UNIVERSITY OF GOTHENBURG

DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

# SPATIAL AND TEMPORAL DYNAMICS OF PHYTOPLANKTON BIOMASS IN WETLANDS LOCATED IN MINERAL AND PEAT SOIL IN SOUTHERN SWEDEN

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Photo by Stuart Matthews, 2024 from the research project “Exploring greenhouse gas emissions in wetlands: rewetted organic soils vs constructed agricultural ponds.”

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## Abstract

Rewetting wetlands for biodiversity and climate change mitigation is on the Swedish agenda, however, temporal and spatial patterns in relation to wetland soil type are yet not resolved. This thesis studied the temporal dynamics of phytoplankton biomass, measured as chlorophyll-a concentration, over the course of one year, as well as how wetland characteristics (eg size, catchment area, land uses, water physicochemical parameters and nutrients) influence phytoplankton biomass and water quality, with a specific focus on wetlands located in different soil-types, mineral soil *vs* peat soil. The study included 10 wetlands in southern Sweden, five on mineral soil, and five on peat soil. Sampling occurred 14 times from February to November 2025. The total amount of phytoplankton biomass was estimated by quantifying spectrophotometrically chlorophyll a (Chl-a) concentration after pigment extraction. This resulted in 417 observations of Chl-a. Results showed the seasonal dynamics of Chl-a concentrations being higher during the warmer seasons (spring, summer and autumn) than the cold season (winter). Regarding the spatial variability, Chl-a concentrations were higher in wetlands on mineral soil (9.83 ± 16.6) than on peat soil (4.59± 9.61). Wetlands on mineral soil are characterized for having higher concentrations of phosphorus and total nitrogen in water, and both parameters correlate positively with Chl-a. Higher levels of nutrients in wetlands located on mineral soil relate to agricultural land uses in the catchment, suggesting that for the studied wetlands, the main source of nutrients enrichments in the wetlands is coming from the runoff from the catchment and not leaking from the soil. The results also showed that wetlands with smaller size, smaller catchment and higher cropland percentage in the catchment had a positive effect to Chl-a. Altogether, this study shows a temporal dynamic of phytoplankton biomass and that wetlands on mineral soils tend to have a higher phytoplankton biomass and nutrient levels, which can be seen as poor water quality, than those on peat soil. Therefore, rewetting on peat soil should be prioritized.

**Keywords:** phytoplankton, Chlorophyll a, wetlands, water quality, mineral soil, peat soil

## Sammanfattning

Restaurering av våtmarker för biologisk mångfald och begränsning av klimatförändringar står på den svenska agendan, men tidsmässiga och rumsliga mönster i relation till våtmarkernas jordtyp är ännu inte hittade. Denna avhandling studerade den tidsmässiga dynamiken hos fytoplanktonbiomassa, mätt som klorofyll-a-koncentration, under loppen av ett år, samt hur våtmarkens egenskaper (t.ex. storlek, avrinningsområde och markanvändning, vattnets fysikaliska-kemiska parametrar och näringsämnen) påverkar fytoplanktonmassan och vattenkvalitet, med ett specifikt fokus på våtmarker belägna i olika jordtyper, mineraljord och torvjord. Studien omfattade 10 våtmarker i södra Sverige, fem med mineraljord och fem med torvjord. Provtagningen genomfördes 14 gånger från februari till november 2025. Den totala mängden fytoplanktonbiomassa uppskattades genom att kvantifiera klorofyll a (Chl-a) -koncentrationer spektrofotometriskt efter pigmentextraktion. Detta resulterade i 417 observationer av Chl-a. Resultaten visade att den säsongsmässiga dynamiken hos Chl-a-koncentrationerna var högre under de varmare årstiderna (vår, sommar och höst) än under den kalla årstiden (vinter). Beträffande den rumsliga variationen var Chl-a-koncentrationerna högre i våtmarker på mineraljord (9,83 ± 16,6) än med torvjord (4,59 ± 9,61). Våtmarker på mineraljord kännetecknas av att ha högre koncentrationer av fosfor och totalt kväve i vatten, och båda parametrarna korrelerar positivt med Chl-a. Högre nivåer av näringsämnen i våtmarker belägna på mineraljord relaterar till jordbrukets markanvändning i avrinningsområdet, vilket tyder på att för de studerade våtmarkerna kommer den huvudsakliga källan till näringsberikning i våtmarkerna från avrinningsområdet och inte läckor från jorden. Resultaten visade också att våtmarker med mindre storlek, mindre avrinningsområde och högre andel odlingsmark i avrinningsområdet hade en positiv effekt på Chl-a. Sammantaget visar denna studie en tidsmässig dynamik av fytoplanktonbiomassa och att våtmarker med mineraljord tenderar att ha högre fytoplanktonbiomassa och näringsnivåer, vilket kan ses som dålig vattenkvalitet, än de med torvjord. Därför bör restaurering av våtmarker med torvjord prioriteras.

**Nyckelord**: fytoplankton, klorofyll a, våtmarker, vattenkvalitet, mineraljord, torvjord

# Introduction

## 1.1 Wetlands Importance

There is a lot of different definitions of what a wetland is. According to the Ramsar Convention on Wetlands (Ramsar, Iran, 1971) a wetland is "areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters". They include inland habitats of a wide variety, for example floodplains and lakes as well as human made wetlands such as dams and reservoirs (Ramsar Convention Secretariat, 2016).

But there is also a widely used definition from the Swedish EPA (Environmental Protection Agency). It stems from VMI (Swedish EPA's nationwide wetland inventory/naturvårdsverkets rikstäckande våtmarksinventering), and it reads as follows: "Wetlands are areas where the water table for the main part of the year is close below, at, or above the ground level, including vegetation-covered lakes. A site is called a wetland when at least 50% of the vegetation is hydrophilic, i.e. water-loving. An exception is periodically flooded shores along lakes, seas and rivers, which are classified as wetlands despite a lack of vegetation" (Andersson, 2012; Löfroth, 1991). This study is focused on inland wetlands in southern Sweden, with permanent water, and freshwater that is flowing. Inland wetlands are often considered one of the most poorly studied and underreported ecosystems on Earth, even though they are critically threatened.

How did we get to this point? Well, during the 19$^{th}$ century, Swedish wetlands were extensively drained to make room for agricultural land. This led to significant ecological degradation and resulted in Sweden loosing important ecosystems and nutrient sinks that naturally removed carbon and nutrients from the environmental circulation (Fisher & Acreman, 2004). Due to this Sweden has under the last decades adopted a more holistic approach to wetland restoration, which is guided by national environmental goals (Sveriges miljömål, n.d.). These efforts to rewet Swedish wetlands are further reinforced by the EU Nature Restoration Regulation, which requires member states to restore degraded ecosystems, where wetlands are included (European Commision, 2024). Sweden has a goal of rewetting wetlands to have a better chance at achieving climate mitigation to reach the goal of becoming climate neutral until the year 2045. However, Sweden don't have a national target of the amount of land that should be rewetted, or **what kind of land** should be rewetted (Norion Consult, 2025).

What do we know about wetlands in general and why do we put this effort into restoring them? Well, wetlands are ecosystem that acts as a major carbon sink by absorbing large amounts of atmospheric $CO_2$ (and also other nutrients, such as phosphorus and nitrogen) (Fisher & Acreman, 2004; Mitsch et al., 2013). Their high carbon sequestration capacity is because of high primary productivity and the slow decomposition of organic matter. Carbon storage varies with environmental factors such as for example vegetation, hydrology, soil conditions, climate and age (Mitsch et al., 2013; Villa & Bernal, 2018; Zhang et al., 2025). Wetlands contribute to a lot of important ecosystem services that has been taken for granted historically. Examples of important ecosystem services that wetlands help us with is as previously mentioned, C sequestration but also services as water supply and quality and wildlife habitat. The wildlife habitat is especially important due to wetlands being characterized by anaerobic processes. This means that specific vegetation and animal life can thrive there and are specifically adapted to that environment (Pouyat et al., 2020).

Wetlands receive inputs of water (and with it, nutrients and organic matter) from the catchment surrounding the wetland. Wetlands also discharge outputs to the groundwater and nearby waterways and uplands through the soil with the help of hydraulic forces. The catchment therefore have an important role in how much organic matter and nutrients move around and exists in the

wetland, and due to the availability of nutrients and organic matter in wetlands, they are also hotspots for phytoplankton blooms and cyanobacteria. (Pouyat et al., 2020). More of this in 1.2.

One major initiative to help us get more information about wetlands is the REWETT project, which is led by the University of Gothenburg and funded by Swedish Geological Survey (SGU) (University of Gothenburg, 2025). This project focuses on investigating how the act of rewetting a wetland affects factors such as carbon dynamics. Particularly greenhouse gas emissions like methane, across different soil types. This present study on seasonal variability of phytoplankton biomass in mineral and peat soil wetlands forms a part of this REWETT project.

### 1.1.1 Wetland soil types, size and catchment characteristics influence water quality

Wetlands consist of what we call hydric soils, which is soils that has been formed under flooding or saturation during the growing season and in turn develop anaerobic tendencies. These hydric soils can be classified into certain soil types, e.g.d organic soils and mineral soils (Pouyat et al., 2020). In this study we will be focusing on mineral soil, and a type of organic soil called peat soil. Soil is made up of both mineral material such as sand and clay, and organic matter, together with air and water. Mineral soil is mostly made up of sand and clay, and a low amount of organic matter, <15%. It also has characteristic mottling which organic peat soils lack. Organic peat soil is similarly built up of sand and clay but has an organic layer at the surface that is 50 cm or more. Peat soil can occasionally be more than 10 m deep (Pouyat et al., 2020; Scottish Government, n.d.; United States Department of Agriculture, Natural Resources Conservation Service, 2016).

Nutrient enrichment from soil in wetlands differs depending on what type of soil it is due to the soil-types different characteristic. There is, however, not a lot of knowledge regarding how the wetlands soil characteristics, as for example soil type (peat or mineral) affect the phytoplankton mass in wetlands. What we do know is that peat soil can become an effective medium for retention nitrogen, phosphorus and suspended solids (example iron) and specifically phosphorus adsorption capacity remains at a high level in wetlands with peat soil (Karjalainen et al., 2016). The retention of phosphorus is mediated by uptake from the vegetation, adsorption, precipitation and microbiological assimilation that exists in the wetland (Karjalainen et al., 2016).When it comes to mineral soil wetlands, they tend to have a lower organic matter concentration, porosity, water holding capacity and cation exchange capacity compared to organic peat soil wetlands. The mineral soil also has a greater soil bulk density, nutrient availability and pH compared to organic soil which affects the soil ability to influence water quality (Mitsch & Gosselink, 2015; Pouyat et al., 2020).

As previously mentioned in 1.1, the catchment around an aquatic environment (like wetlands) has an important role regarding water quality. According to the study by J. Li et al., 2020, the more agricultural land there is in catchment around lakes the more phosphorus there is in the aquatic environment. For examples, Lake Krageholmssjön in Sweden had 65% agricultural land in the catchment, and the phosphorus level where $127 \pm 60$ µg/l, which supports that higher the percentage of agricultural land in the catchment, the higher the nutrients levels, including phosphorus, in the aquatic environments of the catchment (J. Li et al., 2020). Phosphorus is a key parameter for phytoplankton growth and eutrophication (more of this in 1.3.1) and Ritter et al., 2002 showed that around 80% of the phosphorus that is present in the aquatic environments is there due to human activities (Ritter et al., 2002).

## 1.2 Phytoplankton- What is it and why is it important, how does it affect water quality

Phytoplankton are microscopic single-celled, photosynthetic organisms and live in the water. Due to their need for sunlight to be able to photosynthesize effectively, phytoplankton must live near the water surface . Phytoplankton is a primary producer in the aquatic ecosystem's food chain and contribute a quarter or more of the biomass of the world's vegetation. Apart from forming the base of the aquatic food webs, phytoplankton is also important because they produce a large portion of Earth's oxygen, cycle nutrients like nitrogen and phosphorus and influence water quality and ecosystem productivity. Phytoplankton have a rapid reproduction and are sensitive to environmental changes and is a critical indicator that reflects water quality in lakes and wetlands (Roy et al., 2011).

Phytoplankton consists of a lot of diverse species, that includes both prokaryotic and micro eukaryotic organisms (Bhat et al., 2015; Roy et al., 2011). The main phytoplankton groups are usually classified based on their pigmentation, cell structure, and ecological characteristics. The most important eukaryotic groups in freshwater include: **Bacillariophyta**, also known as diatoms or brown algae, and **Chlorophyta** also known as green algae. Groups like **Cyrptophyta, Dinophyta, Euglenophyta, Chrysophyta or Desmids** are less dominant. There is also a prokaryotic group that is very abundant in phytoplankton, which is **Cyanophyta**, also known as cyanobacteria or blue-green algae. (X. Li et al., 2022; Roy et al., 2011).

