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Humic Matter and Phytoplankton Nutrient Limitation in a Changing Environment

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Abstract

Climate change leads to increased inputs of terrestrial dissolved organic carbon (DOC) and associated nutrients to freshwaters, potentially affecting the risk for eutrophication. While the effect of higher DOC concentrations on primary productivity (PP) due to light attenuation and increased competition by bacteria is rather well studied, the effect of DOC on PP due to effects on nutrient availability is still unclear. Therefore, laboratory incubation experiments with natural phytoplankton communities were performed to investigate the effect of DOC on phytoplankton growth under different phosphorus (P) and iron (Fe) regimes, under exclusion of light and grazing effects. The experiments were conducted with water originating from two sites with differing DOC character within Lake Mälaren, the third largest lake in Sweden. P (ambient, 50 µg L^{-1} added), Fe (ambient, 400 µg L^{-1} added) and DOC (low, ambient, high) concentrations were crossed in all possible combinations giving 12 different treatments. Chlorophyll *a* concentrations and initial and final chemical conditions were analysed after 7.5 days of incubation.

Specific growth rates were by far highest in P addition treatments, showing that the phytoplankton community in Mälaren was mainly limited by P. Simultaneous addition of P and Fe further stimulated algae growth, indicating a co-limitation of Fe and P. The addition of Fe alone had no or a negative effect on growth rates, which might be explained by a strong binding of P to precipitated Fe. The effect of DOC on primary production depends on nutrient regime. Under ambient P conditions, DOC enhanced growth rates, probably due to a concurrent increase in nutrients associated to DOC. Under P-rich conditions, higher DOC concentrations resulted in lower growth rates compared to ambient DOC treatments. Fe showed a significant interaction effect with DOC under ambient P conditions. The direction of this effect depends on DOC quality, indicating that the different chemical composition of DOC influences Fe bioavailability differently. To determine which DOC type has a higher binding capacity for Fe, more research in primarily Fe-limited systems is needed. The study shows that PP in Lake Mälaren is mainly regulated by P and partly by Fe and DOC. Moreover it demonstrates that DOC affects the availability of Fe to phytoplankton. It is concluded that increased DOC concentrations result in higher primary productivity, but a lower sensitivity of phytoplankton to P inputs.

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1 Introduction

1.1 Ecological consequences of eutrophication, climate change and brownification

Of the world´s total water volume, only about 2.6 % consists of freshwater. 99.7 % of the total freshwater resources are locked in glaciers, ice caps or deep groundwater pools. Only the remaining 0.3 % of freshwater is accessible as surface water in lakes, rivers and wetlands. However, this tiny fraction of total freshwater resources is of great importance as a habitat for organisms, but also a main resource for human consumption. In lakes, algae that are living in the open water body, the phytoplankton, is a very important component of food webs. They are an essential food source for higher trophic levels and largely determine fish production (Kalff, 2002). However, the enrichment of lakes with nutrients (eutrophication) may result in the excessive growth of phytoplankton and potentially harmful algae blooms. Eutrophication can cause oxygen deficiency, fish kills and loss of biodiversity as well as problems for drinking water production and health, due to toxins produced by cyanobacteria. Important regulating factors for primary production are inorganic nutrients, such as phosphorus (P) and nitrogen (N). Both are required for phytoplankton growth, since phosphorus is needed for DNA, RNA, phospholipids and energy transfer, while nitrogen is needed for nucleic acids and protein synthesis (Conley *et al.*, 2009; Klausmeier *et al.*, 2008). Especially phosphorus has widely been recognized as a key limiting nutrient in freshwater ecosystems, causing eutrophication when overly supplied (Kalff, 2002; Conley *et al.*, 2009; Correll, 1998). Recently, also iron (Fe) has been identified as a cause of algae blooms, since it stimulates the ability of cyanobacteria to dominate the phytoplankton community (Molot *et al.*, 2010; Molot *et al.*, 2014, Sorichetti *et al.*, 2014a, 2014b). However, recent studies indicate that also dissolved organic carbon (DOC) has a major role in determining the productivity of lakes (Karlsson *et al.*, 2009; Finstad *et al.*, 2013). DOC is the fraction of natural organic matter that passes through a 0.45 µm filter (Roulet and Moore, 2006). The DOC pool of lakes consists of autochthonous DOC, which is produced inside the lake via phytoplankton, macrophytes and bacteria, and allochthonous DOC, which is imported from terrestrial soils and wetlands. Autochthonous DOC usually consists of protein-like, low-molecular-weight molecules, while allochthonous DOM consists of high-molecular-weight, humic-like molecules and is of darker colour (Burrows *et al.*, 2013).

