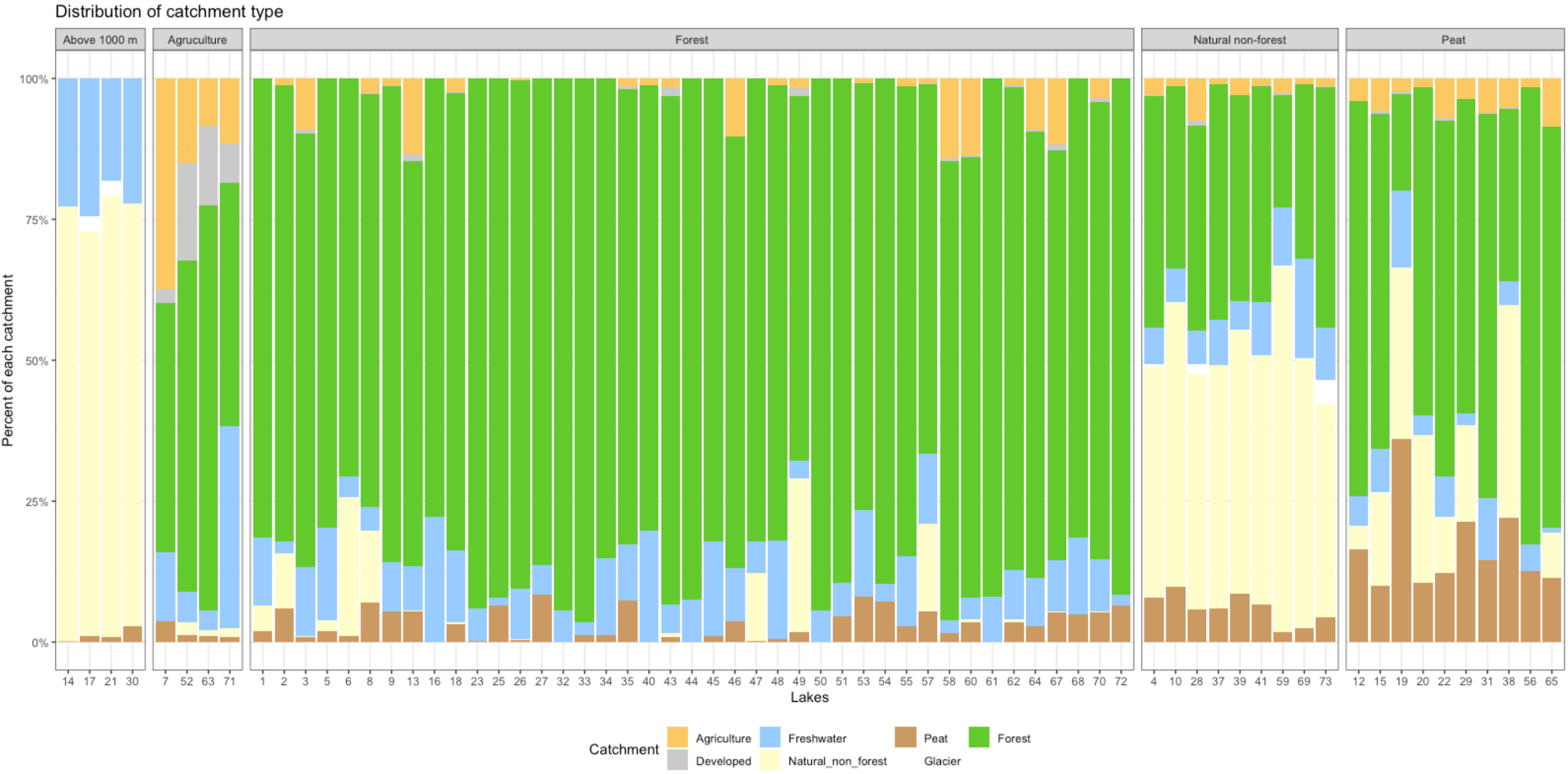


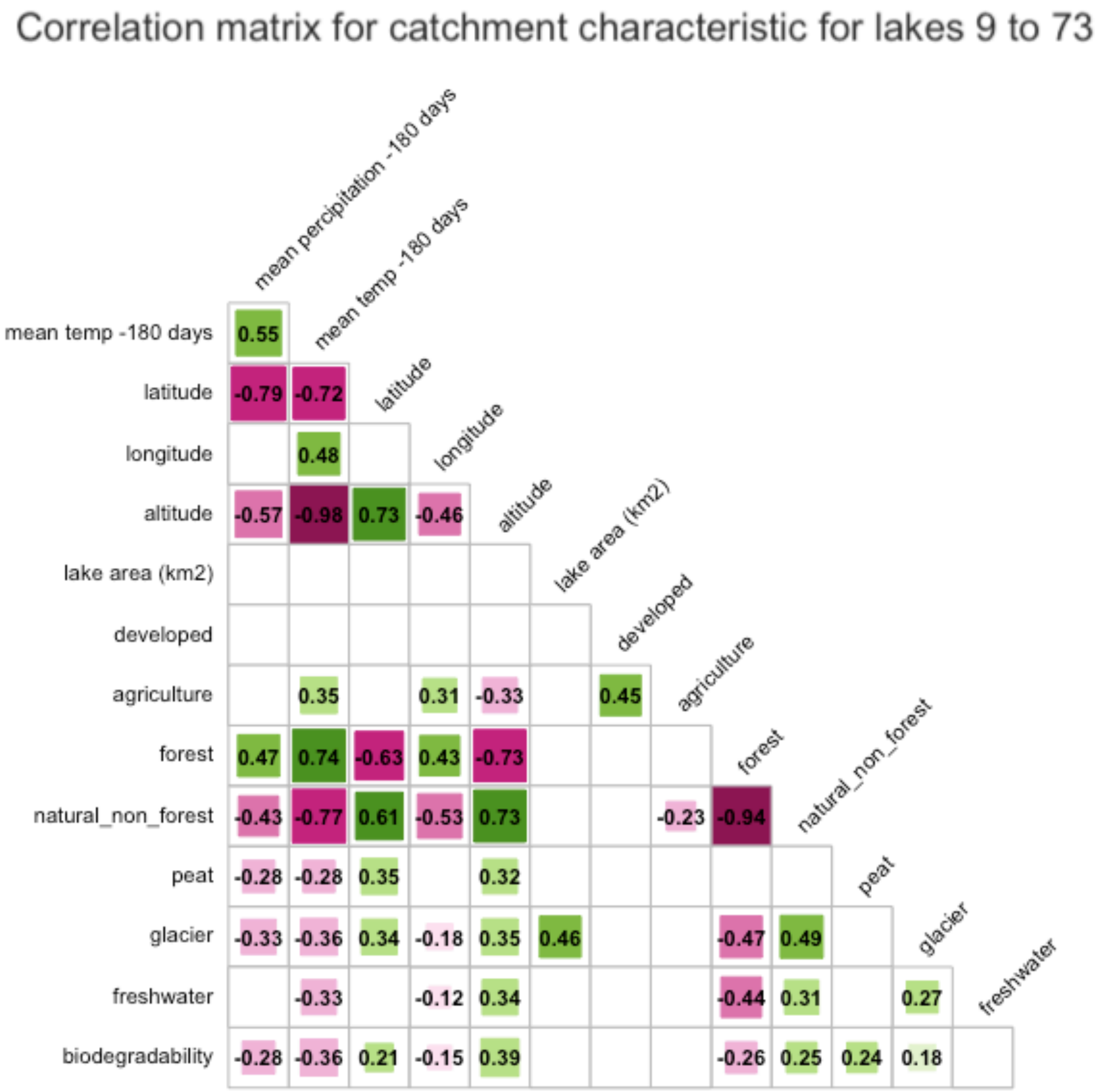
Figure 9. Distribution of categories of catchment cover of lakes grouped after the cluster analysis in Figure 8.

Four lakes (14, 17, 21, and 30) have catchments without forest, this is because they are above the tree-line, at over 1000 meters above mean sea level (cluster 1). Lake 28 that was previously found to be in the wrong cluster and has been moved to natural non-forest (cluster 2). Lakes 7 and 71 were not moved as they would not fit better in another cluster but would need their own. Lakes 1 to 4, 6 and 8 are geographically close, as shown in Figure 3, and were thus expected to have similar catchment coverage ratios. Lakes 1, 2, 3, 6 and 8 do show similarities with high amount of forest and limited agriculture, all having a small amount of fresh water and peatlands (cluster 3, forest). Lake 4 is an exception in itself, having very high values for most of the chemical parameters that were measured. For example, the concentration of DOC is twice as high as its neighbors. Tree of the lakes (11, 36, 66) have missing land cover data and are therefore not included.

These figures (8 and 9) show the large variance among the studied catchments. Most of the catchments do have some forest, natural non-forest, freshwater, and peat, but with varying ratios.

4.4 Catchment characteristics and the effect on biodegradability

A correlation analysis is conducted in order to assess if catchment characteristics are empirically correlated to the biodegradability of DNOM. Figure 10 shows the correlation matrix between the different catchment characteristics and whether they correlate to the biodegradability. Including, average precipitation from the sampling day to six months back, average temperature changes from the sampling day and six months back in time (i.e 180 days); longitude, latitude and altitude of the lake; lake area, as well as the catchment types, i.e. developed land, agriculture, forests, natural non forests, peat, glaciers, freshwaters, and absolute biodegradability. Only statistically significant correlations ($p < 0.05$) are shown.