In wetlands, lakes, rivers and oceans, phytoplankton abundance is often estimated using chlorophyll-a, a green pigment involved in photosynthesis. Higher chlorophyll concentrations usually indicate more phytoplankton biomass (Roy et al., 2011). In this thesis chlorophyll pigments (chlorophyll-a, b and c) will be used to quantify the abundance of total phytoplankton, diatoms, and green algae, respectively.

## 1.3. Chlorophylls

Chlorophylls are critical pigments in photosynthesis, as they allow cyanobacteria, algae and plants to absorb energy from light (PAR). Chlorophyll-a is the primary light-harvesting pigment responsible for photosynthesis and serve as the universal reaction centre for all oxygen-producing photosynthetic organisms. The chlorophyll-a is present in the plants and algae's chloroplast, which is a specialized organelle where the photosynthesis takes place. Chlorophyll exists in the thylakoids, which is disc- shaped structures within the chloroplast. Thylakoids enclose quantasomes, which contains the chlorophyll pigment, and are therefore the center for the photosynthesis (A.M. Humphrey, 1980; Maclachlan & Zalik, 1963; Mandal & Dutta, 2020; Roy et al., 2011; Terashima & Inoue, 1985).

Chl-a is the more abundant and universally present pigment in all phytoplankton groups, and for this is commonly used marker for the total phytoplankton biomass in a waterbody. While Chl-a is the most prevalent, phytoplankton also contain several accessory pigments that help them absorb different wavelengths of light and protect against intense sunlight. These accessory pigments can sometimes dominate specific taxonomic groups. Chlorophyll-b and chlorophyll-c are accessory pigments that expand the light-absorption spectrum of chlorophyll-a. Chlorophyll-b is abundant in Chlorophyta (green algae) and for this it is used in this thesis as marker for Chlorophyta. Chlorophyll-c is built up of chlorophyll c1 and c2 is a marker for brown algae (more specifically, the types of algae within "red algal linage"), such as Bacillariophyta (also known as diatoms) and Cryptomonads. Bacillariophyta has in previous studies showed to be more abundant in colder

months compared to Chl-b, which has showed to be more abundant in the warmer months (Avrahami et al., 2025; Barlow et al., 2013; Jinkerson et al., 2024; Roy et al., 2011).

## 1.4 Factors influencing Phytoplankton growth

Photosynthetic Active Radiation (PAR) is the section of the spectral light between 400 nm-700 nm which is needed by plants and algae for doing the photosynthesis (McCree, 1971). PAR and temperature influences both phytoplankton's community and their structure, as well as their growth and reproduction rate. PAR and temperature usually have maximum capacity at the surface of the water, where phytoplankton biomass tend to thrive (A. Ahmed et al., 2022; Borovkova et al., 2026; X. Li et al., 2022; Neun et al., 2025). This means that there is a connection between phytoplankton growth and the season of the year. The total phytoplankton abundance, biomass and primary production are generally higher in warmer waters during the summer, then in colder water during spring and autumn (Borovkova et al., 2026; X. Li et al., 2022).

Another example of important factors that influence phytoplankton and chlorophyll's growth is the enrichment of nutrients, such as nitrogen and phosphorus. Over-enrichment of nitrogen and phosphorus in soil leaks into lakes and wetlands and causes an accelerating eutrophication (Hamilton et al., 2016; Neun et al., 2025). Eutrophication is an accelerated buildup of organic matter due to overgrowth of algae, plants and bacteria, which greatly affects the water quality and can harm the ecosystem (Southern California Coastal Water Research Project, 2025). This excess of organic material causes a higher growth of phytoplankton. Efforts to reduce non-point sources of phosphorus have resulted in significant decline of phytoplankton biomass in lakes, which also shows that there is a connection between  and phytoplankton biomass (Paerl et al., 2020). It is however not only soil leaks that affects the phytoplankton biomass that exists. The catchment composition plays a role as well as human factors.

The environment around lakes as well as human factors such as if the land around is undeveloped, developed or agricultural has been proven having a consistent effect on phytoplankton/chlorophyll growth due to water runoff into the aquatic environment, which also is a principal driver of eutrophication (Gentine et al., 2022; Southern California Coastal Water Research Project, 2025). For example, if there are more farmland and cropland around the lakes, the water runoffs have led to nutrients enrichments in the aquatic environment in the catchment and in turn more algae growth, and therefore a higher amount of Chl-a. One reason for this is farmlands frequent use of fertilizers that leaks into the water. Impervious surfaces, such as roads and parking lots and even forests have however not as strong connection to algae and chlorophyll growth in the lake. So, if there is more cropland percentage in the catchment, there is higher chance that there will be phytoplankton in the aquatic environment (Cao et al., 2022).

The age of the aquatic environment can also have an impact on the species of phytoplankton that are present. It has been observed in a previous study that phytoplankton biomass in younger wetlands, are higher than in older wetlands. Older wetlands, (over four years) have lower amounts of chlorophyll which implies that there is a more established nutrient cycling process in older wetland, as well as vegetation that limit the phytoplankton growth (Sanyang, 2025).

## Aim

This **study aims** to increase the understanding on the spatial and temporal variability of phytoplankton biomass in Swedish wetlands, and how these patterns relate to wetland characteristics- with emphasis on wetlands soil-type- and water quality parameters. Poor understanding of phytoplankton ecology in Swedish inland wetlands limits effective conservation and ecosystem management. The outcome of this study can be used to help prioritize which type of wetlands should be rewetted, for example either wetlands located in mineral soil, or wetlands located in peat soil.

The main research questions that will be addressed are:

- How does total phytoplankton biomass vary temporally throughout the year?
- Which wetland characteristics best explain variation in total phytoplankton biomass
- Which water parameters are most important for explaining changes in total phytoplankton biomass?

## Hypothesis

It is hypothesized that the total phytoplankton biomass and composition in the wetlands will vary over the year due to the variability of environmental factors associated with seasonality such as light and temperature. It is expected that the total phytoplankton biomass as well as Chlorophyta will be higher in the warmer months, but Bacillariophyta is expected to be higher during the colder months.

There is also an expectation that surface water in wetlands that are in peat soil (with higher organic matter) will be more reach in nutrients and organic carbon because of soil leachate, than wetlands located in mineral soil. The higher amount of nutrients in the water column is expected to promote the phytoplankton biomass growth. Therefore, there is an expectation that the total phytoplankton biomass will be higher in wetlands located in organic peat soil compared to wetlands located in mineral soil.

## Material and method

### 2.1. Sampling sites and field dataset

A total of 10 different wetlands in southern Sweden (figure 1) were included in this study. Five wetlands were in mineral soil and five in peat soil. A one-year sampling campaign was performed from February 2025 to November 2025 to account for temporal variability. In total each wetland was sampled 14 times.

Surface water was collected at three different positions in each wetland, from the inlet of the wetland to the outlet. The three different positions where named P07 (inlet), P05 (center) and P02 (outlet). This was done to make sure that the radius of the wetland was covered. A total of one liter of water was collected at 20-250 cm depth from the surface using transparent amber glass bottles. An inflated sampling raft was used to avoid disturbing the sediments. For each sample, around 150 ml of water was filtered through glass fiber filter (GF/C filters, 25 mm) with a syringe.

Each filter where den removed from the holder and dried with paper, folded and transferred to a previously labelled Eppendorf tube. The volume of water filtered was written on the tube with permanent marker. After that the tubes were stored in a car freezer at -20 degrees °C, and then again -20 degrees °C in the lab in Natrium, Faculty of Sciences and Technology, University of Gothenburg. The samples where then stored in the freezer until extraction and analysis.


*Figure 1 Map showing the location of the 10 wetlands included in this study, in the south of Sweden.*

## 2.2. Wetland Characterisation/Water sampling and Analysis

Surface water samples were collected to be analysed for dissolved organic carbon (DOC), dissolved inorganic nutrients ($P\text{-}PO_4$ phosphate, $N\text{-}NH_4$ ammonium and $N\text{-}NO_3$ nitrate), total nitrogen (TN) and total phosphorus (TP). Water was filtered through 0.45 µm filter and stored at -20 degrees °C. In situ water quality parameters, including pH, temperature, dissolved oxygen (DO) and electric conductivity (EC) were recorded using a multi portable probe parameter reader (e.g. Hach LDO10101). The light irradiance (PAR) at the surface water (20 cm depth) was ensured with a portable light meter (Li-COR).

### 2.2.1 $P\text{-}PO_4$ (Phosphorus)

The method that was used to determine the concentration of $P\text{-}PO_4$ was the Bran & Leubbe method G-287-03 using the instrument Autoanalyzer 3 HR from Seal Analytical (2011). This is an automated procedure for the determination of $P\text{-}PO_4$ in the water. The $P\text{-}PO_4$ reacts with molybdate, and antimony followed by reduction with ascorbic acid at a pH<1 which forms a blue-coloured complex. This reduced blue phospho-molybdenum complex is colorimetrically read at 880 nm and the detection limit is set at 0.4 µg $P\text{-}PO_4$/L. This method was performed by the department of forest ecology and management at the Swedish University of Agricultural Sciences (SLU). However, the $P\text{-}PO_4$ was only analysed from surface water samples that were collected in the outlet of the wetlands (position P02), but the values will be interpreted for position P05 and P07 too.

### 2.2.2 N-$NH_4$ (Ammonium)

To determine the N-$NH_4$ the Bran & Leubbe method G-171-96 was used using the Autoanalyzer 3 HR from Seal Analytical (2011). This is also an automated procedure to determine the amount of N-$NH_4$ that exists in the water. N-$NH_4$ reacts with salicylate at high pH (>11.5) and forms a blue-green complex that is colorimetrically read at 660 nm. EDTA is used as a complexing agent to prevent precipitation of calcium and magnesium hydroxides and sodium nitroprusside is used to enhance the sensitivity of the method and the detection limit is set at 0.3 µg N-$NH_4$/L. This method was also performed by the department of forest ecology and management at the Swedish University of Agricultural Sciences (SLU). The N-$NH_4$ was also only analysed from surface water samples that were collected in the outlet of the wetlands (P02) but the values will be interpreted for position P05 and P07 too.

### 2.2.3 N-$NO_3$ (Nitrate)

The method that was used to determine the N-$NO_3$ concentration in the water was the SS-EN ISO 13 395 modified for Bran & Leubbe Method G-284-08 Rev. 4. using the instrument Autoanalyzer 3 HR from Seal Analytical (2011). The difference with this method is that it calculates the sum of both N-$NO_3$ (nitrate) and N-$NO_2$ (nitrite) in the water. This method is also an automated procedure for the determination of the sum of N-$NO_3$ and N-$NO_2$ in the water. Nitrate is reduced to nitrite at pH 7.5 in a copprerized cadmium reduction coil. This nitrite plus any nitrite in the water react under acidic conditions with sulfanilamide to form a diazo compound which then couples with N-1-naphthylenediamine dihydrochloride (NEDD) to form a reddish-purple azo dye that is colometrically read at 550 n and the detection limit is set at 0.4 µg/l. This method was as well as the two previous methods performed by the department of forest ecology and management at the Swedish University of Agricultural Sciences (SLU) and the N-$NO_3$ was only analysed from surface water samples that where collected in the outlet of the wetlands (P02), but the values will be interpreted for position P05 and P07 too.

### 2.2.4 Dissolved Oxygen and Total Nitrogen

The method that was used to determine dissolved oxygen and total nitrogen was a modified SS-EN 1484. Inorganic carbon was removed by acidifying the sample and bubbling it with inert gas before analysis. The sample was then combusted at 720°C with a platinum catalyst. Carbon in the sample formed carbon dioxide during combustion, which was measured by IR absorbance. The combusted sample was then passed on to nitrogen analysis by chemiluminescence. Nitrogen compounds in the sample formed NO during combustion. When NO was oxidized by ozone to $NO_2$, light was produced, which was then measured by a non-dispersive infrared detector (NDIR). The analyser measures total organic carbon and total nitrogen. For analysis of dissolved organic carbon and total dissolved nitrogen, the sample was filtered through a membrane filter with a pore size of 0.45 µm before analysis. This method was performed by the Earth science department at the University of Gothenburg.

## 2.3. Pigments Extraction and Analysis

The total phytoplankton biomass and the relative abundance of phytoplankton groups Chlorophyta (green algae) and Bacillariophyta (diatoms and brown algae) were determined by pigments analysis quantified spectrophotometry following the method of Jeffrey and Humphrey 1975 (Jeffrey & Humphrey, 1975). Chlorophyll-a was used as a marker for the total phytoplankton biomass that was present in the samples, Chlorophyll-b was used as a marker for the abundance of green algae

present in the samples, and Chlorophyll-c was used as a marker for the abundance of brown algae, diatoms and cryptomonads that where present in the samples (see table 1).