Anthropogenic climate change is predicted to go along with increased carbon dioxide (CO_2) concentrations, higher temperatures and changes in hydrology and run-off due to higher precipitation, draughts and more extreme weather events (IPCC, 2007). As a consequence of climate change the input of nutrients and DOC into aquatic ecosystems may change, potentially effecting primary productivity (PP) and hence the risk for eutrophication (Bengtsson *et al.*, 2012). Several studies across Europe and North America report an increase in DOC loads to aquatic ecosystems during the last 30 years (Larsen *et al.*, 2011; Tian *et al.*, 2013;

Weyhenmeyer and Karlsson, 2009; Weyhenmeyer *et al.*, 2014). Multiple possible reasons have been presented in literature, which are mainly connected to climate change and declines in acid deposition. The increased DOC inputs could be a result of increased production of DOC in terrestrial ecosystems caused by elevated CO_2 concentrations stimulating plant productivity or by climate warming increasing decomposition rates (Lepistö *et al.*, 2008; Freeman *et al.*, 2001). An additional explanation is an increase in DOC leaching from soils caused by higher precipitation and runoff (Tranvik and Jansson, 2002; Erlandsson *et al.*, 2008; Hongve *et al.*, 2004). Also the reverse acidification due to decreased anthropogenic sulphur emissions during the last decades, could explain brownification, since the increasing pH mobilizes DOC bound to the soil (Monteith *et al.*, 2007). Besides DOC, also an increase in iron concentration has been observed. The increase in iron is often correlated to the increase in terrestrial DOC inputs, but the positive trend for iron is larger than that for DOC. This indicates that DOC and Fe export is controlled by similar but not identical mechanisms (Kritzberg and Ekström, 2012; Neal *et al.*, 2008).

Given the expected change in stoichiometry in aquatic ecosystems, it is of great importance to understand how primary productivity will respond to increasing DOC concentrations under different nutrient availability regimes. Understanding the interactions between P, Fe and DOC and their effects on primary productivity will help making better predictions about aquatic ecosystems and water quality, under different future climate change scenarios. There are multiple ways how P, Fe and DOC can affect primary productivity, which are reviewed in the following section.

1.2 Effects of DOC, P and Fe on primary productivity

1.2.1 Effects of P, Fe and DOC and their interactions

First, P and Fe are essential nutrients stimulating phytoplankton growth (Fig. 1a). P is often considered as the main limiting nutrient, regulating primary productivity of lakes (Schindler, 1977; Correll, 1998; Kalff, 2002). Besides phosphorus also other macro-nutrients and micro-nutrients such as Fe can control primary production (Sterner, 2008). Iron can be a main limiting nutrient or co-limiting together with P (Vrede and Tranvik, 2006; Boyd *et al.*, 2007). Since the solubility of Fe is very low under oxic conditions, Fe either precipitates as iron oxides (ferrihydrite) or binds to organic compounds (Kalff, 2002). Under aerobic conditions Fe can also bind to phosphate and precipitate as ferric phosphate minerals, reducing the amounts of bioavailable P and Fe (Moore and Reddy, 1994) (Fig. 1c).

DOC can bind to nutrients such as P and Fe, thus controlling their bioavailability (Fig. 1d) (Porcal *et al.*, 2009). There is evidence that the binding of DOC to P, makes P less available for phytoplankton, thus leading to a decrease in primary productivity (Guilford *et al.*, 1987;

Drakare *et al.*, 2003). There are also studies concluding that DOC decreases the availability of iron due to complexation (Imai *et al.*, 1999; Guilford *et al.*, 1987). However, more recent studies provide evidence that DOC acts as a natural organic chelator, enhancing the solubility of Fe and preventing it from precipitating with P. By enabling Fe to remain in solution DOC makes Fe accessible for phytoplankton (Vrede and Tranvik, 2006; Maloney *et al.*, 2005; Hassler *et al.*, 2011). Moreover, as a Fe-scavenging strategy phytoplankton can release iron-chelating organic compounds (siderophores) to keep iron dissolved and being able to access it (Benderliev, 1999; Benner, 2011). Whether the binding of DOC to nutrients, makes them more or less bioavailable and to which extent phytoplankton is able to access the nutrients bound to DOC, is still unclear.