Correlation matrix for catchment characteristic for lakes 9 to 73

	mean percipitation -180 days	mean temp -180 days	latitude	longitude	altitude	lake area (km2)	developed	agriculture	forest	natural_non_forest	peat	glacier	freshwater
mean temp -180 days	0.55												
latitude	-0.79	-0.72											
longitude		0.48											
altitude	-0.57	-0.98	0.73	-0.46									
lake area (km2)													
developed													
agriculture		0.35		0.31	-0.33		0.45						
forest	0.47	0.74	-0.63	0.43	-0.73								
natural_non_forest	-0.43	-0.77	0.61	-0.53	0.73			-0.23	-0.94				
peat	-0.28	-0.28	0.35		0.32								
glacier	-0.33	-0.36	0.34	-0.18	0.35	0.46			-0.47	0.49			
freshwater		-0.33		-0.12	0.34				-0.44	0.31		0.27	
biodegradability	-0.28	-0.36	0.21	-0.15	0.39				-0.26	0.25	0.24	0.18	

Figure 10. Correlation matrix (r) for biodegradability with catchment characteristics for lakes 9 - 73. Dark pink shows a high negative correlation and dark green shows a high positive correlation. Only statistically significant (p<0.0?) correlations are shown.

Figure 10 shows that the biodegradability of DNOM has a negative correlation (r) to forest cover at -0.26 and positive correlation to cover of natural non forests at 0.25 and peat at 0.24. This means that for this data set a catchment with a lot of natural non forest and peat generate DNOM with a high absolute biodegradability, while a lot of forest give a low biodegradability. Biodegradability was assumed to be negatively correlated to the amount of peat and positively correlated to the amount of forest. A catchment with high amounts of natural non forest inherently means there is a low amount of forest, as seen with a correlation at -0.94. As explained in Chapter 2.5.1, high forest coverage was not expected to provide DNOM with higher biodegradability. This discrepancy can be linked to the high proportion of conifer trees in these watersheds. They make more recalcitrant organic matter than deciduous forest, which is less degraded in soils because of nitrogen limitation. Furthermore, autochthonous DNOM in boreal lakes is used more for assimilation than respiration (Hessen, 1992), this can give lower biodegradability. Figure 10 also shows that altitude (0.39) has a positive correlation to the biodegradability. And it has a high positive correlation to natural non forests at 0.73.

Agriculture and developed land have a positive correlation at 0.45 ($p < 0.05$), meaning a lot of agriculture usually follows a high amount of developed land.

For lakes 1 to 8 the biodegradability (Appendix Figure F) shows a clear positive correlation to forests with an $r = 0.48$. Meaning a high percentage of forests in the catchment gives high absolute biodegradability. For both sets altitude, at 0.39 for lake 9 to 73 and 0.45 for 1 to 8, shows correlation to biodegradability. This is most likely because the DNOM in lakes in high altitude is more often LMW DNOM and therefore easier to degrade. A correlation matrix for all lakes (1 to 73) was also made (Appendix Figure G), though no variables was significantly ($p < 0.05$) correlated to the absolute biodegradability.

These catchment characteristics parameters that were significantly correlated to biodegradability were further studied with a new cluster analysis. Figure 11 shows the two clusters resulting from the grouping based on the significant variables in Figure 10. The optimal number of clusters is two (Appendix Figure I).

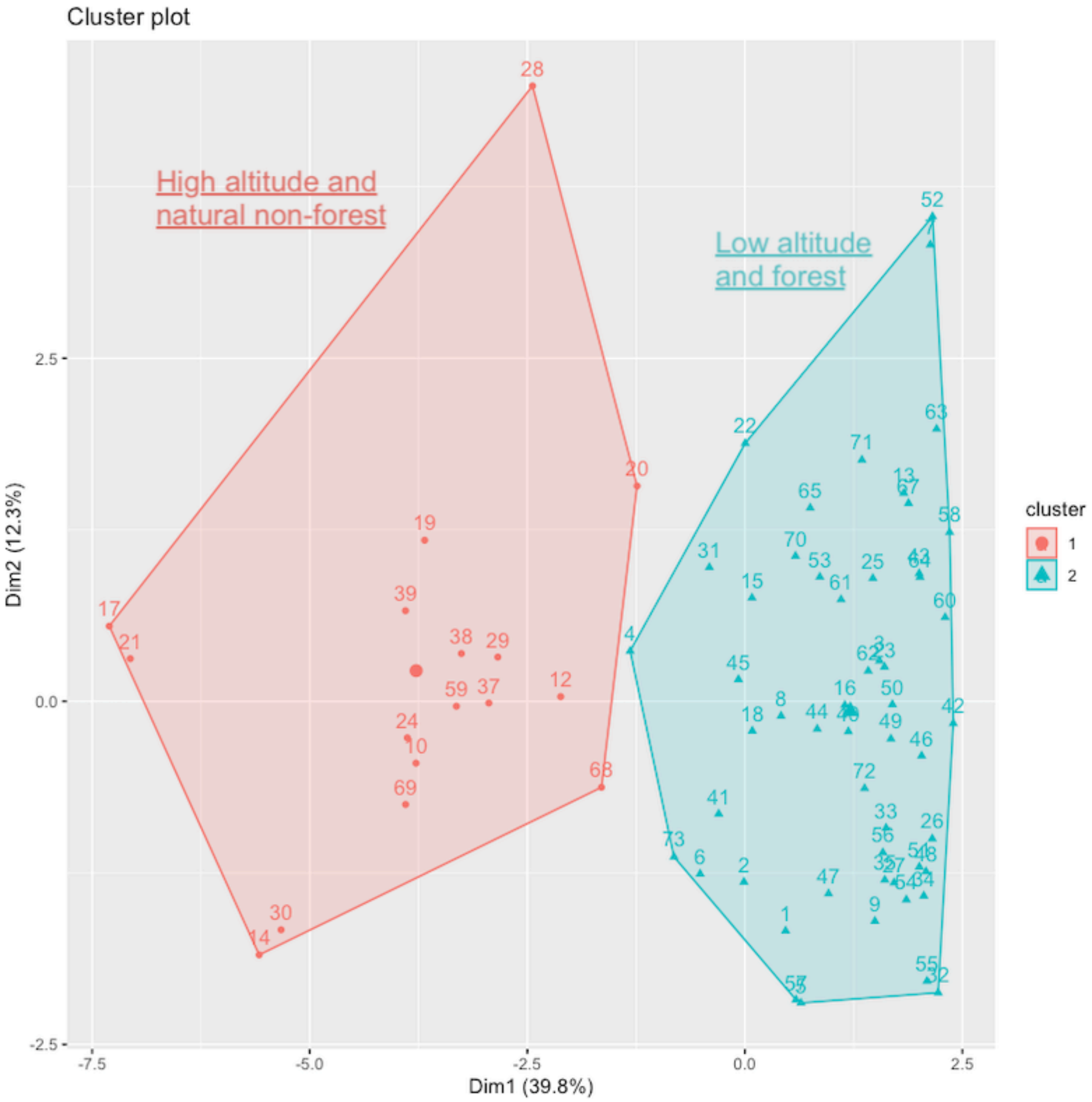


Figure 11. Cluster analysis for catchment characteristics Grouping the lakes together with the most important different parameters. K-mean quality partitioning at 32%, Appendix Equation B.

From Figure 11 only two groups or clusters are relevant: cluster 1 with 17 lakes and cluster 2 with 53 lakes. Cluster 1 dominated by a high altitude and a high percentage of natural non-forest, while cluster 2 groups lakes with lower altitude and a high percentage of forests in the catchment. Using a silhouette plot (Appendix Figure I), two lakes were found to be in the wrong cluster, this was lake 12 and 68. And one lake was in between the two clusters, this was lake 20. The high altitude waters can be first order watercourses only staying for a short period of time and therefore not being degraded in the cold waters. They often are also clear, shallow waters which gives room for photo-degradation, creating more LMW DNOM which is easier to degrade. Since these lakes are generally oligotrophic and generally nitrogen limited, it could also be a priming effect, meaning that adding nutrients to nutrient limited lakes is affecting the primary production and giving high empirical correlation to biodegradability.

Since catchment type and altitude are the main clusters in Figure 11, a PCA with these parameters and biodegradability of DNOM is shown in Figure 12.