*Table 1 Selected target pigments as proxy of all algal groups (Jeffrey & Humphrey, 1975)*

| Pigment family | Pigment | Marker freshwater phytoplankton groups |
|---|---|---|
| Chlorophylls | Chlorophyll *a* | All phytoplankton groups |
| | Chlorophyll *b* | Chlorophyta (green algae) |
| | Chlorophyll *c* | Bacillariophyta (diatoms) |

The chlorophyll pigments were extracted by resuspending the frozen GF/C filter in 4 mL of solvent (90% acetone, 90/10 acetone/water v/v, see table 2) using scintillation vials. The frozen filters were resuspended in solvent, and then the samples where vortexed for 10s. After the samples where vortexed, they were put in a sonification bath at 4°C for 3 min. The ultra-sonification bath breaks down the cell walls so that the chlorophyll pigments in the cells get released and absorbed into the solvent. The samples where then incubated overnight for about 20-24 h at 4°C in the darkness to ensure complete pigment extraction. After the overnight incubation, the samples where once again vortexed for 10s. All these steps were done in the darkness to avoid pigment degradation due to chlorophylls sensitivity to the light.

Then the particles/debris from the extracts had to be removed. This was done by filtrating the extract through GFC filters. First the filter where wet with 1 mL of solvent (90% acetone) before the filtration of the extract. This was done to ensure that the filter didn't move around, and to make sure that all of the solvent where used. A total of 1 mL of the extract was pipetted into a reduced glass cuvette with the length of 1 cm. It was the immediately measured according to the wavelengths below (Figure 2) using the spectrophotometer Evolution 60, ThermoScientific, and the program VISIONlite, protocol Acetone chlorophylls Phytoplankton.mfx. Two cuvettes were used at the same time: one for the blank and one for the sample. Both cuvettes were closed off with parafilm to avoid evaporation of the solvent (90% acetone).

To avoid cross contamination between samples, the cuvettes were rinsed with acetone between each measurement, and the acetone leftovers were poured out. The excess pigments extract was stored in small glass vials in -20°C, to be used in case of emergency. The readings from each measurement and absorbance spectra (see table 2) were recorded in an excel sheet, and the chlorophyll concentration (µg/l) where calculated using equations from Jeffrey and Humphrey (1975) (Figure 2)

*Table 2 Table showing the absorbances measurements for chlorophyll a, b and c quantification (Lichtenthaler, 1987).*

| Wavelength | nm | Marker |
|---|---|---|
| **1** | 664 | Chl-a |
| **2** | 647 | Chl-b |
| **3** | 630 | Chl-c |
| **4** | 750 | Turbidity |

$$C_a = [11{,}85(A_{664} - A_{750}) - 1{,}54(A_{647} - A_{750}) - 0{,}08(A_{630} - A_{750})]\left(\frac{V_r}{V_f \times L_c}\right)$$

$$C_b = [-5{,}43(A_{664} - A_{750}) + 21{,}03(A_{647} - A_{750}) - 2{,}66(A_{630} - A_{750})]\left(\frac{V_r}{V_f \times L_c}\right)$$

$$C_c = [-1{,}67(A_{664} - A_{750}) - 7{,}6(A_{647} - A_{750}) + 24{,}52(A_{630} - A_{750})]\left(\frac{V_r}{V_f \times L_c}\right)$$

*Figure 2 Spectrophotometric equations for pigments quantifications. f = original sample volume filtered in the field [ml], Vr = volume of solvent used to resuspend the filter [ml], Lc = length of cuvette [cm] (Jeffrey & Humphrey, 1975)*

### 2.4. Statistical Analysis.

All the data was compiled into an Excel spreadsheet. Data analysis and data visualization (box plots, point plots, bar plots, etc) were done in R-studio. Normality tests were done to see the distribution of each variable, as well to see potential outliers. The normality tests that were done was Q-Q-plots, histograms, boxplots and Shapiro-Wilk tests (alpha level=0.05). The conclusions that could be drawn from these normality tests was that the Chl-a data was not normally distributed.

Data transformation was done to see if normality could be achieved. Logarithmic transformation (log+1) where done in this case. Some values were significantly improved by the logarithmic transformation, but some were not. This led to the conclusion that the data analysis would be made with non-parametric tests.

Before the non-parametric tests could be done, all the data's zeros were transformed to half the limit of detection. The data analysis tests that where made were Kurskal-Wallis's test, to check for differences in Chl-a concentrations between different wetland characteristics to check for differences in Chl-a, Chl-b and Chl-c among seasons and to check for differences in water quality descriptors and different soil types. A Dunn's multiple comparisons test was done to see which of the seasons had statistical difference in Chl-a concentration. Then a Spearman rank correlation coefficient (rho) where used to assess if the relationship between Chl-a and environmental variables were strong or weak, as well as positive or negative.

An R-studio package used in the data analysis was the tidyverse package, more specifically the libraries **ggplot2**, **readr**, **dplyr**, **forcats** and the **tidyverse** library itself. These were used for plotting, reading the CSV files, data wrangling and factor handling. Standalone libraries were also used in the statistical analysis. Libraries that worked as plotting extensions included **lemon**, which provided ggplot layout tools; **scales**, which handled with axis and label formatting; **ggpubr**, which provided publication-ready plots; **GGally**, which added ggplot extensions; and **pheatmap**, which enabled heatmap visualisation. Other libraries that was useful during the statistical and data-transformation steps were **DescTools**, which provided descriptive statistics; **bestNormalize**, that was used for data normalization; **rstatix**, which tidied up statistical tests; **boot**, which supported bootstrapping; and **performance**, which gave model diagnostics.

## Results

### 3.1. Summary statistics and main wetland characteristics

A total of 16 variables were considered and included in the data analysis. The variables that were included represented wetland characteristics, seasonality markers, nutrient levels and phytoplankton pigments (see table 3). Due to the data not being normally distributed, even when transformed, the data has been kept in its raw form, and non-parametric tests has been done. The observations of the variables are highly different due to each of the ten wetlands being sampled several times over the course of the year. When it comes to amount of phytoplankton pigment observations, three of the 420 samples were excluded. This was because no samples could be taken from one of the wetlands (wetland 4 to be specific) on one occasion due to bad weather conditions.

*Table 3 Summary of wetland characteristics, seasonality markers, nutrient levels and phytoplankton pigments results from the wetlands.*

| *Variable* | *Variable name* | *Unit* | *N* | *Min* | *Max* | *Median* |
|---|---|---|---|---|---|---|
| ***Wetland characteristics*** | | | | | | |
| *Organic matter* | OM | % | 10 | 2.57 | 92.41 | 12.7 |
| *Wetland Size* | Size | Ha | 10 | 0.19 | 12.16 | 1.75 |
| *Catchment Area* | Area | Ha | 10 | 12 | 11604 | 129 |
| *pH* | pH | | 135 | 4.64 | 9.59 | 7.42 |
| *Electrical conductivity* | EC | µS/cm | 135 | 41.5 | 1407 | 219.2 |
| ***Seasonality markers*** | | | | | | |
| *Temperature* | Temp | °C | 135 | 0.9 | 29.4 | 15.8 |
| *Photosynthetic active radiation* | PAR | µmol/ $m^2$/s | 138 | 9.3 | 1940 | 603.85 |
| ***Nutrients*** | | | | | | |
| *Dissolved Organic Carbon* | DOC | mg/l | 417 | 4.22 | 40.01 | 11.90 |
| *Ammonium* | N-NH4 | µg/l | 139 | 3.47 | 6756 | 12.35 |
| *Nitrate* | N-NO3 | µg/l | 139 | 2.72 | 3487 | 10.60 |
| *Total Nitrogen* | TN | mg/l | 417 | 0.151 | 11.18 | 0.859 |
| *Phosphorus* | P-PO4 | µg/l | 135 | 0.44 | 50.39 | 2.94 |
| *Dissolved oxygen* | DO | mg/l | 134 | 3.19 | 17.58 | 9.22 |
| ***Chlorophylls*** | | | | | | |
| *Chlorophyll-a* | Chl-a | µg/l | 417 | 0 | 183.21 | 3.71 |
| *Chlorophyll- b* | Chl-b | µg/l | 417 | 0 | 46.42 | 0.75 |
| *Chlorophyll-c* | Chl-c | µg/l | 417 | 0 | 11.17 | 0.64 |

### 3.2 Main wetland characteristics

Organic matter in the sediment differs depending on soil type (see figure 3a). Wetlands with peat soil did have a higher percentage of organic matter in the sediment compared to wetlands with mineral soil. Wetland size differs from wetland to wetland. There is no specific pattern that says that wetlands with mineral soil have a larger area than wetlands with peat soil and vice versa (see figure 3b). There are big differences between catchment areas for the different wetlands. The difference is however **not** due to soil type. Some wetlands had over 11 000 Ha of Catchment area, while others had around 30 ha (see figure 3c). Wetlands with mineral soil had in general a more cropland percentage in the catchment (except wetland 33) compared to wetlands with peat soil (see figure 3d).

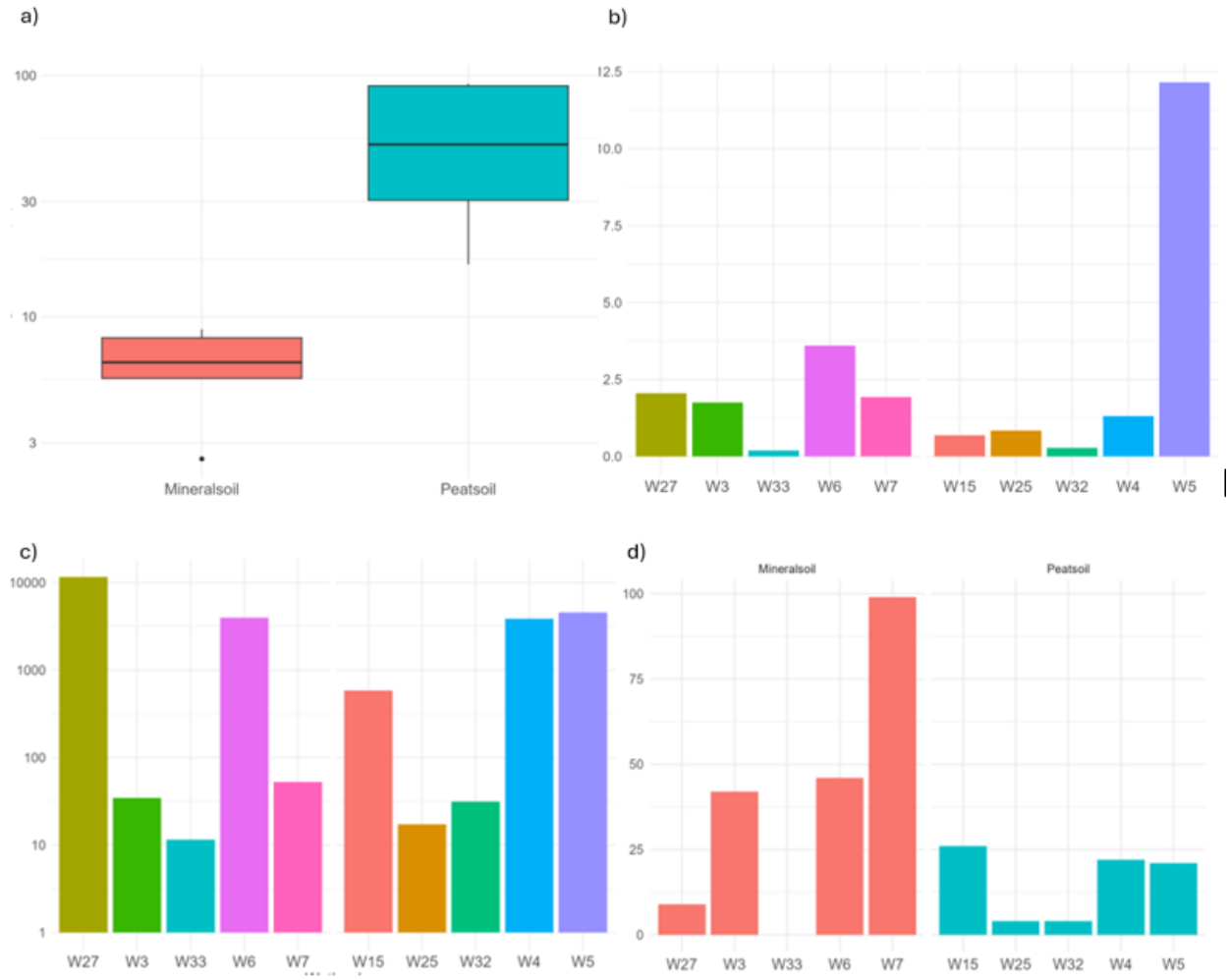


*Figure 3 Main wetland characteristics results a) Boxplots showing the organic matter in the sediment. b) The different wetland sizes (ha), c) Catchment area for each wetland. X-axis is in log-scale to better visualise the differences. d) Cropland percentage in catchment per wetland, red bars represent wetlands with mineral soil, blue bars represent wetlands with peat soil.*

### 3.3 Seasonal variability of phytoplankton biomass

The Chl-a concentration varied greatly during a year, both in wetlands with mineral soil (see figure 4) and wetlands with peat soil (see figure 5). The main difference is that wetlands with mineral soil in general have higher concentration of Chl-a compared to wetlands with peat soil (see figure 4 and 5.