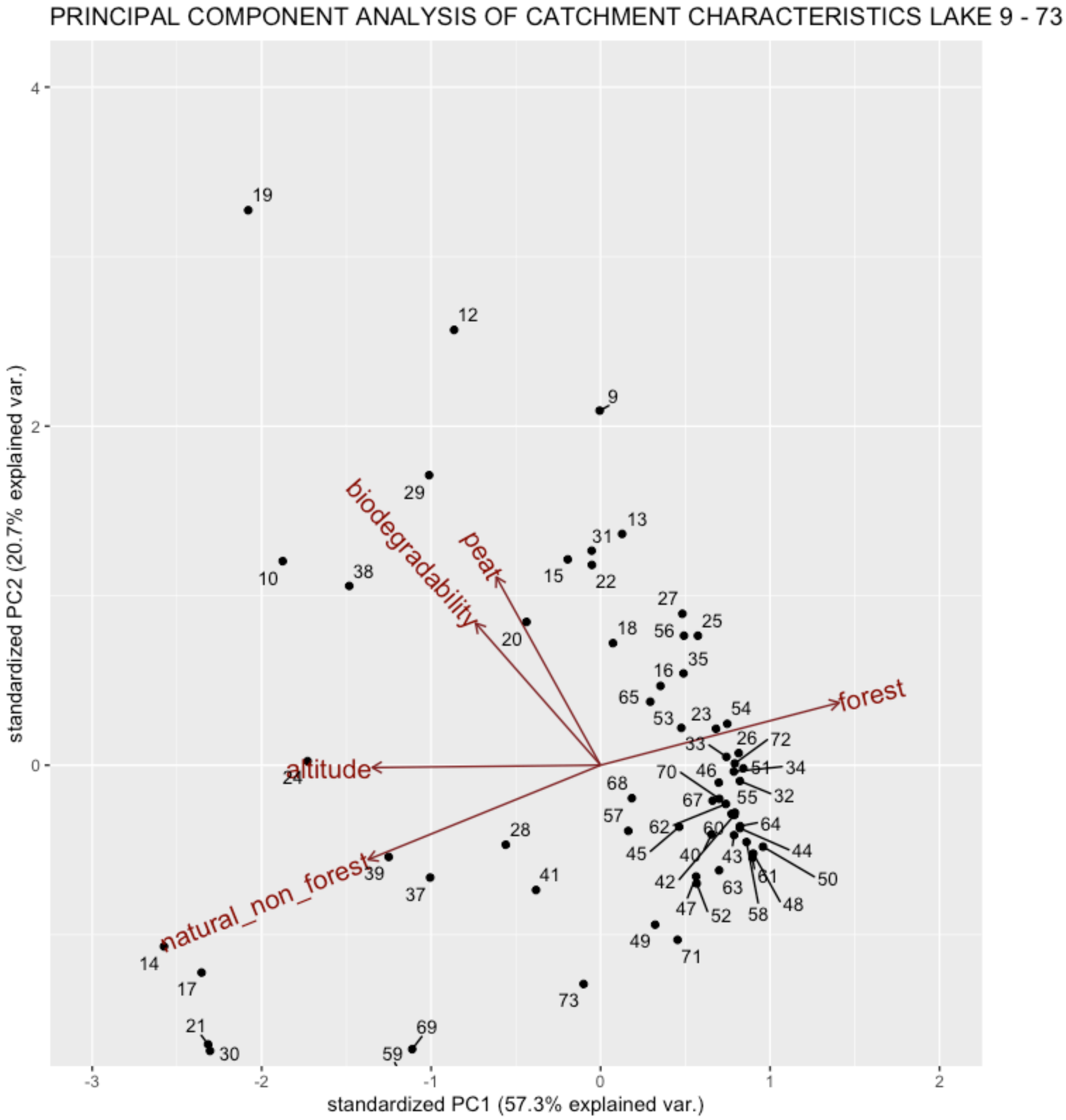
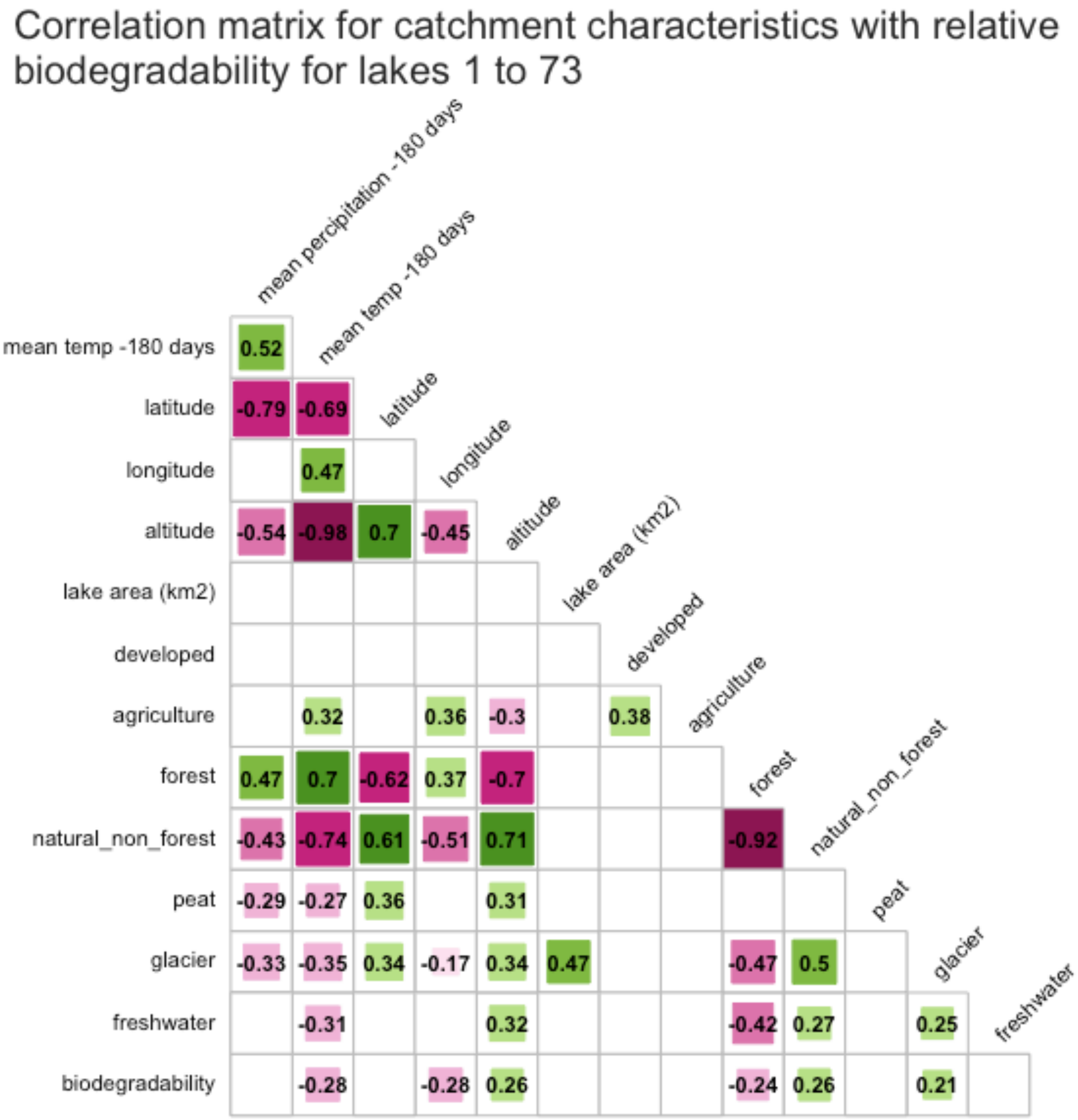


Figure 12. PCA of natural non-forest, altitude, biodegradability of DNOM, peat, and forest with PC1 and PC2 explaining 78 % of the variance.

From Figure 12 biodegradability and altitude is positively correlated along both principal components, as we have seen in the other analyses as well (Figure 10 and Figure 11). Peat also is highly positively correlated to biodegradability; this is most likely because it is the absolute biodegradability that is assessed. What this means is that the correlation to peat is very dependent on the amount of DNOM in the catchment. Therefore, using the relative biodegradability can give a clearer picture of how peat correlates to biodegradability of DNOM (Chapter 4.4.1). Forests are inherently highly negatively correlated to natural non-forests. Surprisingly natural non-forests are not correlated to biodegradability as we have seen in the other analyses, this could be because it is so strongly negatively correlated to forests. Therefore, PC1 showing a forest to non-forest gradient, with PC2 showing a more peat dependent gradient, biodegradability of DNOM gets put in the middle as it is dependent on all.

4.4.1 Catchment characteristics with biodegradability relative to the amount of dissolved natural organic matter

The biodegradability normalized by the DOC expresses the relative biodegradable quality of the DNOM. A correlation matrix for the relative biodegradability of DNOM for catchment characteristics is depicted in Figure 13.



Correlation matrix for catchment characteristics with relative biodegradability for lakes 1 to 73

	mean percipitation -180 days	mean temp -180 days	latitude	longitude	altitude	lake area (km2)	developed	agriculture	forest	natural_non_forest	peat	glacier	freshwater
mean temp -180 days	0.52												
latitude	-0.79	-0.69											
longitude		0.47											
altitude	-0.54	-0.98	0.7	-0.45									
lake area (km2)													
developed													
agriculture		0.32		0.36	-0.3		0.38						
forest	0.47	0.7	-0.62	0.37	-0.7								
natural_non_forest	-0.43	-0.74	0.61	-0.51	0.71				-0.92				
peat	-0.29	-0.27	0.36		0.31								
glacier	-0.33	-0.35	0.34	-0.17	0.34	0.47			-0.47	0.5			
freshwater		-0.31			0.32				-0.42	0.27		0.25	
biodegradability		-0.28		-0.28	0.26				-0.24	0.26		0.21	

Figure 13. Correlation matrix (r) for catchment characteristics with relative biodegradability for lakes 1 to 73. Dark pink shows a high negative correlation and dark green shows a high positive correlation. Only statistically significant (p<0.05) correlations are shown.

The correlation matrix (r) shows that natural non-forest at 0.26 and altitude at 0.26 are the catchments characteristics that are strongest positively correlated to the relative biodegradability. The relative forest cover is negatively correlated at -0.24, along with mean temperature for the last 180 days and longitude at -0.28. Neither developed land nor agriculture are significantly ($p < 0.05$) correlated to relative biodegradability. Peat is also no longer significant with $r = -0.08$ and a $p = 0.7$. This tells us is that the explanatory value of peat, that for the absolute biodegradability was 0.24, was mainly because of its correlation to DNOM. Peat is generally more likely to be negatively correlated to biodegradability than positive, which was the expected outcome.

Then a PCA is made similarly to Figure 12, but without peat as it is not significantly correlated in the correlation matrix in Figure 14, i.e. including natural non-forest, altitude, relative biodegradability (bdg), and forest, for lakes 1- 73.

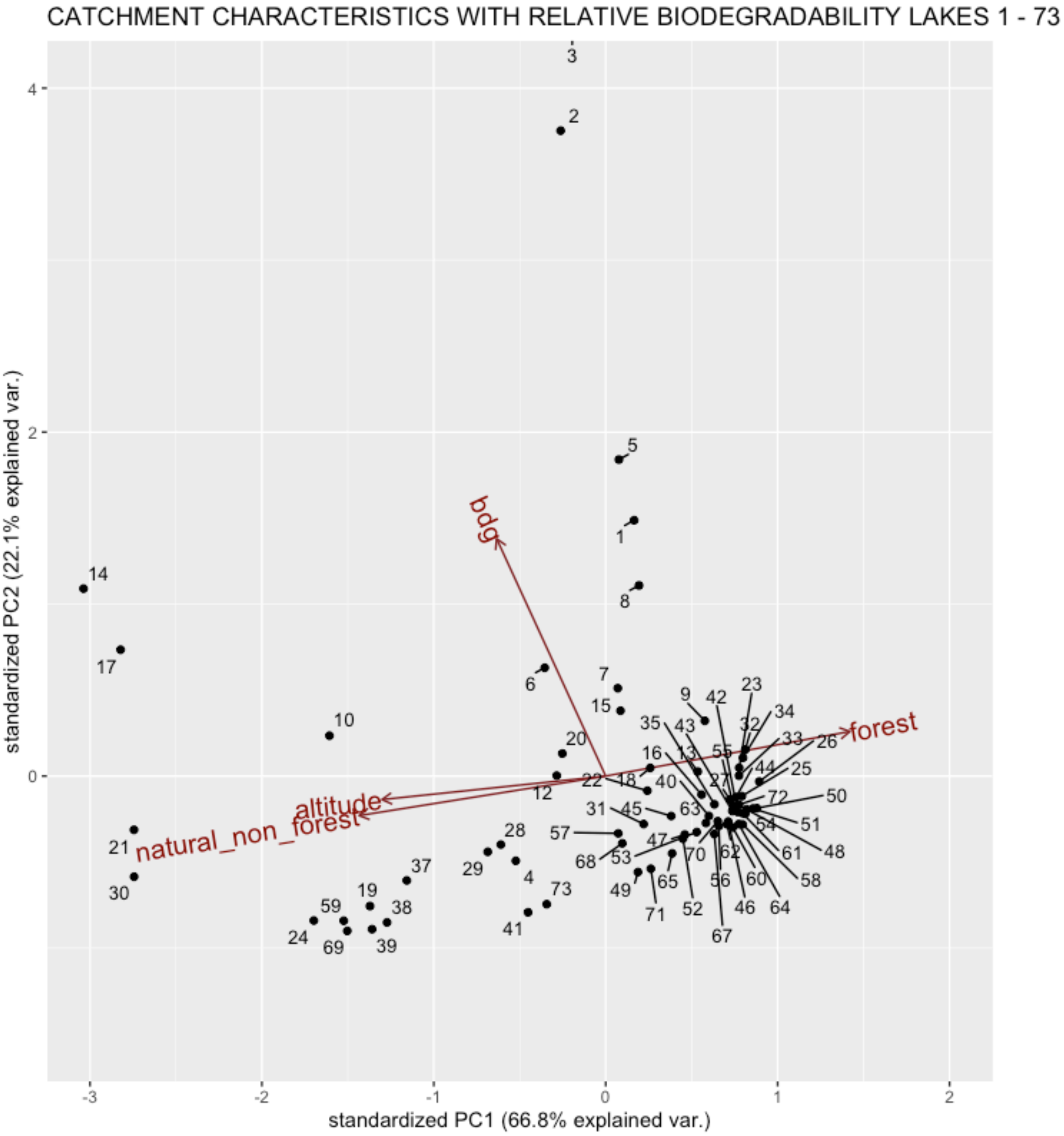


Figure 14. PCA with forest, non-forest, altitude, and relative biodegradability (bdg) of DNOM with PC1 and PC2 explaining 88.9 % of the variance.

Relative biodegradability (bdg) is balanced between the forest or no forest cover. Natural non-forest is highly correlated to altitude. The lakes generally cluster around forest cover, this is also what has been seen in Figure 9 and 11, where there are most lakes with forest cover governing the catchment. Again, whether there is a forest cover or not is the most important parameter for the biodegradability of DNOM.

4.4.2 Comparison of absolute and relative biodegradability of dissolved natural organic matter

Comparing the correlations of catchment characteristics to the absolute and relative biodegradability of DNOM provides a clearer view of what is governing the amount and quality of DNOM. This is shown in Figure 15.

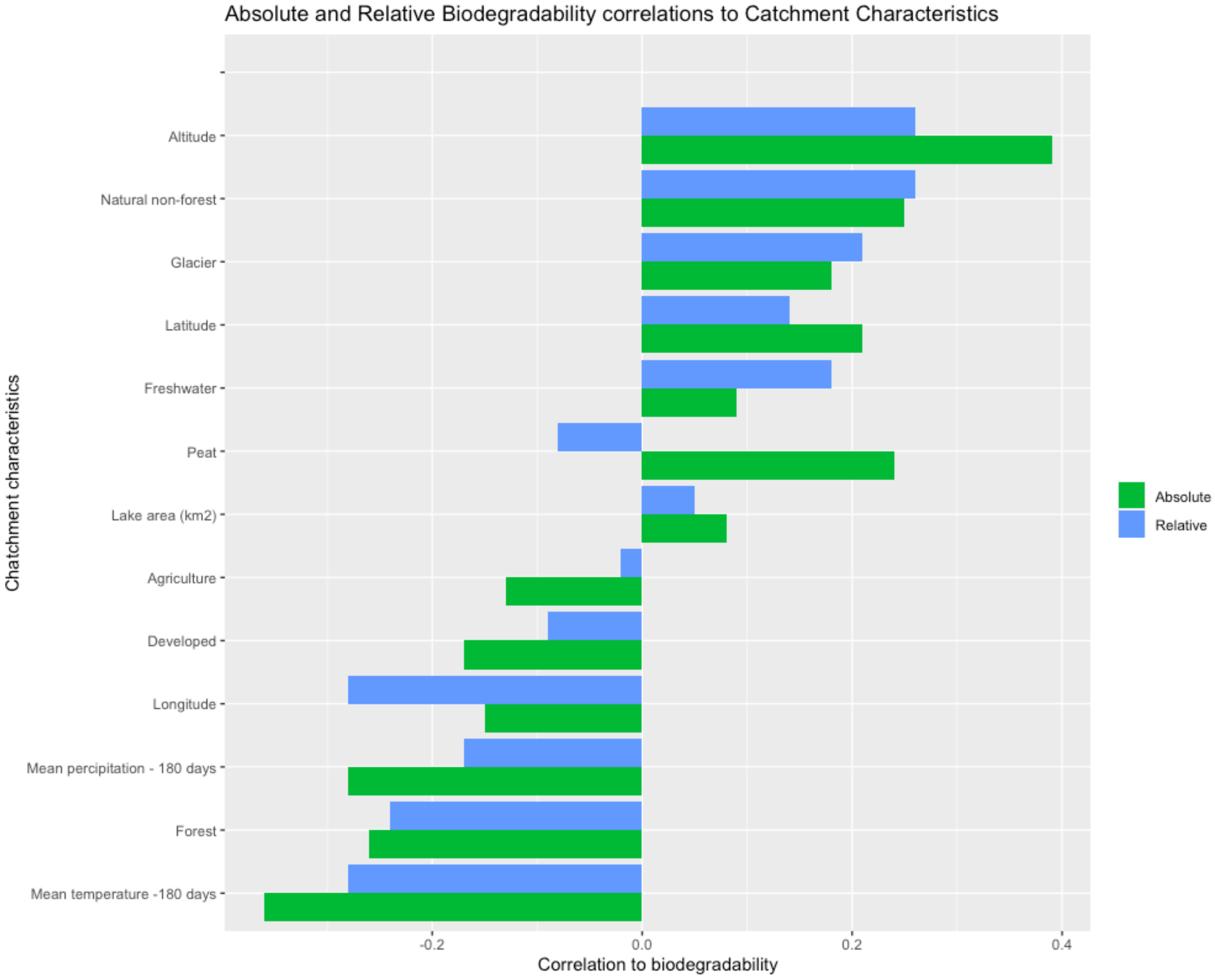


Figure 15. Absolute and relative biodegradability comparison for correlations to catchment characteristics. The highest positive correlations are shown first, then the low correlations, and finally the negative correlations. The absolute biodegradability is shown in green and the relative biodegradability in blue.

The main difference between the absolute and relative biodegradability is its correlation to peat. In the relative biodegradability the peat coverage is no longer dependent on the amount of DNOM. As expected, peat is negatively correlated to relative biodegradability, since peat generally has more HMW allochthonous DNOM, making it harder to degrade and therefore giving lower relative biodegradability. Relative and absolute biodegradability is otherwise rather equal. With altitude, and natural non-forest as the highest positively correlations, and with mean temperature the last 180 days and forest as the highest negatively correlated. This means that generally the forest or non-forest cover, and altitude is highly indicative of the biodegradability of DNOM.

5 Conclusion

In this study empirical correlations are assessed between spatial differences in biodegradability of the DNOM and its characteristics, the chemistry of the water matrix, and the site and catchment characteristics. The aim was to deduce the main chemical processes governing the absolute and relative biodegradability of DNOM.