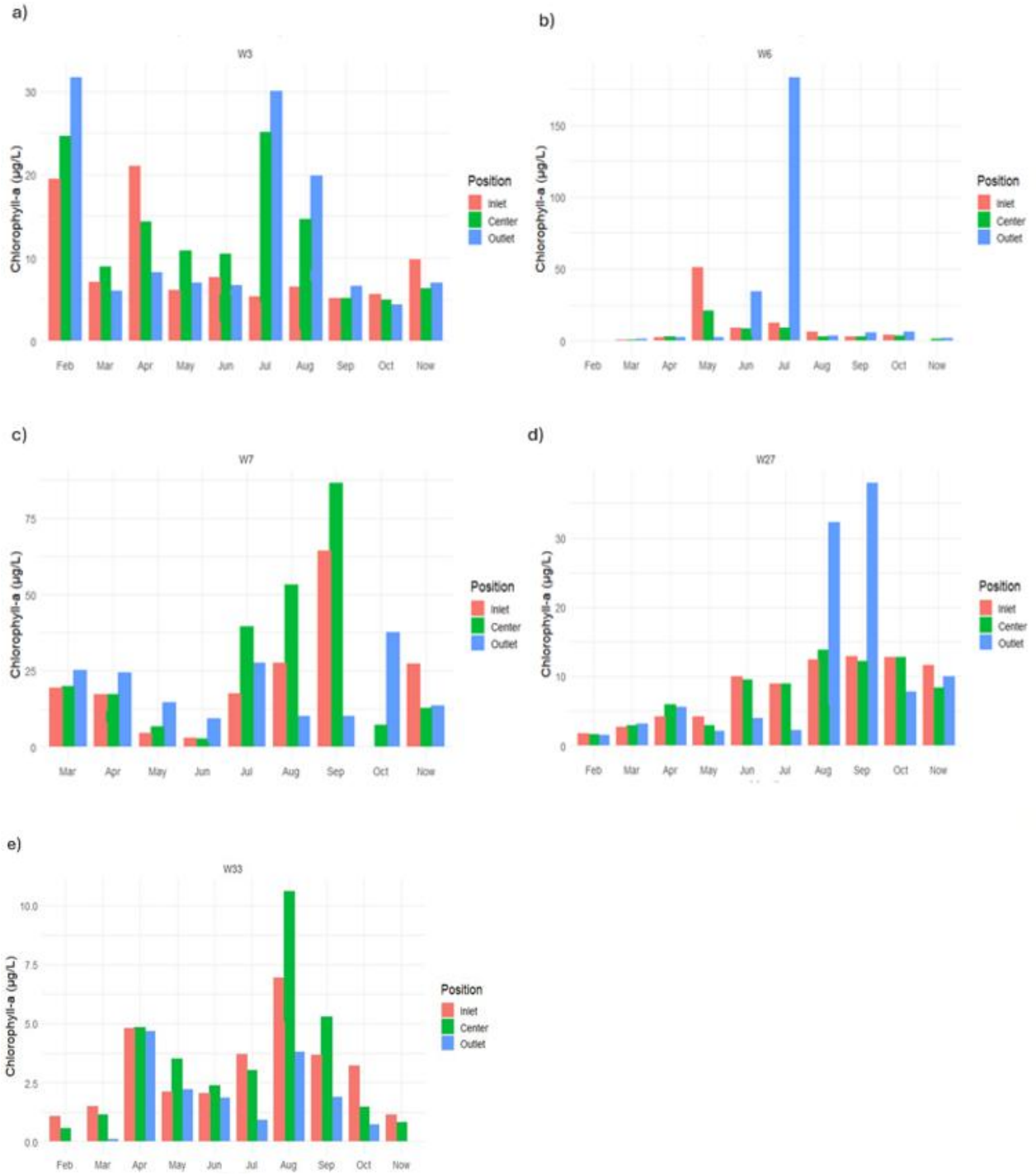


*Figure 4 Bars represent Chl-a concentration in the five wetlands located in mineral soil. The bars represent the positions inlet (red), center (green) and outlet (blue) under the sampling year (February-November). a) Wetland 3, b) Wetland 6, c) Wetland 7, d) Wetland 27, e) Wetland 33*

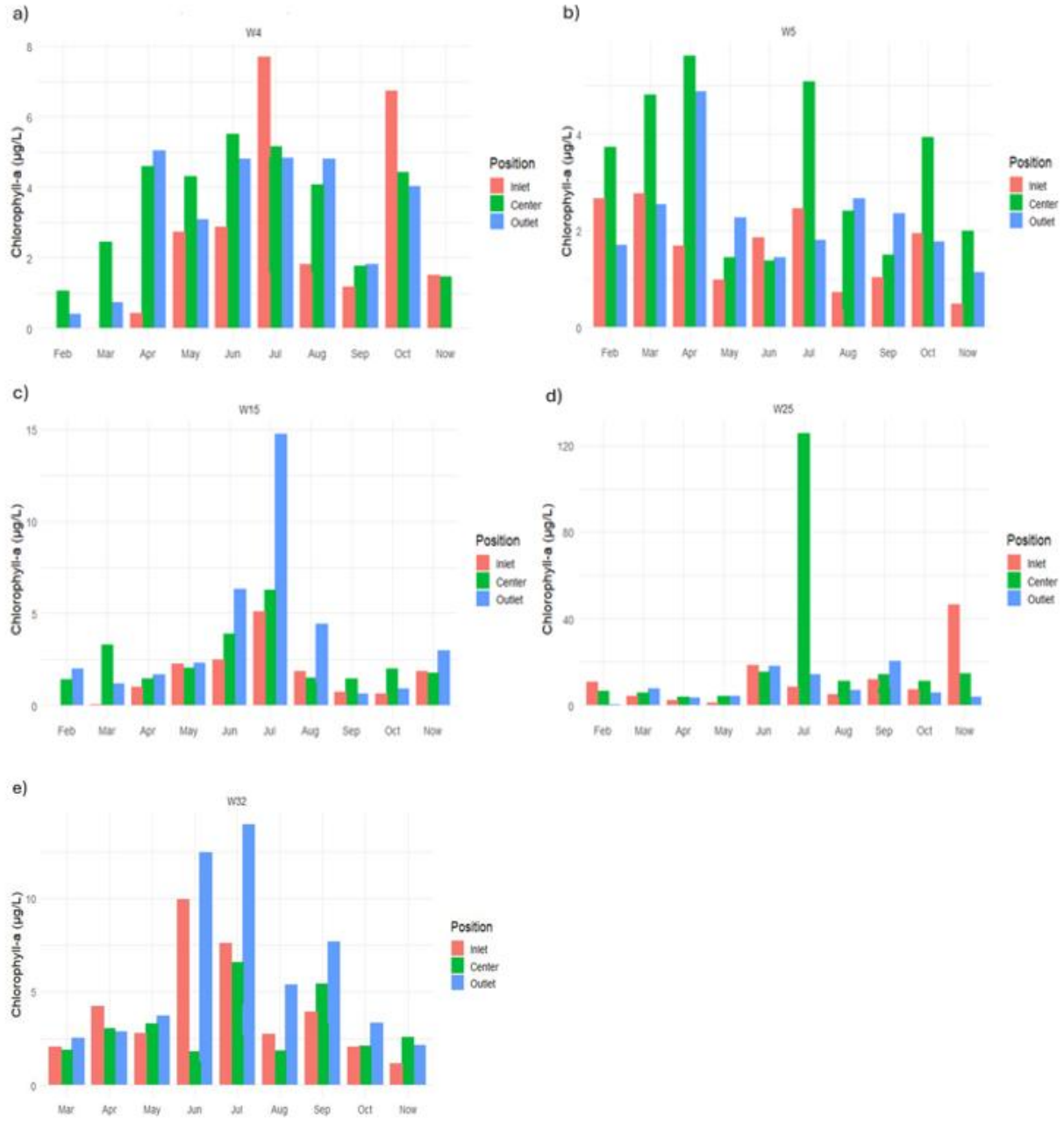


*Figure 5 Bars represent Chl-a concentration in the five wetlands located in peat soil. The bars represent the positions inlet (red), center (green) and outlet (blue) under the sampling year (February-November). a) Wetland 4, b) Wetland 5, c) Wetland 15, d) Wetland 25, e) Wetland 32*

The relationship between Chl-a and seasons is statistically significant (p-value <0.05; see figure 6, table 4). So, there is strong evidence that Chl-a differs between the seasons. The Dunn's test also indicated that there is a difference in Chl-a between the winter months and the spring, summer and autumn months (figure 6). The relationship between Chl-b and seasons is not statistically significant (p-value >0.05) and do not show a meaningful seasonal variation (see figure 7a, table 4). So, the differences that might occur is likely to be random. The relationship between Chl-c and seasons is not statistically significant either (p-value>0.05; see figure 7b, table 4). So Chl-c is, like Chl-b, approximately about the same amount across all seasons.

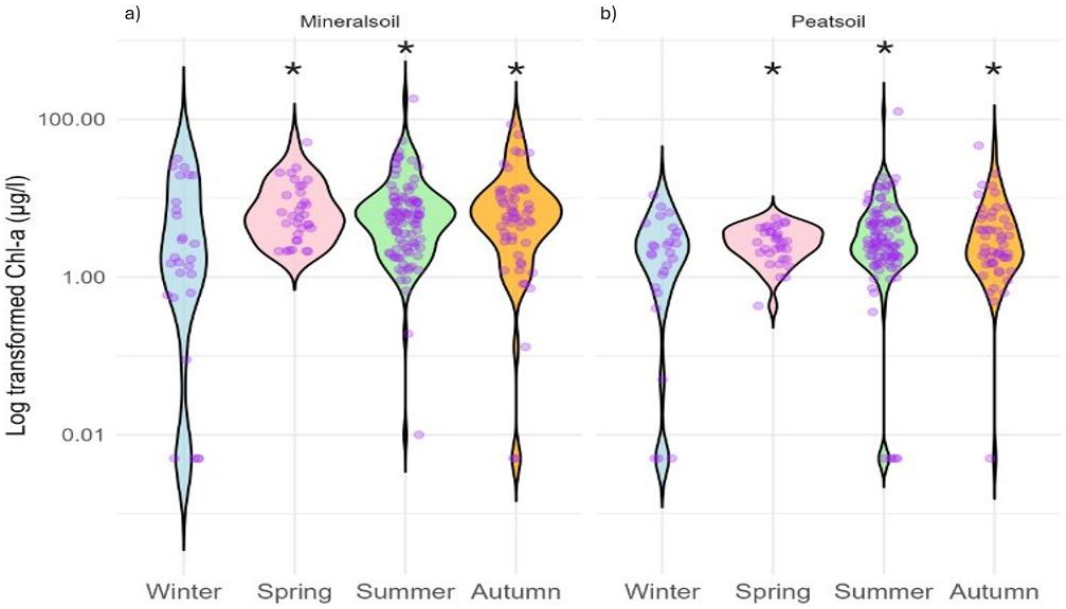


*Figure 6 Violin plots showing Chl-a concentrations during the different seasons of the year, winter, spring, summer and autumn. The asterisk (*) on the top of the violin indicate a significant result between the seasons winter and the others obtained after Dunn's multiple comparisons test (p<0.05). a) Shows the distribution in wetlands with mineral soil. b) Shows the distribution in wetlands with peat soil.*