This study shows that there is a significant ($p < 0.05$) empirical correlation between the biodegradability of DNOM and site and catchment characteristics. The spatial variation in absolute biodegradability of the DNOM was not clearly explained by the water chemistry. Though, some of the variance in biodegradability could be explained by the level of DOC as a proxy for the amount of DNOM. And a lower biodegradability could be found in lakes with a higher trophic level. The degree of forest cover is a key explanatory factor for the differences in biodegradability of the DNOM. Both high cover of forest and low cover (natural non-forest) had relatively strong correlations, at -0.26 and 0.25. Peat coverage was also found to be an important explanatory factor, having relatively high positive correlation (0.24) to the absolute biodegradability of DNOM. But assessing the biodegradability of DNOM relative to the amount of DOC showed that amount of peat in the catchment was highly governing for DNOM concentrations. And peat was found to not be significantly ($p < 0.05$) correlated to relative biodegradability. The catchment characteristic that had the highest explanatory value on the absolute and relative biodegradability of DNOM was the altitude. Altitude reflects multiple characteristics of the catchment that affect the DNOM, that again affects the biodegradability of DNOM. Lakes at high altitudes have more LMW DNOM having an important effect on the biodegradability of DNOM.

Based on these findings we can speculate that as climate change continues with increasing temperatures, the tree-line will be affected and the biomass will continue to increase. This will lead to more DNOM in high altitude lakes, though this DNOM will be more HMW so more difficult to degrade. Though it now is relatively easy to degrade

DNOM at higher altitudes, there is also a low concentration of DNOM. So even if the DNOM will become harder to degrade there will likely be more emissions of GHG. If we assume that this “recalcitrant” organic matter from trees is not degraded because of the lack of nutrients, the greening will produce a pool of organic matter that only needs a boost to be degraded.

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Appendix

Outliers

Outliers in the data set for biodegradability, using boxplot, all lakes with biodegradability higher then 10 are outliers, meaning lake 1-8 are outliers for this data set.

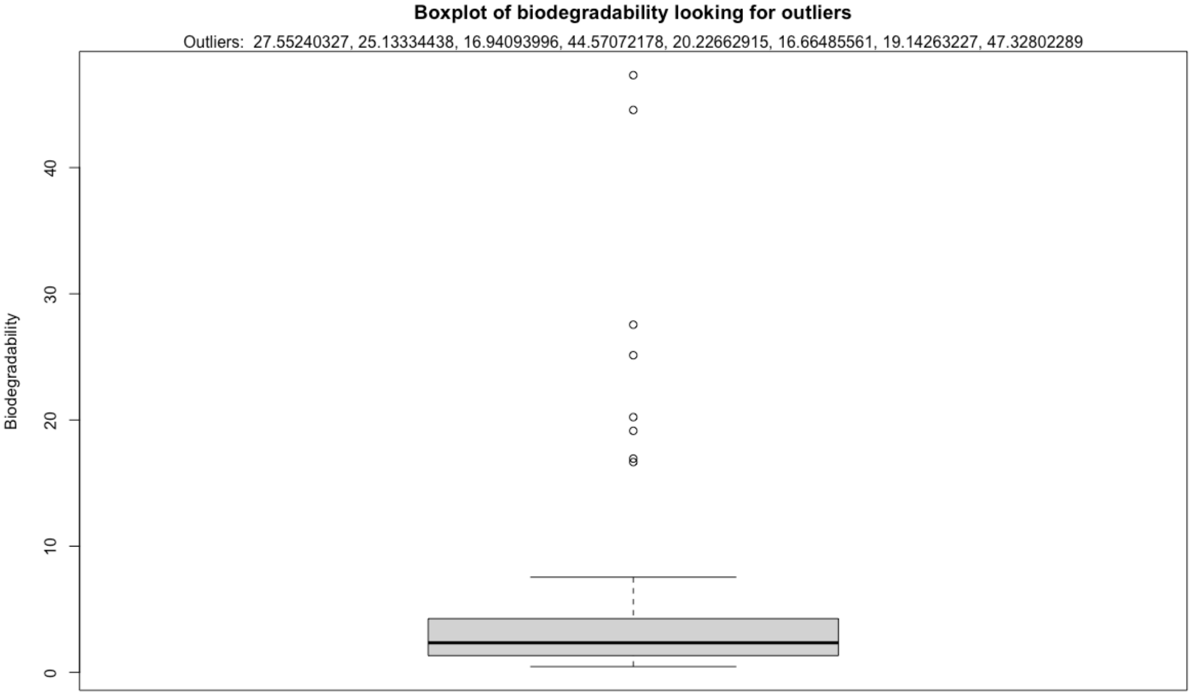


Figure A. Outliers in biodegradability

Water chemistry

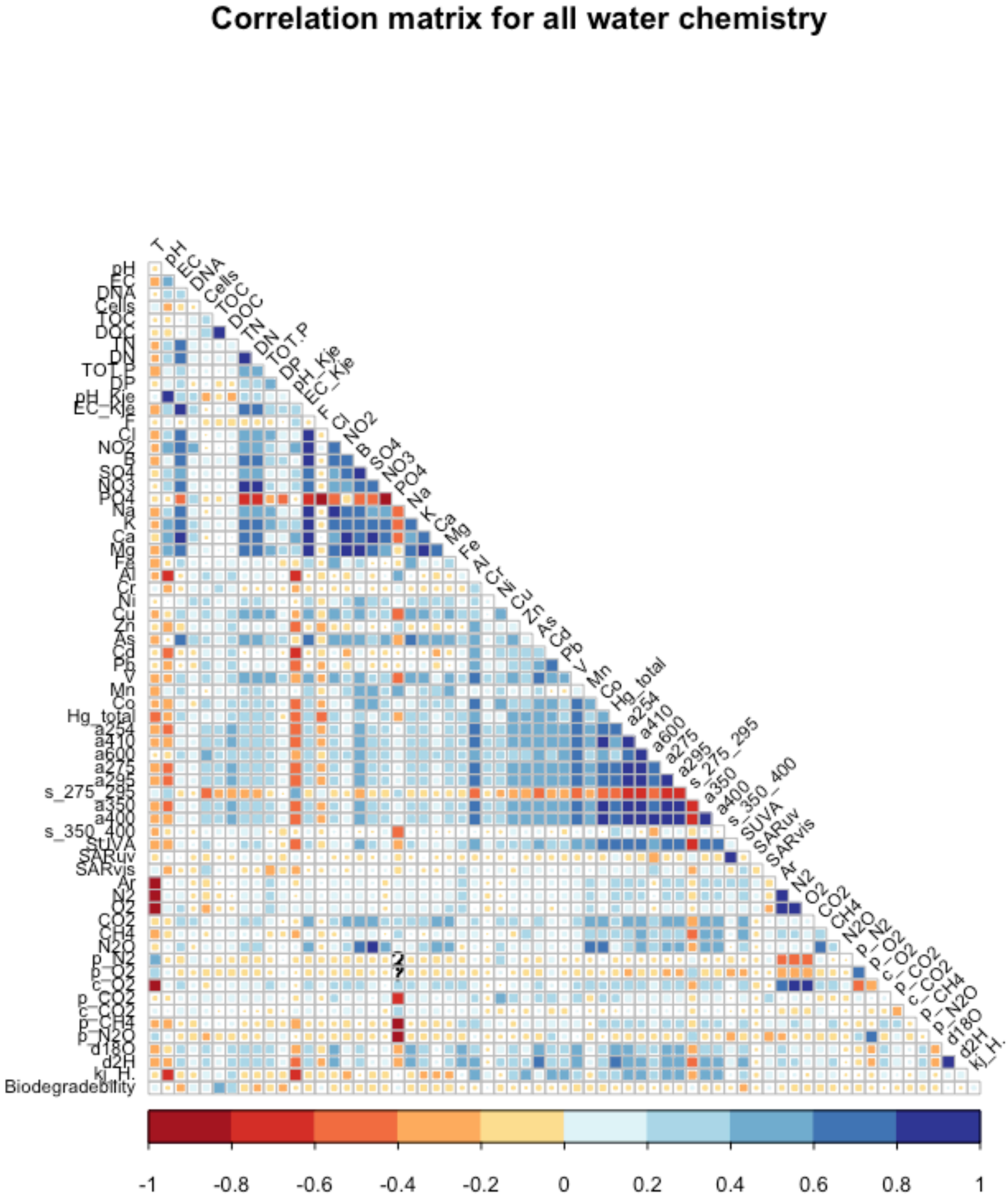
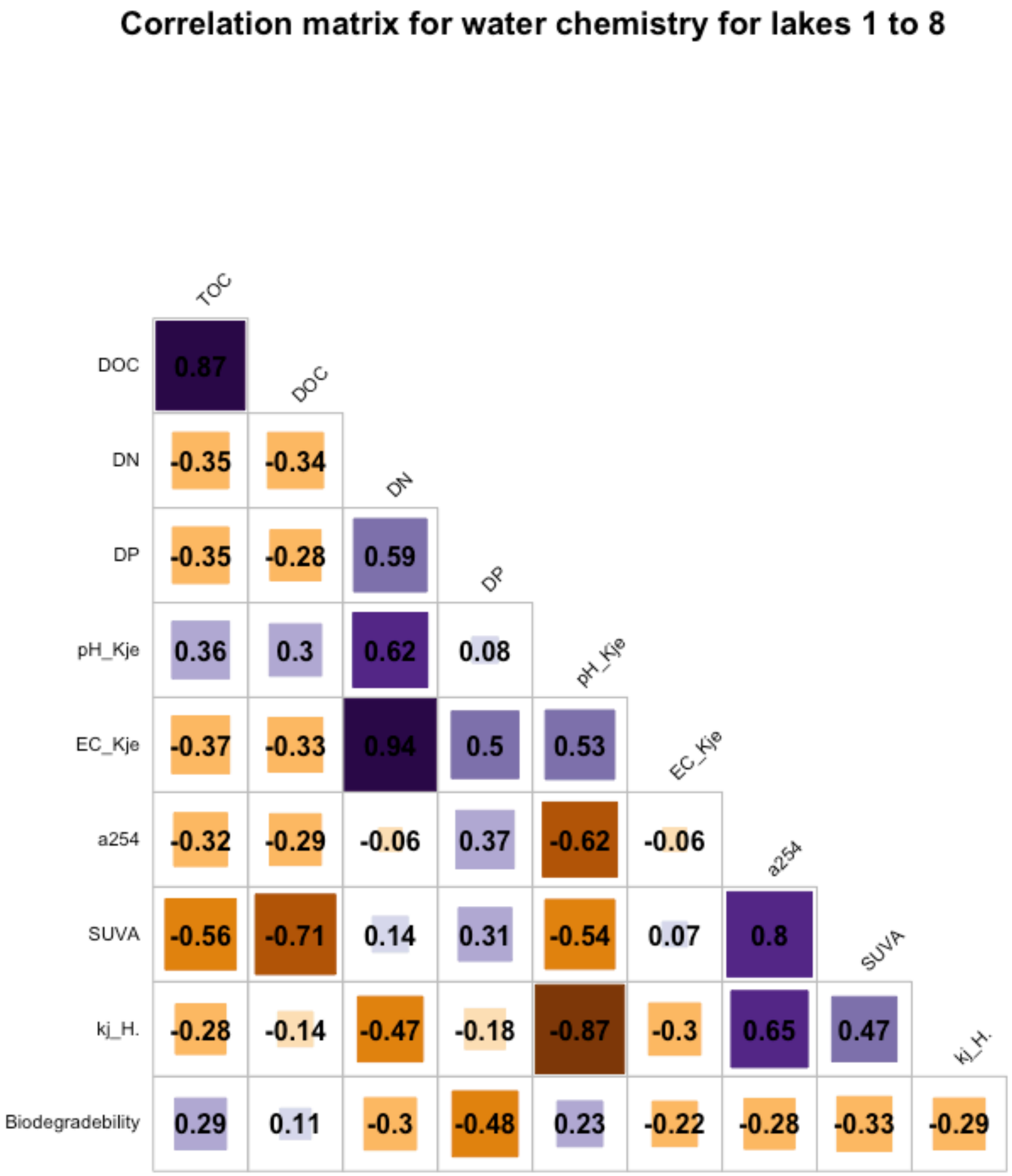