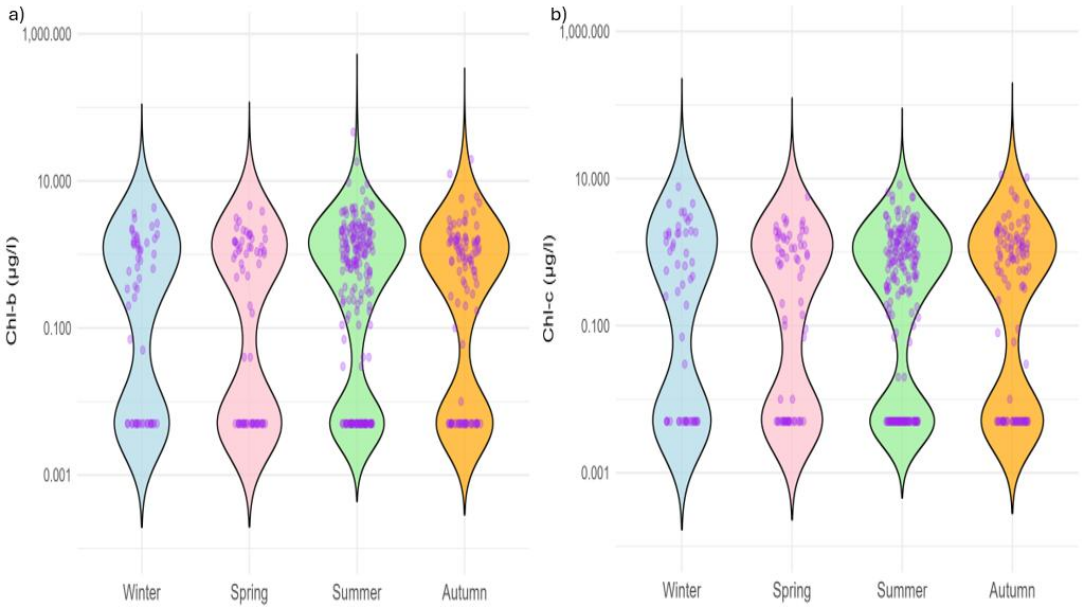


*Figure 7 Violin plots showing Chl-b and Chl-c concentrations during the different seasons of the year, winter, spring, summer and autumn. a) Chl-b raw data, y-axis log transformed b) Chl-c raw data, y-axis log transformed*

*Table 4 Statistic results from the Kruskal-Wallis test about temporal variability of, chl-a, chl-b and chl-c among seasons. * stands for p<0.05, ** stands for p<0.01, *** stands for p<0.001, NS stands for p>0.05*

| ***Source*** | **Chi-squared** | **p-value** |
|---|---|---|
| *Chl-a* | 14.13 | ** |
| *Chl-b* | 4.5918 | NS |
| *Chl-c* | 35122 | NS |

**Spearman correlation plots for Chl-a and temperature, as well as Chl-a and PAR**

The Spearman correlation coefficient (rho) shows that there is a small positive correlation between the Chl-a, temperature and PAR (figure 8a and b). The result indicates that the correlation is highly statistically significant (rho=0.1966, p<0.001), so the relationship between temperature and Chl-a is unlikely to randomly change. The result for PAR is also statistically significant (rho=0.1423496, p <0.05). So, when there are higher temperature and PAR, there will be more Chl-a in the wetlands. The correlations are however weak (rho is closer to 0 than to 1), so temperature and PAR are not the only variables that affects the Chl-a concentrations in the wetlands.

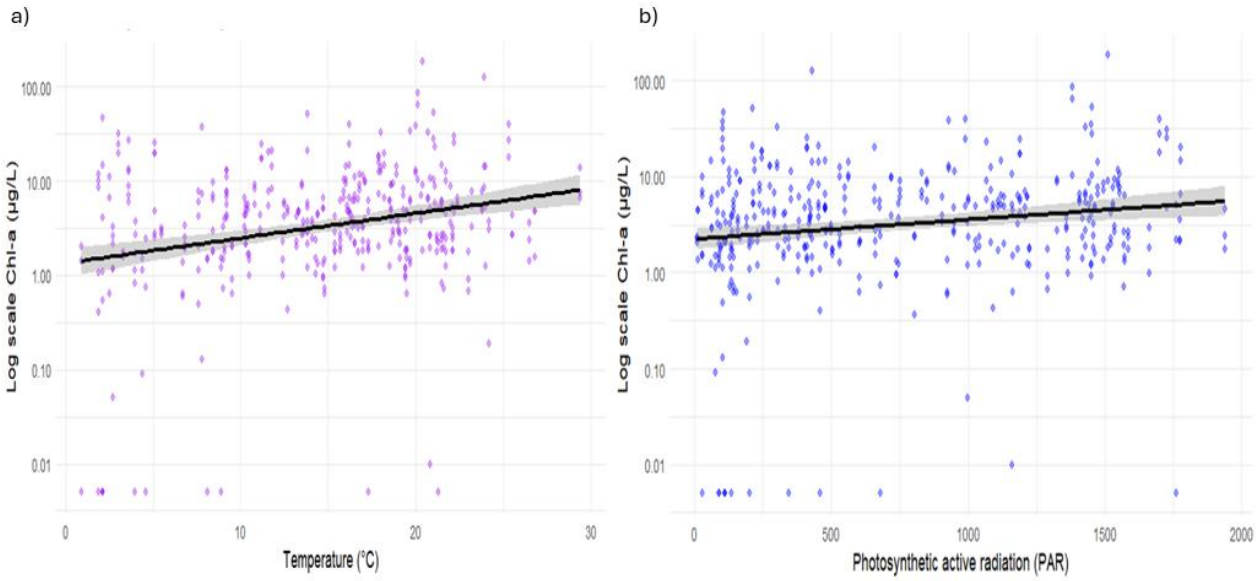


*Figure 8 Spearman correlation plot between Chl-a and Temperature and PAR. a) Temperature p-value= 7.215e-05, rho=0.1966. b) Photosynthetic active radiation (PAR) p-value=0.003703, rho=0.1423496*

### 3.4 Chlorophyll a in relation to wetland characteristics

Chl-a concentration was higher in mineral soil (9.83 ± 16.6 µg/l) than in peat soil (4.59± 9.61 µg/l; figure 9a, table 5). Chl-a concentration was higher in wetlands with a small size (7.78±14.5) than in bigger wetlands (2.10±1.31; figure 9b, table 5). Chl-a concentration was higher in wetlands with small catchment area (9.25±13.6) compared to larger catchment areas (5.17±13.8; figure 9c, table 5). Chl-a concentration was higher in wetlands with cropland as a land use in the catchment above 40 % (12.8±20.4) than in wetlands with lower cropland in the catchment (4.83±8.79; figure 9d, table 5). However, the position in the wetland do not influence the amount of Chl-a that exists in the wetland at all (figure 10, table 5). This indicates that there is no evidence that Chl-a differs significantly between inlet, center and outlet of the wetland.

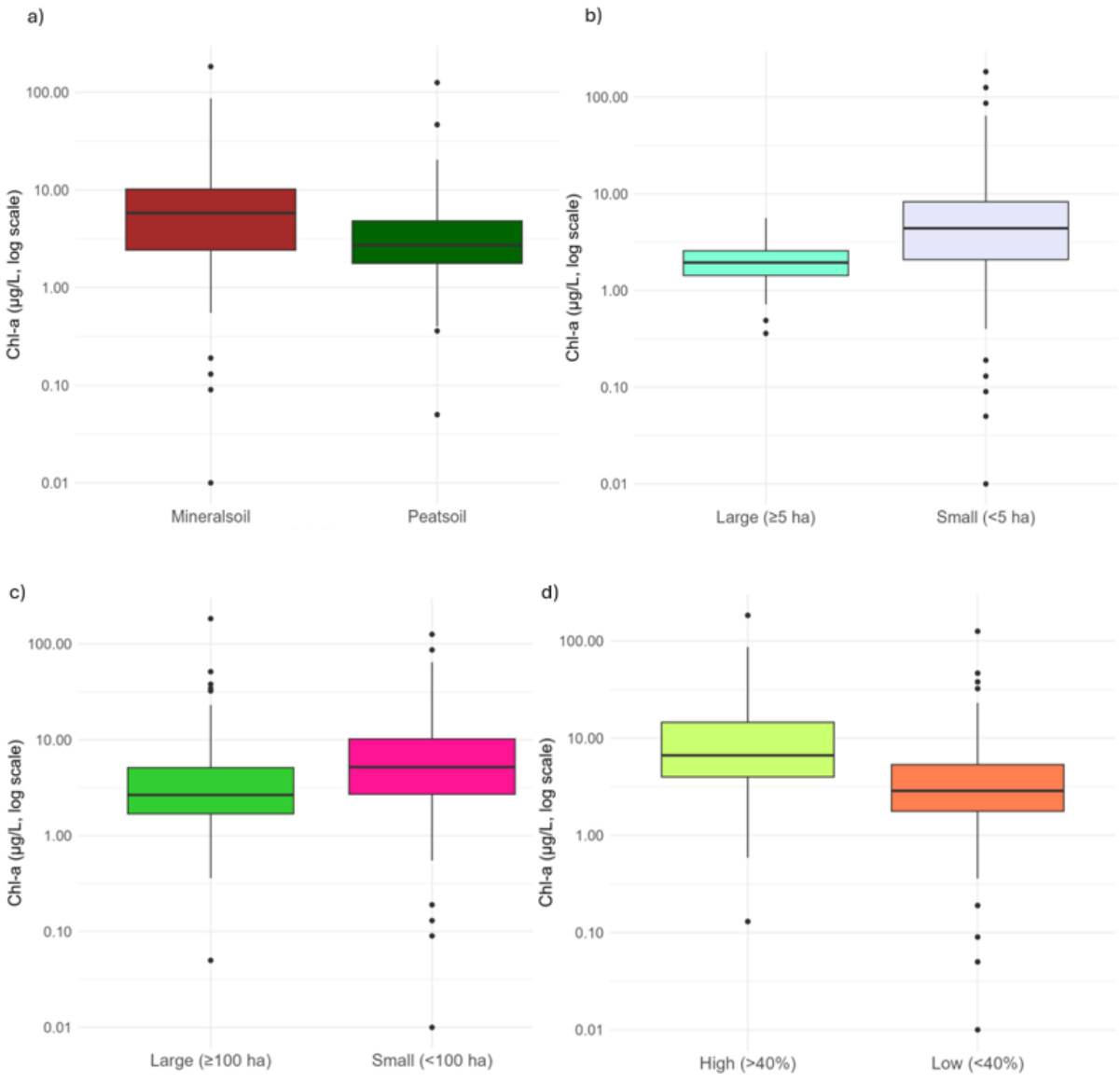


*Figure 9 Boxplots showing the differences in Chl-a with different wetland characteristics. a) Chl-a and soiltype, b) Chl-a and Wetland size c) Chl-a and Catchment area d) Chl-a and Cropland Percentage*

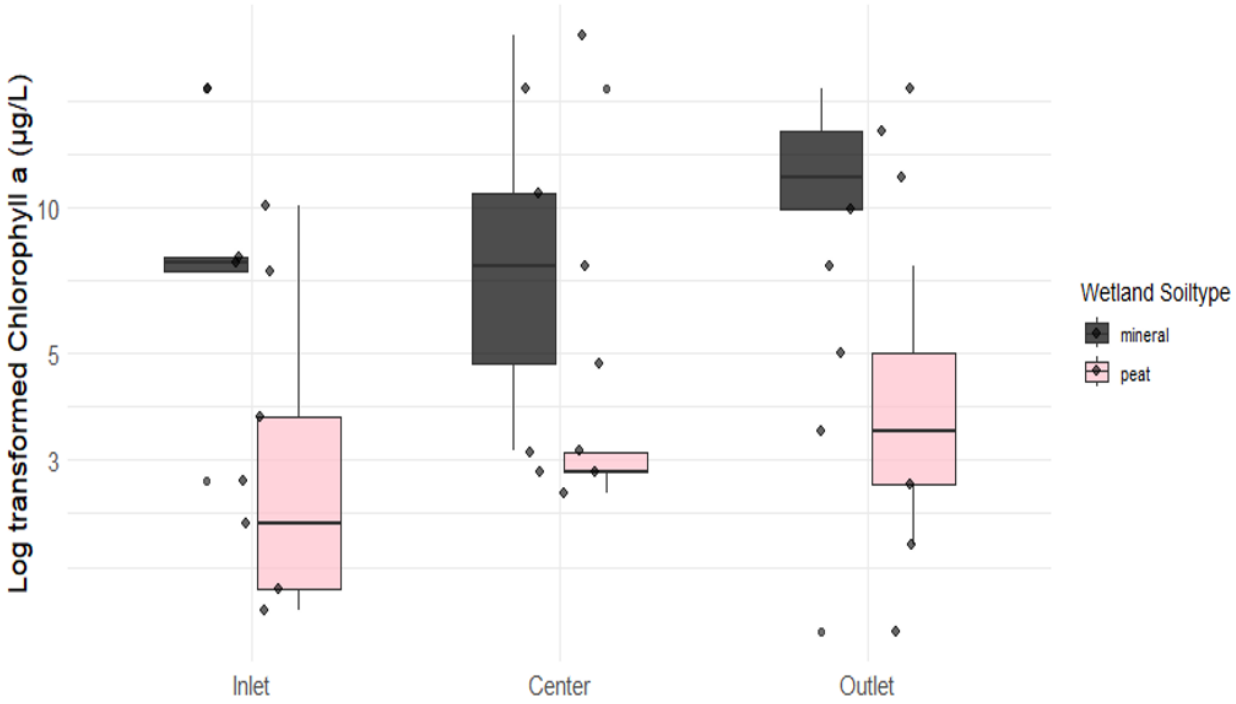


*Figure 10 Average Chl-a at Inlet, Center and Outlet by soiltype*

*Table 5 Statistic results from the Kruskal-Wallis's test showing the relationship between Chl-a and Wetland characteristics. * stands for p<0.05, ** stands for p<0.01, *** stands for p<0.001, NS stands for p>0.05.*

| ***Source*** | **Chi-squared** | **p-value** |
|---|---|---|
| *Wetland Soiltype* | 35.2 | *** |
| *Wetland Position* | 3.14 | NS |
| *Wetland Size* | 26.263 | *** |
| *Catchment Area* | 41.757 | *** |
| *Cropland (%)* | 91.931 | *** |