Figure B. Correlation matrix for all water chemistry for all the lakes



	TOC	DOC	DN	DP	pH_Kje	EC_Kje	a254	SUVA	kj_H.
DOC	0.87								
DN	-0.35	-0.34							
DP	-0.35	-0.28	0.59						
pH_Kje	0.36	0.3	0.62	0.08					
EC_Kje	-0.37	-0.33	0.94	0.5	0.53				
a254	-0.32	-0.29	-0.06	0.37	-0.62	-0.06			
SUVA	-0.56	-0.71	0.14	0.31	-0.54	0.07	0.8		
kj_H.	-0.28	-0.14	-0.47	-0.18	-0.87	-0.3	0.65	0.47	
Biodegradebility	0.29	0.11	-0.3	-0.48	0.23	-0.22	-0.28	-0.33	-0.29

Figure C. Correlation matrix for water chemistry for lakes 1 to 8 with selected variables.

Catchment characteristics

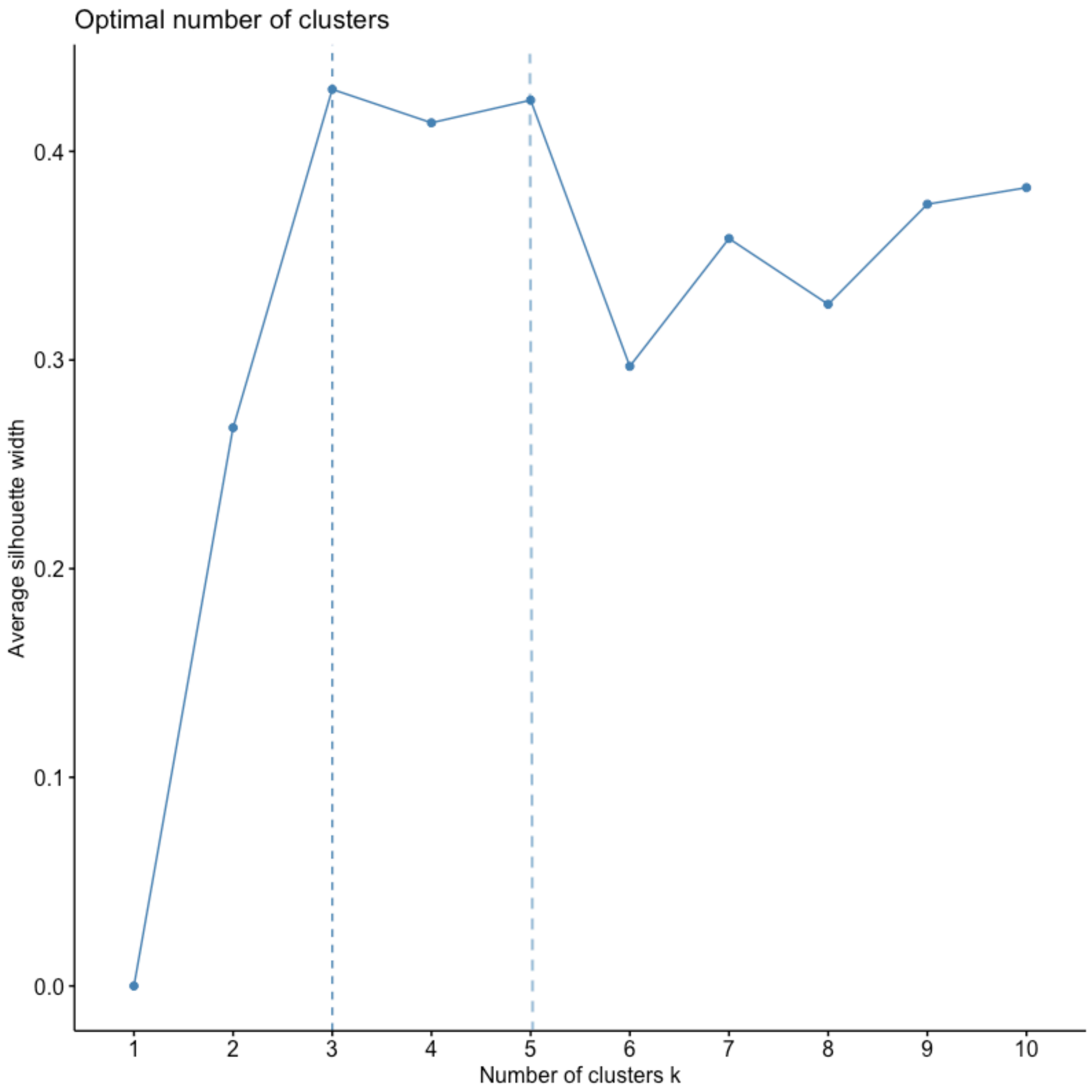


Figure D. Plot determining the optimal number of clusters,

Gives optimal number of clusters as 3, or secondly as 5.

The quality of the k-means partition was found by calculating the percentage of the Total sum of squares (TSS) is explained by the partition using equation, where BSS stand for Between Sum of Squares. The higher the percentage, the better the quality, because it means that between each cluster is large, and there is good separation of the clusters.

Equation A. Quality of the k-means partition

$\frac{BSS}{TSS} * 100$ = 63.07196 % in this cluster analysis.