### 3.5 Relationships between wetland characteristics, water physical chemical parameters and Chlorophyll a.

Wetlands with mineral soil have a higher amount of phosphorus (7.90±9.66), TN (1.15±1.30) and EC (301±243) compared to wetlands with peat soil, phosphorus (3.52±3.87), TN (0.822±0.434) and EC (219±140; figure 11a, 11b and 11c; table 6). DO had a weaker but still significant difference between the different soil types, mineral soil average 9.33±2.90, peat soil average 9.95±2.37 (table 6). However, the amount of DOC, pH, Ammonium and Nitrate seems to be around the same in both wetlands with mineral soil, and wetlands with peat soil (table 6).

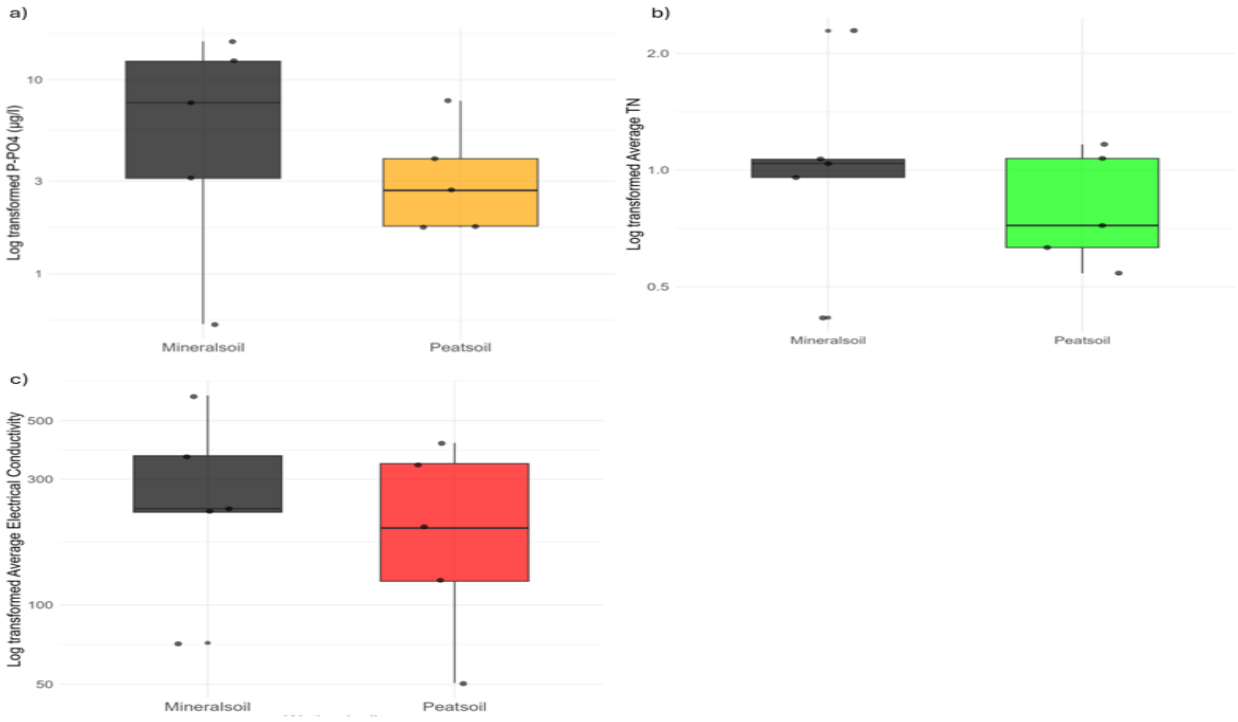


*Figure 11 boxplots showing the differences of wetland characteristics and chemical parameters between the different soil types. a) Mean Phosphorus b) Mean TN c) Mean EC*

*Table 6 Statistic results from the Kruskal-Wallis test showing differences between wetland characteristics and soil type. * stands for p<0.05, ** stands for p<0.01, *** stands for p<0.001, NS stands for p>0.05.*

| ***Source*** | **Chi-squared** | **p-value** |
|---|---|---|
| ***Phosphorus*** | 5.2038 | * |
| *Ammonium* | 0.0078262 | NS |
| *Nitrate* | 0.35032 | NS |
| ***Total Nitrogen*** | 18.942 | *** |
| *Dissolved organic carbon* | 5.9437e-06 | NS |
| *pH* | 1.3759 | NS |
| ***Electrical conductivity*** | 10.621 | ** |
| *Dissolved oxygen* | 3.9843 | * |

The factors that Chl-a have a positive correlation with is Phosphorus (rho=+0.17, p<0.05), TN (rho=+0.19, p<0.001), DOC (rho=+0.21, p<0.001) and pH (rho=+0.19, p<0.001). So, if there are more phosphorus, TN, DOC and pH in the wetlands, there is more Chl-a. The correlation between these factors and Chl-a is however weak (see figure 12).

However, there are also nutrients that have negative correlation to Chl-a. Ammonium (rho=-0.31, p<0.01) and Nitrate (rho=-0.33, p<0.001; see figure 12) both have a negative, but statistically significant correlation with Chl-a. So, if there is more of these nutrients in the wetlands, the Chl-a will be affected negatively and decrease. Ammonium and Nitrate have a moderately strong effect on Chl-a (see figure 12).

Electrical Conductivity (EC) however don't have a meaningful correlation with Chl-a. It showed a very weak, but non-significant negative correlation (rho=-0.04, p-value<0.05). So, if EC increases, Chl-a will either increase or decrease consistently. EC don't tell anything regarding the Chl-a concentration. DO didn't have a significant correlation with Chl-a either (rho=-0.08, p-value>0.05). This means that, like EC, if DO increases or decreases, the Chl-a will not be affected.

Temperature and PAR also have a positive correlation to Chl-a, but both correlations are however relatively weak, which has already been concluded (see figure 8 for more info about these specific factors)

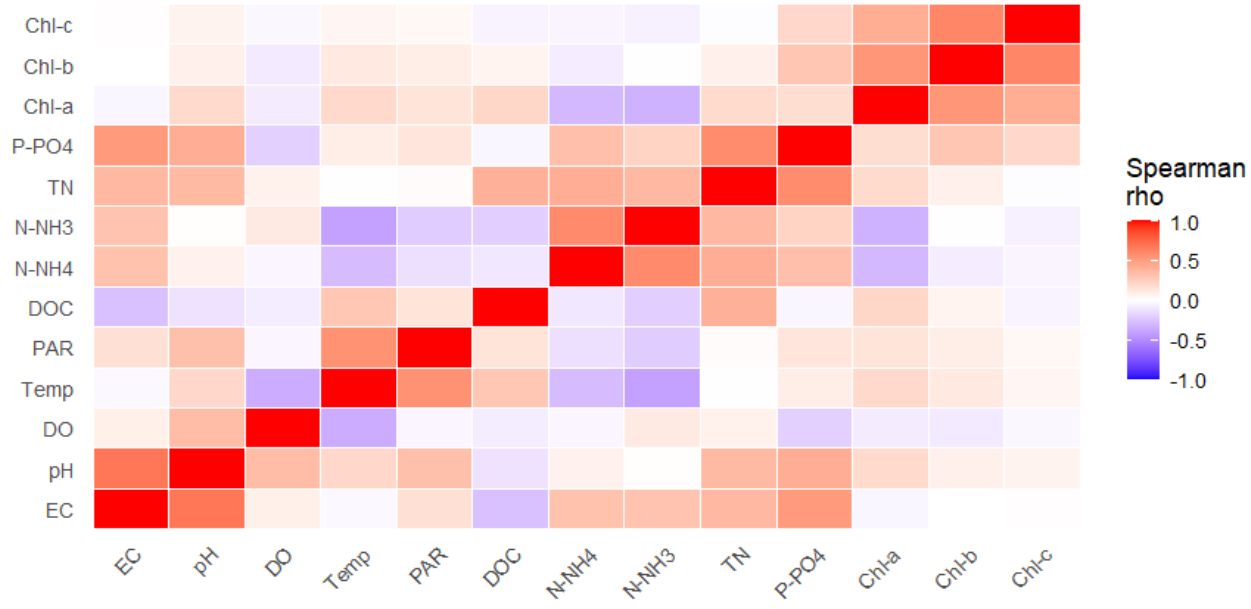


*Figure 12 Heatmap of Spearman correlation matrix for wetland nutrients, seasonal markers and Chlorophyll.*

# Discussion

## 4.1 Summary

Knowledge of the temporal and spatial factors influencing phytoplankton biomass and water quality in wetlands is essential for their conservation and management. In this study, we examined how phytoplankton biomass, measured as chlorophyll- concentration, varies over the course of one year, as well as how wetland characteristics influence phytoplankton biomass and water quality, with a specific focus on wetlands located in different soil-types, mineral soil *vs* peat soil. A total of 10 wetlands in southern Sweden with different soil-types and land uses were included in the study. Each wetland was visited 14 times between February and November 2025. Chlorophyll-a as marker for total phytoplankton biomass was extracted with acetone and quantified spectrophotometrically.

## 4.2 Temporal variability of phytoplankton biomass in wetlands located in mineral and peat soil.

Chlorophyll-a concentrations in both wetlands located in mineral and peat soil showed a seasonal variability, being higher but comparable during the warmer seasons of the year (spring, summer and autumn) than the colder season of the year (winter). The results align with previous studies in wetlands located in Northeast China and India, where the total phytoplankton abundance, biomass and primary production was observed to be higher during warmer seasons like spring, summer and autumn (ex Borovkova et al (2026), Li et al (2022) and Bhat et al 2015). The seasonal markers of temperature in water and light availability in form of PAR (photosynthetic active radiation) were found to be positively correlated with Chl-a concentrations, suggesting that these factors have a positive effect on Chl-a concentrations. Our results can be compared to Cao et al (2022) study, which also concluded that temperature had a positive correlation to Chl-a in wetlands. What makes this study different from Cao et al (2022) is that in this study there is a focus on water temperature, while in Cao et al (2022)'s study they focused on air temperature. But both temperature variables could be concluded had a positive correlation to Chl-a. This result proves the hypothesis that the total phytoplankton biomass (Chl-a) will be higher in the warmer months due to increase of temperature and PAR. The reason why there is a positive correlation between temperature and PAR with Chl-a is due to the temperature enhancing the phytoplankton biomass and activating their metabolism, while higher PAR irradiance promotes phytoplankton biomass. So, phytoplankton depends on light both for photosynthesis and growth.

Neither the Chl-b (marker for Chlorophyta, green algae) nor Chl-c (marker for Bacillariophyta, brown algae and diatoms) concentrations showed a significant temporal variability among seasons, and no significant correlation could be observed with either temperature or PAR. This means that the hypothesis that Chlorophyta has higher concentration in the summer months, and Bacillariophyta having higher concentrations in the winter months must be rejected. But the hypothesis can be partly proven due to the fact that the total phytoplankton biomass, Chl-a, could be seen having a higher concentration during the summer months and having positive correlations to seasonal markers. The results for Chl-c differ from previous studies that concluded that diatoms (Chl-c) dominated in aquatic environments during cold conditions and nutrient rich environments, which are more abundant during the winter months due to the seasonal nutrient mixing (Avrahami et al., 2025; Barlow et al., 2013).