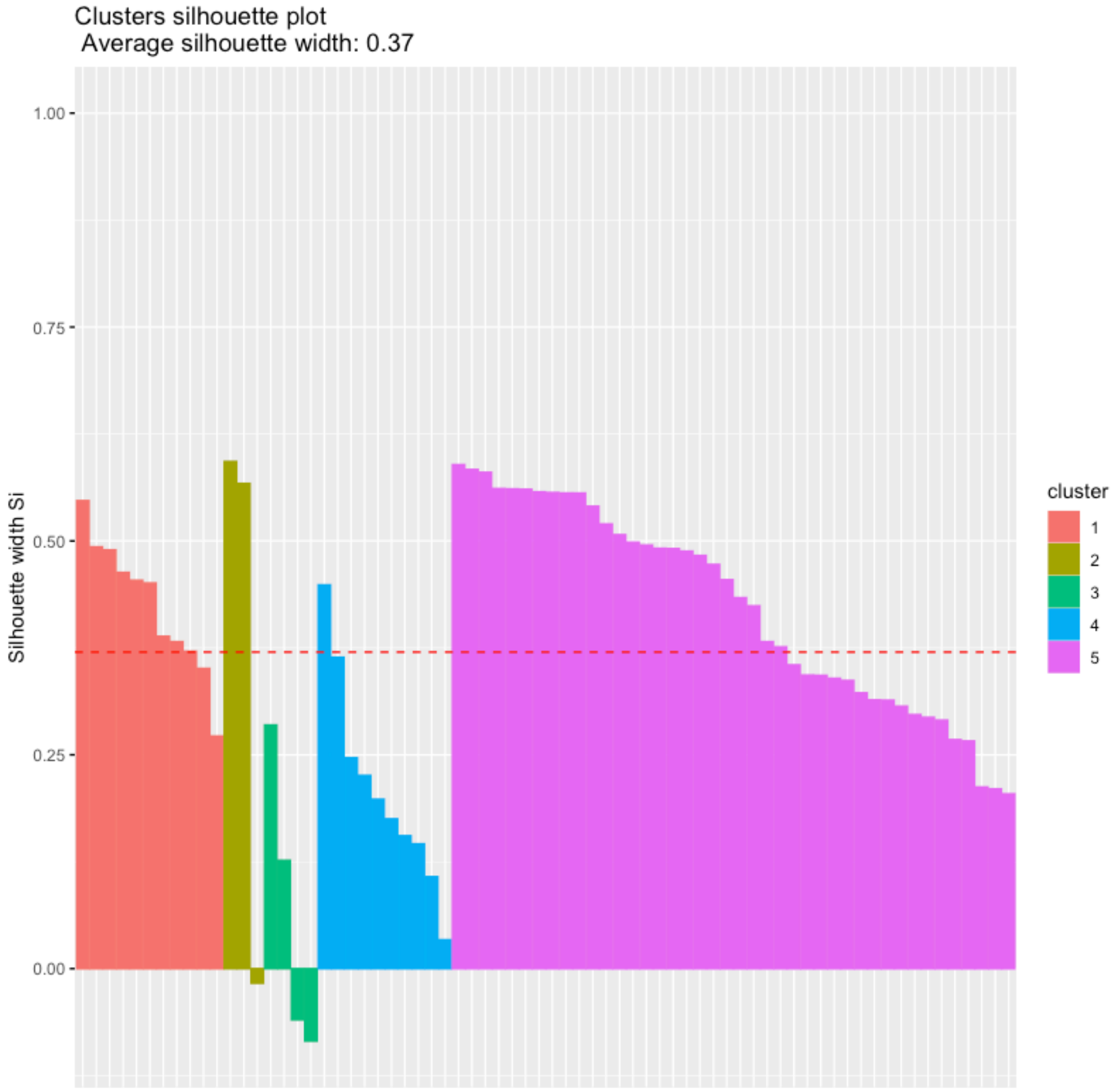
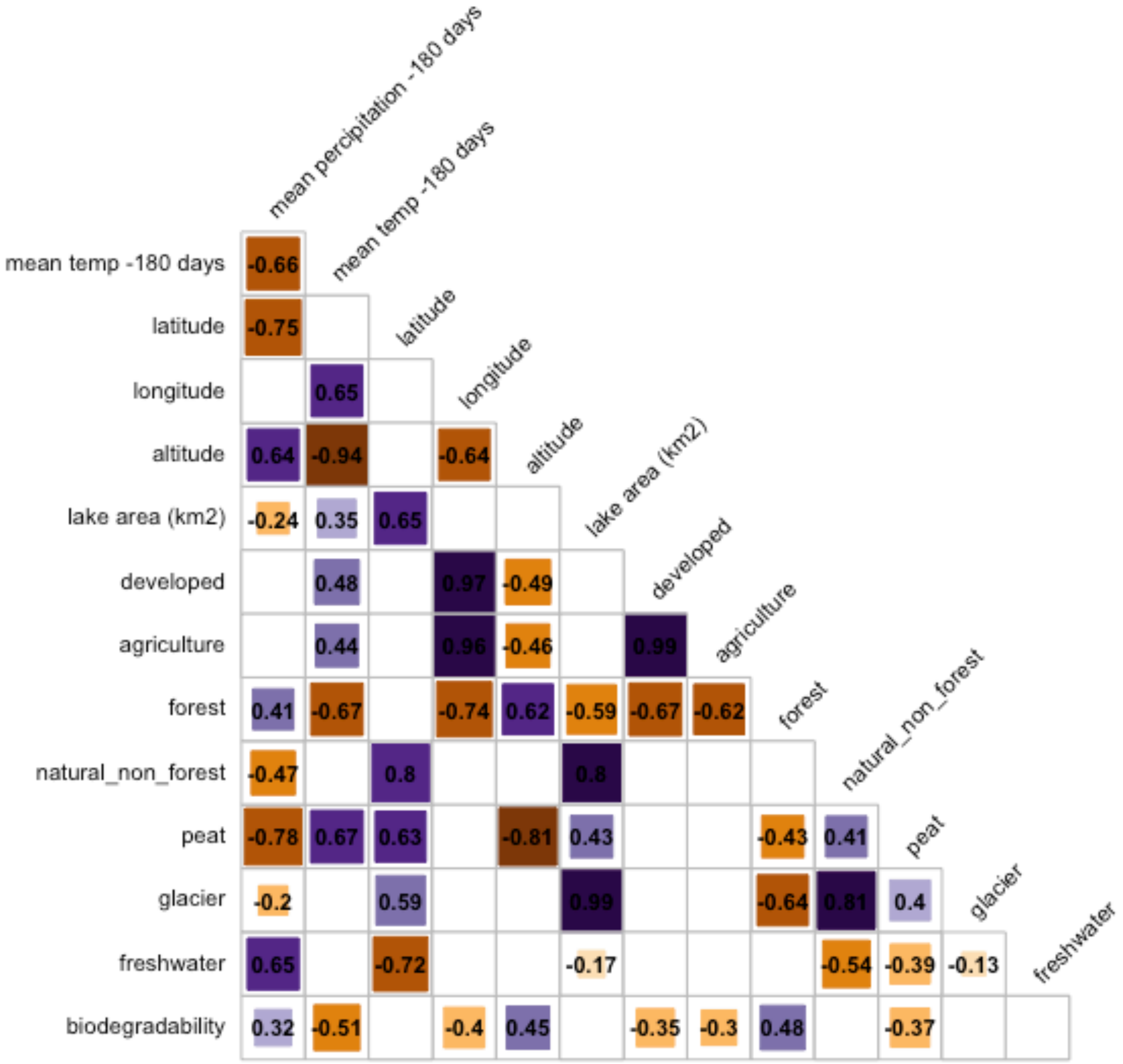


Figure E. Cluster silhouette plot, determining whether the groups are clustered correctly.

Here three observations are underneath 0 meaning they are placed in the wrong group. And none are over 0.75 so they are not perfectly grouped.

Correlation matrix for catchment characteristics for lakes 1-8



	mean percipitation -180 days	mean temp -180 days	latitude	longitude	altitude	lake area (km2)	developed	agriculture	forest	natural_non_forest	peat	glacier	freshwater
mean temp -180 days	-0.66												
latitude	-0.75												
longitude		0.65											
altitude	0.64	-0.94		-0.64									
lake area (km2)	-0.24	0.35	0.65										
developed		0.48		0.97	-0.49								
agriculture		0.44		0.96	-0.46		0.99						
forest	0.41	-0.67		-0.74	0.62	-0.59	-0.67	-0.62					
natural_non_forest	-0.47		0.8			0.8							
peat	-0.78	0.67	0.63		-0.81	0.43			-0.43	0.41			
glacier	-0.2		0.59			0.99			-0.64	0.81	0.4		
freshwater	0.65		-0.72			-0.17				-0.54	-0.39	-0.13	
biodegradability	0.32	-0.51		-0.4	0.45		-0.35	-0.3	0.48		-0.37		

Figure F. Correlation matrix for all catchment characteristics for lakes 1-8.

Forest is the most positively correlated to biodegradability, meaning these 8 lakes with very high biodegradability have catchment with more forested areas.

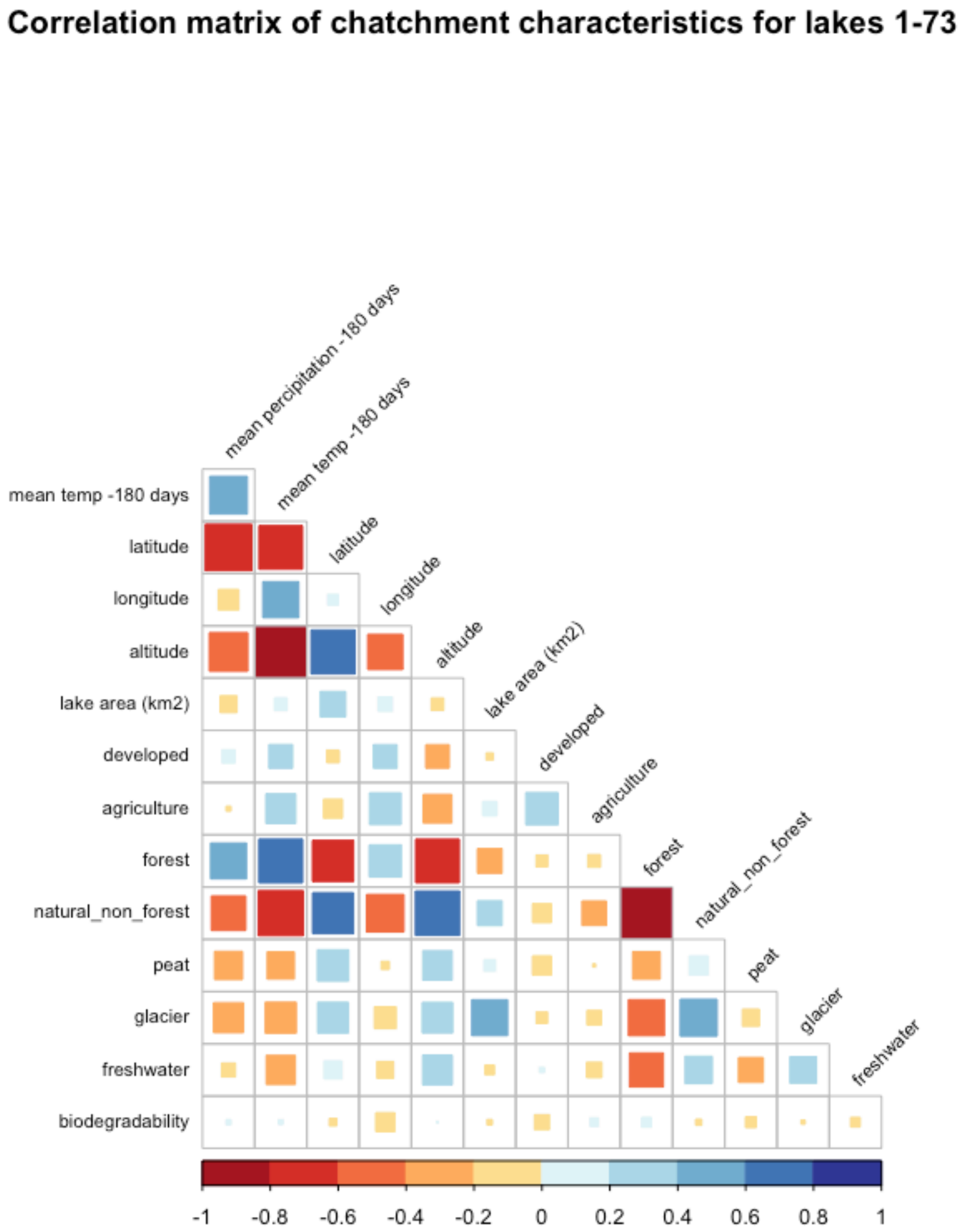


Figure G. Correlation matrix for all catchment characteristics with all lakes, including the 8 lakes that are statistic outliers.