Why the result differs in this study may be due to the low amount of Chl-c that was observed in the samples. There were a lot of zero results for Chl-b and Chl-c (see figure 7), and the lack of data might have affected the correlation results between the seasonal markers and the pigment markers. One reason for the Chl-b and Chl-c having a lot of zero results in the wetland samples is that they

are not as abundant in all phytoplankton groups the same way that Chl-a is. There are photosynthetic organisms that only contain either Chl-b, Chl-c or none at all (Roy et al., 2011). Other phytoplankton species can rely on other accessory pigments. One example is cyanobacteria, that don't have any Chl-b or Chl-c at all. It does have Chl-a, but the most abundant pigment marker in cyanobacteria is phycobilisome, which works closely with Chl-a by absorbing and transferring light energy to it and can be particularly abundant (Yu et al., 1999).

If there were higher concentrations of the Chl-b and Chl-c in the wetland samples the results might have been different and this is worth looking into in future studies. Other pigments such as lutein and fucoxanthin could also be observed to target Chlorophyta and Bacillariophyta, respectively, in future studies. Lutein and Fucoxanthin have a higher accuracy as markers for Chlorophyta and Bacillarophyta. However, for detecting these pigments other methods than spectrophotometry is required. They are usually quantified with HPLC, which were out the scope of this study (Roy et al., 2011).

### 4.3 How wetland characteristics impact water quality and phytoplankton biomass

Overall, this study shows that wetlands located on mineral soil had higher Chl-a concentrations than wetlands located on peat soil, as well as higher concentrations of nutrients such as phosphorus and total nitrogen. In these wetlands, with higher Chl-a concentrations and nutrient loads, electrical conductivity (EC) was also higher. EC reflects higher ion or nutrient availability in aquatic environments, so the results indicate that there are more ions and nutrient availability in mineral soils. Although, these wetlands had higher levels of dissolved oxygen (DO). This can be linked to higher productivity in wetlands with mineral soil due to photosynthesis producing more oxygen.

Overall, the average Chl-a in the observed wetlands is lower than those that are detected in high eutrophic state wetlands, regardless of soil type. A previous study concluded that eutrophic wetlands in Northern Alberta, Canada, could have >20 µg/l of Chl-a concentration during high chlorophyll wetland months, and <15 µg/l during low chlorophyll wetland months (Norlin et al., 2005). However, when comparing the Chl-a concentration in mineral wetlands with lakes that have poor water quality and high eutrophication, the mineral wetlands can be considered eutrophic. Lakes with Chl-a concentrations above 10 µg/l has in previous studies been considered having poor water quality and high eutrophication, while lakes with Chl-a concentrations below 6 µg/l has been considered having good water quality and low eutrophication (Borgström et al., 2026). Therefore, wetlands with peat soil in this study presented an average Chl-a concentration of 4.59± 9.61 µg/l, which from a “lake” perspective can be considered having better water quality. While the wetlands with mineral soil in this study presented an average Chl-a concentration of 9.83 ± 16.6 µg/l, which can be considered having bad water quality. But from an eutrophicated wetlands perspective, the results suggests that both mineral soil and peat soil wetlands have good water quality and low eutrophication.

Several studies have reported that phosphorus and total nitrogen show a positive correlation with Chl-a concentration due to them being key nutrients for phytoplankton growth (Hamilton et al 2016, Paerl et al (2020), Cao et al (2022)). Phytoplankton needs phosphorus to incorporate it into Adenosine triphosphate (ATP), to be able to carry energy within the photosynthesis cycle. Nitrogen is used by the phytoplankton to build up amino acids and build up protein so that they can grow, and most important, nitrogen is part of the molecular structure of chlorophyll, so the positive correlation result between Chl-a and total nitrogen in this study makes sense (A.M. Humphrey, 1980; Mandal & Dutta, 2020). Increased nutrient availability can therefore stimulate phytoplankton biomass, resulting in higher Chl-a concentrations. The source of nutrients enrichment in the studied wetlands seems to have an external origin, probably inputs from the catchment. That means that the main enrichment of nutrients that do exist in the wetlands likely don’t come from the leachate from the soil, but rather from the environment around the wetlands. Otherwise, we would have

observed higher nutrient loads in peat soils, which are rich in organic matter associated with nitrogen and phosphorus.

Other factors related to phytoplankton biomass and water quality are the wetland size and the percentage of cropland (agriculture) in the catchment. Our results align with those from Cao et al (2022)'s study, which also showed that the aquatic environments size matter and concluded that if the size is smaller, there will be more Chl-a. The difference compared to this thesis is that Cao et al (2022) focuses on lakes in eastern China, and not wetlands. But the same conclusion can be drawn in this study. The cropland percentage in the catchment also had a positive correlation to how big the Chl-a concentration was in the wetlands. If there is a higher cropland percentage in the catchment, there is more Chl-a in the wetlands. This information can be connected to previous studies like J. li et al (2020) and Ritter et al (2002) that concluded that if there is more agricultural land (like for example cropland), a higher use of fertilizers including phosphorus and nitrogen is expected, resulting in higher nutrients inputs from catchments run-off to the surrounding aquatic environment. This aligns with our results, showing that higher nutrient loads in wetlands located in catchments with more than 40 % of the catchment dedicated to agriculture, are those with higher Chl-a concentrations. Ritter et al (2002) showed further that most of the phosphorus that is present in the aquatic environments are there due to human activities, and cropland only exists due to human need for agriculture. Some new information from this study is that even the size of the catchment affects the amount of Chl-a that exists in the wetland. This study shows that a smaller size of the catchment has a positive correlation with Chl-a. So, if we want wetlands that have better water quality, and less algal blooms due to phytoplankton, the wetlands need to be bigger, have larger catchment area and have less cropland percentage in the catchment as well as be located in peat soil.

## 4.4 Limitations of the study

There were also some limitations to this study. The data that was used was only collected from one year, and the sampling effort was not equal between the seasons. The samplings were done more frequently during the summer and spring months. For example, the wetlands were sampled only once in one of the winter months but was sampled three times during one of the summer months. To improve accuracy, it would be desirable to have a similar sampling effort among months or seasons.

Another limit is that the organic matter in the sediment was sampled from wetlands in 2024 as part of a previous study, and not from wetlands sampled in 2025 when all the rest of the data comes from. For the sake of the result, we have considered organic matter in the wetlands to still be similar to the 2024 results. In further studies regarding wetlands' water characteristics effect on phytoplankton biomass, more recent information regarding organic matter could have given a better understanding of the actual results and other conclusions could have been drawn. Therefore, in future studies a need for accurate information regarding organic matter is needed.

There was also a limit in the knowledge of how much phosphorus, ammonium and nitrate there were in total in all the wetlands. There were measurements done, but they were only done at one position in the wetland, the outlet. So, to get a more integrative overview regarding these nutrients in the wetlands, more measurements should be made at all positions where the chlorophyll samples were taken. In other words, measurements of phosphorus, ammonium and nitrate should be done in the inlet and the center of the wetland as well.

Sampled water volumes were enough for chlorophyll-a quantification; however, they were not enough for an environmental risk assessment (ERA). For further studies it is suggested to collect more water, circa 200–500 ml, to have more biomass of the less abundant phytoplankton taxonomic groups.

### 4.5 Other methods

To quantify phytoplankton biomass and taxonomic groups as well as for a more detailed pigments profile for the phytoplankton a HPLC analysis can be done. HPLC stands for High-Performance Liquid Chromatography, and is used to separate, identify and quantify solution dissolved compounds. Chromatography itself is a separation of mixtures into their individual components, while HPLC's is an upgraded version of specifically liquid chromatography built on the same principle of separating mixtures and chemicals. HPLC gives a detailed characterization of complicated samples, which include shape, size, charge, structure, molecular weight, hydrophobicity and hydrophilicity (R. Ahmed, 2024). By doing an HPLC analysis, more detailed results can be constructed, with more information about the pigment profile in the wetland itself.

Other methods to work with in future projects are microscope observations or counting for taxa identification and abundance. These methods can be used to get a better understanding of the pigment profiling and a better understanding about the wetlands' pigment profile and therefore get more detailed results about the different wetlands' water quality.

### 4.6 Conclusions

The result showed that chlorophyll a, a pigment used as proxy for phytoplankton biomass, responds to seasonal variabilities such as light and temperature, and therefore seasonal differences occur. No temporal or seasonal variables were observed based on chlorophyll b and chlorophyll c pigments, partially because high variability and poor seasonal sensibility. Water characteristics such as mineral soil, small size, small catchment area and high percentage of cropland in the catchment led to a higher amount of phytoplankton biomass.

Furthermore, the water quality of these wetlands is characterized for having higher nutrient loads (phosphorus and total nitrogen), higher electrical conductivity and chlorophyll-a concentrations than those located on peat soil. The positive relationship between chlorophyll-a concentration and nutrient levels (phosphorus and TN) support the role of these nutrients in supporting phytoplankton growth and primary production.

More than the differences on soil-type itself affecting the water quality and phytoplankton biomass, our study shows that the land uses of the catchment play an important part. It co-occurs that mineral soils are frequently associated to catchments with a significant proportion of agricultural land. Our study demonstrated that external inputs of nutrients from the catchment via runoffs seem to have a higher influence on the nutrient levels, conductivity and phytoplankton biomass than the potential leaching of nutrients coming from the soil-types. So, the size of the wetland as well as catchment area and composition had a bigger importance to phytoplankton biomass compared to which soil-type the wetland has.

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# Appendix 1

## Chl-a concentration plots

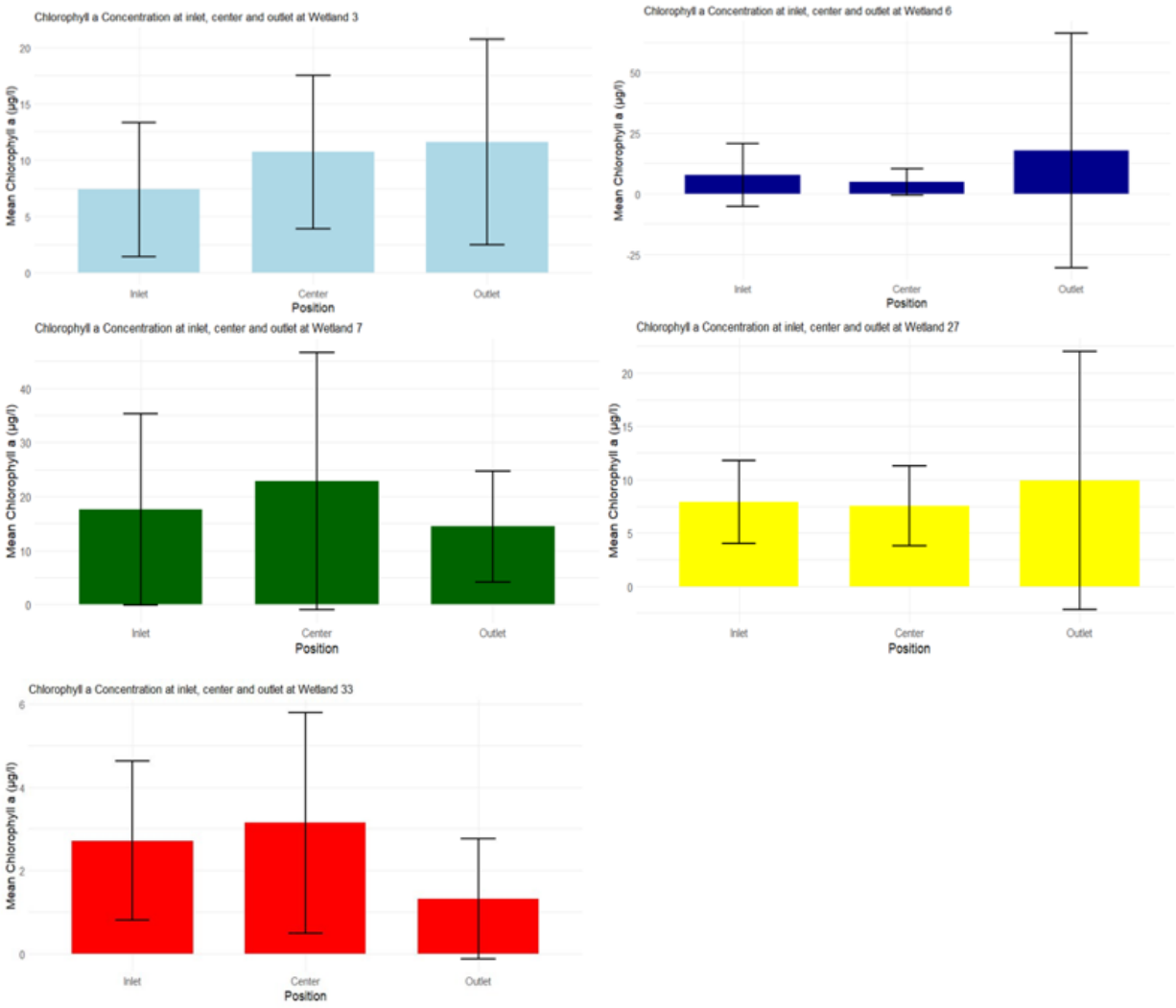


*Figure 13 Chl-a average concentrations in wetlands with mineral soil at inlet, center and outlet.*

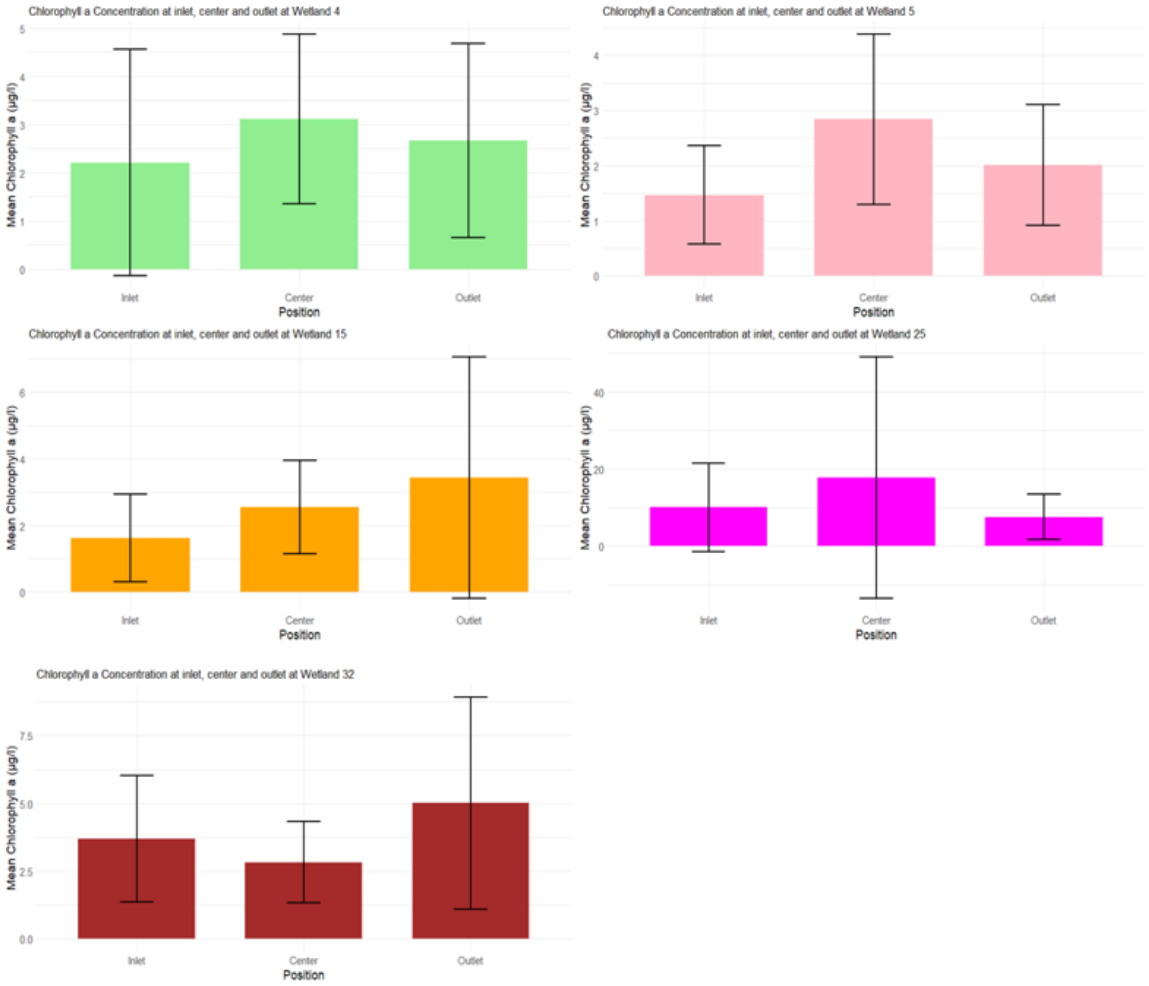


*Figure 14 Chl-a average concentrations in wetlands with peat soil at inlet, center and outlet.*

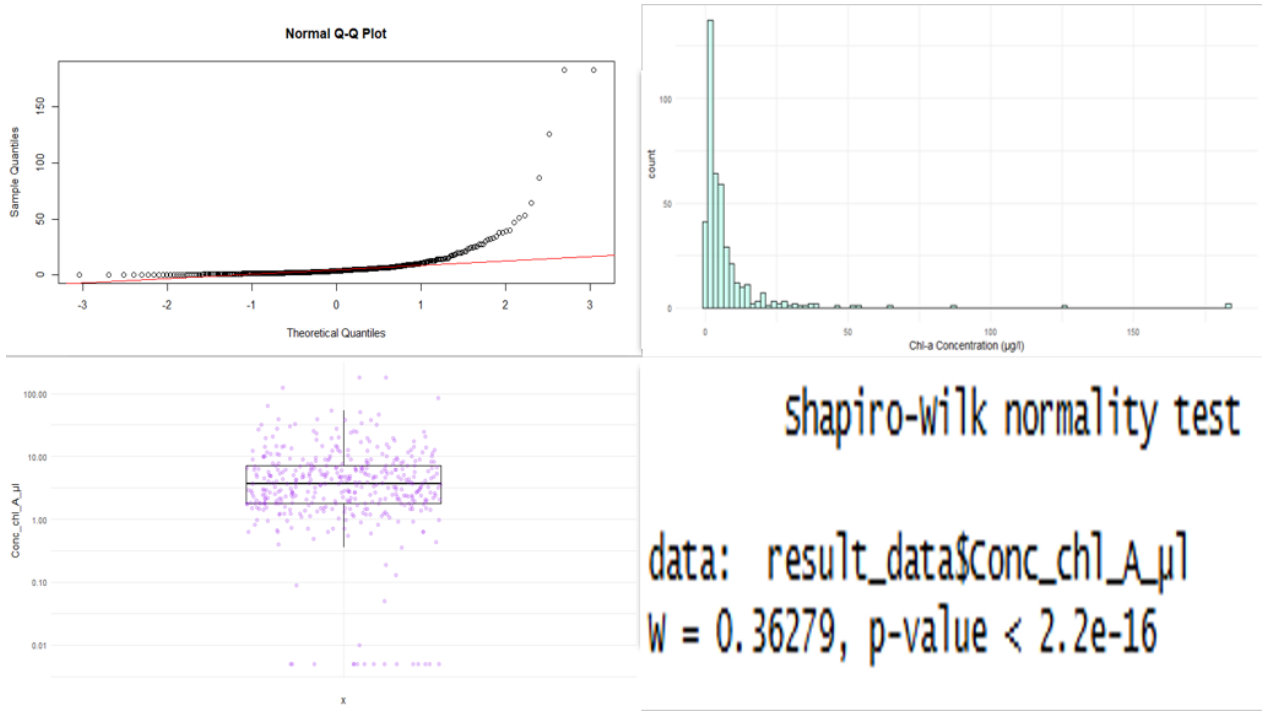


*Figure 15 Chl-a Raw data Normality tests: Q-Q plot, Histogram, Boxplot and Shapiro-Wilk*

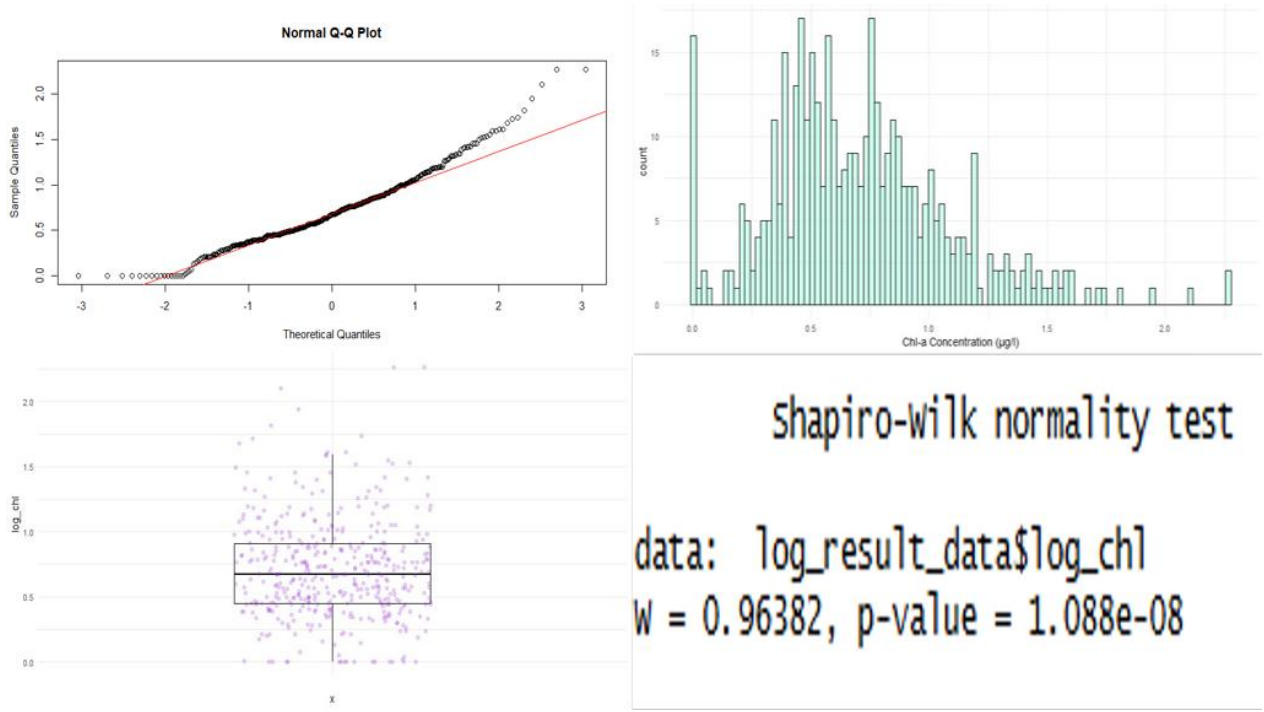


*Figure 16 Log-transformed data Normality test, Q-Q plot, Histogram, Boxplot and Shapiro-Wilk normality test*

# Appendix 2

## Tables for statistical significance tests

*Table 7 Temporal and Seasonal variability for Chl-a, Chl-b and Chl-c*

***Temporal variability***

| *Variable* | *Chl-a min* | *Chl-a max* | *Chl-a mean* | *Chl-a SD* | *Chl-b min* | *Chl-b max* | *Chl-b mean* | *Chl-b SD* | *Chl-c min* | *Chl-c max* | *Chl-c mean* | *Chl-c SD* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Winter* | 0 | 31.7 | 4.5 | 7.23 | 0 | 4.33 | 0.89 | 1.04 | 0 | 7.72 | 1.11 | 1.54 |
| *Spring* | 0.430 | 51.30 | 6.14 | 7.66 | 0 | 4.65 | 0.85 | 1.03 | 0 | 5.72 | 0.85 | 1.05 |
| *Summer* | 0 | 183.21 | 7.86 | 17.0 | 0 | 46.42 | 1.54 | 3.78 | 0 | 8.24 | 1.01 | 1.31 |
| *Autumn* | 0 | 86.49 | 7.93 | 12.7 | 0 | 19.64 | 1.28 | 2.44 | 0 | 11.17 | 1.15 | 1.84 |

*Table 8 Spearman Correlation Analysis between Wetland nutrients and Chl-a*

| Variable | rho | p-value | Strength | Direction | Interpretation |
|---|---|---|---|---|---|
| Phosphorus | +0.17 | 0.04 | weak | positive | More P=slightly more Chl-a |
| Ammonium | -0.31 | 0.0003 | moderate | negative | Higher $NH_4$=lower Chl-a |
| Nitrate | -0.33 | 6.847e-05 | moderate | negative | Higher $NO_3$=Lower Chl-a |
| TN | +0.19 | 0.0001 | weak | positive | More nitrogen= slightly more Chl-a |
| DOC | +0.21 | 1.434e-05 | weak | positive | More DOC= slightly more Chl-a |
| pH | +0.19 | 9.348e-05 | weak | positive | Higher pH=more Chl-a |
| EC | -0.04 | 0.461 | weak | negative | EC increases= Chl-a either increase or decrease |
| DO | -0.08 | 0.10 | weak | negative | Higher DO=Chl-a either increase or decrease |