Having barely any correlation to any of the catchment characteristics when using all lakes and none of the correlations to biodegradability is significant ($p < 0.05$).

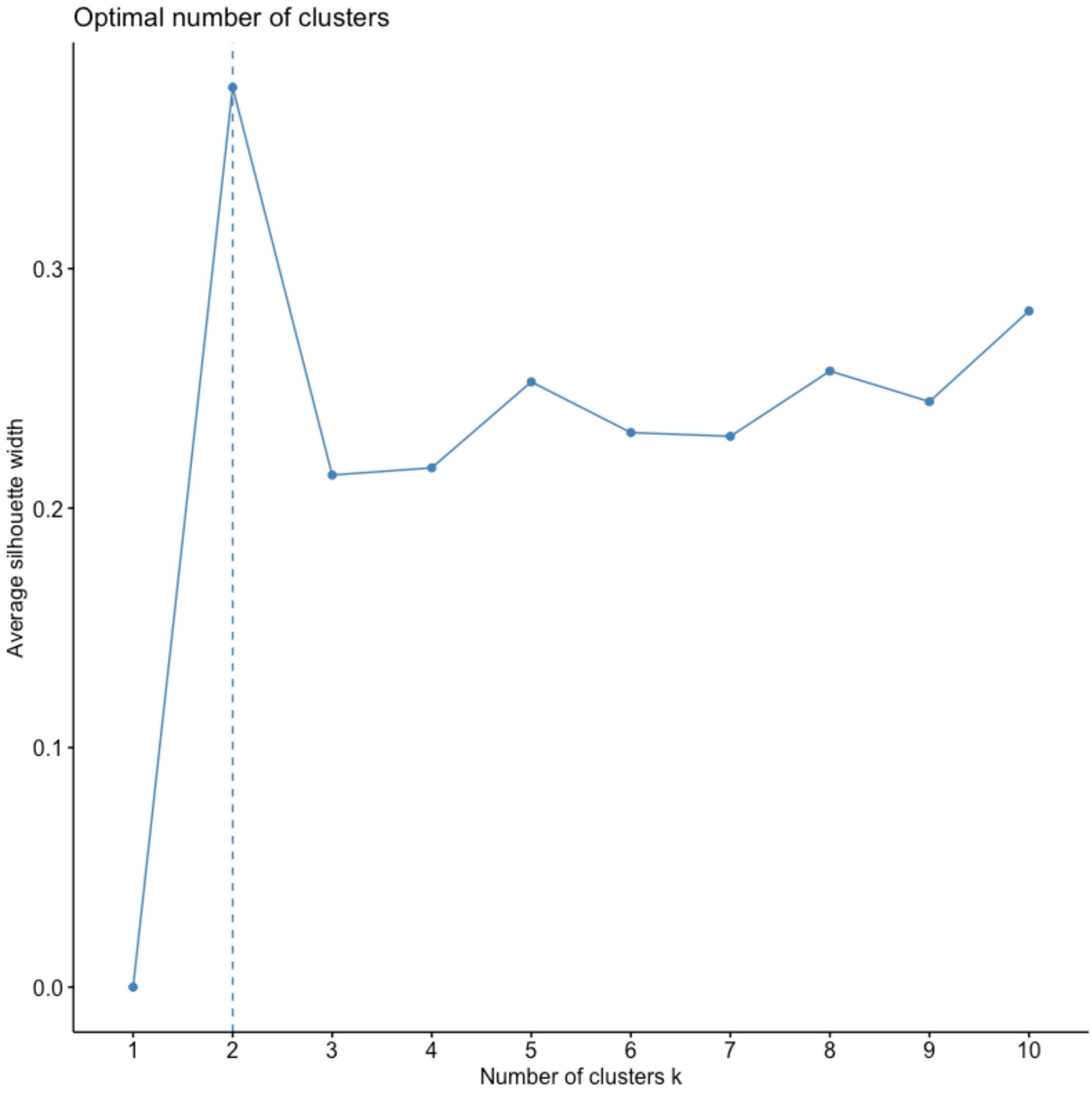


Figure H. Optimal number of clusters for cluster analysis with all catchment characteristics

For the cluster analysis with all the catchment characteristics the optimal number of clusters is 2.

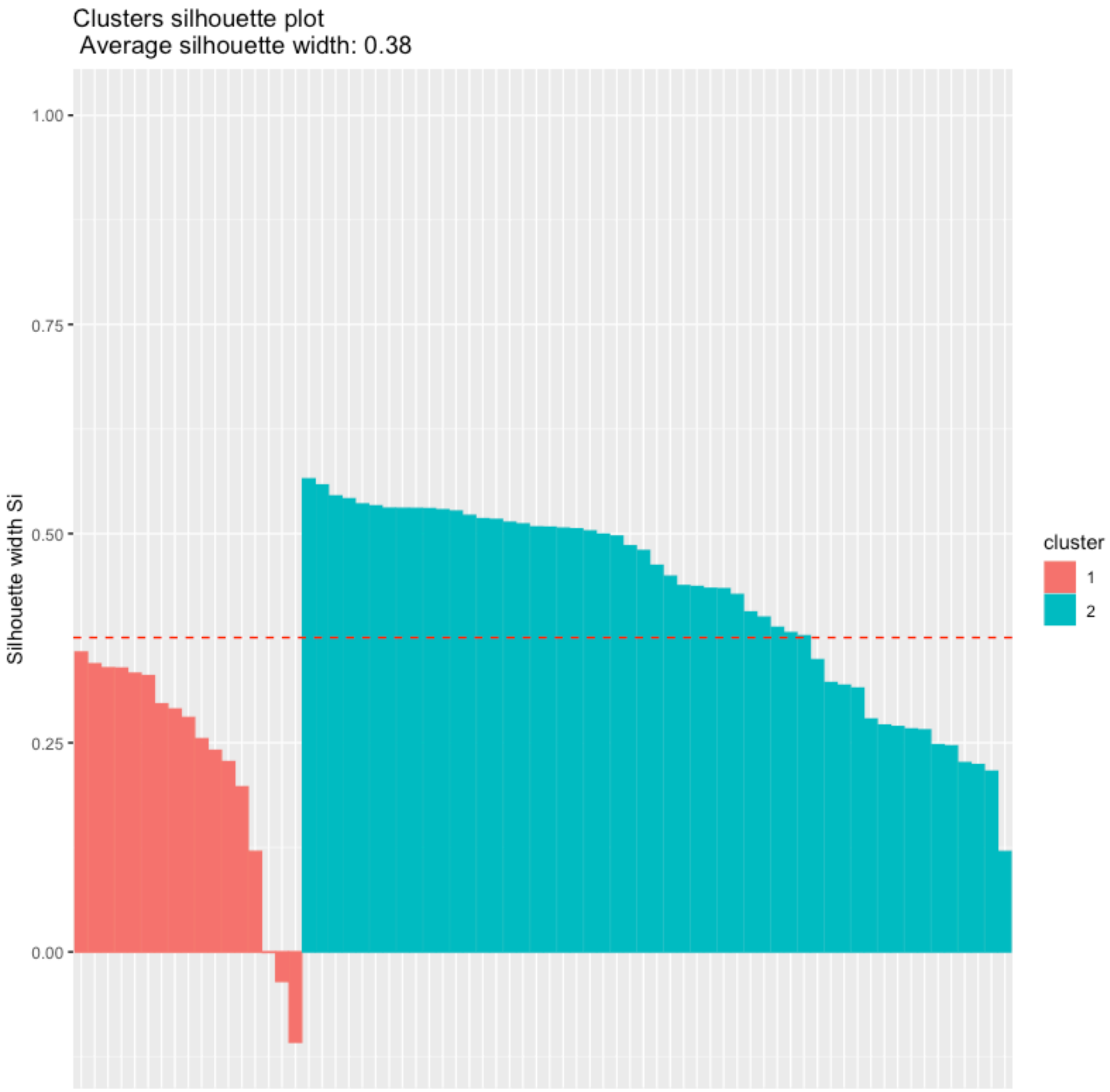


Figure I. Silhouette plot of the cluster analysis determining the accuracy of the cluster placement.

The silhouette plot of the cluster analysis for catchment characteristics shows that 2 of the lakes are placed in the wrong cluster, and should either be in the other cluster or, in a cluster by themselves. And one lake is at zero which means it is between clusters.

The quality of the k-means partition

Equation B. Quality of the k-means partition

$\frac{BSS}{TSS} * 100$ = 31.47252 % in this cluster analysis.

This is a relative low percentage, meaning the clusters are not grouped far apart, which is also visible in Figure 11.