DOC also affects the mobility of nutrients, because it transports nutrients associated to the DOC into the lake (Meili, 1992; Guilford *et al.*, 1987). For instance, there is a correlation between DOC export and P, with a total phosphorus concentration increase of approximately 0.18 µg P L^{-1} per mg C L^{-1} (Thrane *et al.*, 2014). Even though most of the nutrients associated to DOC are initially in organic form and thus not available for phytoplankton, fractions of this pool are eventually transformed into inorganic and bioavailable forms by mineralization, stimulating primary productivity (Vahatalo *et al.*, 2003; Finstad *et al.*, 2013). Hence increased DOC inputs also increase the pool of bioavailable P and Fe (Fig. 1b).

1.2.2 Effect of DOC and Fe on light regime

DOC and Fe can negatively affect primary productivity due to their ability to reduce the penetration of solar radiation into the water column (Fig. 1e). Studies have shown that the light regime is mainly controlled by DOC, often attenuating more than 85 % of photosynthetically active radiation (Forsström *et al.*, 2013; Bengtsson *et al.*, 2012; Bukaveckas and Robbins-Forbes, 2000). Since the optical characteristics of DOC vary depending on their origin, the more coloured allochthonous DOC has a stronger absorbance than autochthonous DOC and thus attenuates light more effectively. Therefore the input of terrestrial DOC is of special importance for the light regime (Sommaruga *et al.*, 1999; Thrane *et al.*, 2014). However, also Fe has been shown to contribute to water colour, thus leading to increased light attenuation. Fe mainly increases absorption when bound to DOC, enabling it to remain in solution (Maloney *et al.*, 2005; Kritzberg and Ekström, 2012; Pullin *et al.*, 2007; Heikkinen, 1990). Fe, DOC and a higher share of coloured DOC contribute to a browner water colour (Köhler *et al.*, 2013; Weyhenmeyer *et al.*, 2014).

By attenuating photosynthetically active radiation, DOC and Fe restrict the depth of the euphotic zone. This light limitation can result in a decline of primary productivity. Karlsson *et al.* (2009) compared small nutrient-poor lakes along a natural DOC gradient and concluded that their productivity was limited by light and not by nutrients. With increasing light attenuation due to higher DOC concentrations, benthic primary productivity decreased. This shows that primary productivity in the benthic habitat is mainly controlled by variations in water colour,

and not so much by nutrients (Karlsson *et al.*, 2009). This is supported by mesocosm experiments, revealing that DOC can decrease the area available for benthic primary production by more than 50% (Forsström *et al.*, 2013). A decrease in primary productivity due to light attenuation by DOC and Fe has not only been observed in benthic habitats, but also in pelagic habitats. In 1998, Carpenter *et al.* manipulated lakes for four years and reported that phytoplankton biomass was negatively correlated to coloured DOC, suggesting that DOC reduces primary productivity due to shading (Carpenter *et al.*, 1998). Also a study in forest lakes reports a decline of the euphotic zone due to DOC inputs, resulting in decreasing phytoplankton biomass (Einem and Granéli, 2010). Moreover, recently published results from boreal lakes found a negative effect of iron and coloured DOC on pelagic primary productivity, due to an increased attenuation of light in the water column (Thrane *et al.*, 2014).

However, in mesocosm experiments Faithfull *et al.* (2011) did not find a decline in pelagic primary productivity or biomass when reducing light conditions, but observed a shift in phytoplankton community. Higher relative abundances of mixotrophic phytoplankton were found in low light treatments, since they can overcome energy limitation by consuming bacteria (Faithfull *et al.*, 2011c). This indicates that phytoplankton communities to some extent can adapt to a reduced light climate. Nevertheless, most studies predict lower primary productivity as a consequence of low light conditions due to increases in water colour.

1.2.3 Effect of DOC on competition for inorganic nutrients

While most phytoplankton cannot use DOC as a nutrient source, DOC serves as substrate for bacterial growth (Fig. 1f). Tranvik (1988) found that bacterial biomass was positively correlated with DOC concentrations in oligotrophic lakes and concluded that bacteria can use DOC as a carbon source (Tranvik, 1988). Also other studies reveal that the productivity of bacterioplankton is largely determined by the amount of DOC in the water and consequently humic lakes can support higher bacterial abundances than clear-water lakes (Roiha *et al.*, 2012; Jones, 1992; Berggren *et al.*, 2009; Lennon and Pfaff, 2005).

Higher microbial productivity can have a strong impact on the competition with phytoplankton leading to a shift from an autotrophic towards a heterotrophic system. Since bacteria and phytoplankton both use inorganic nutrients, they are competing for the same limiting resource. The relief from carbon limitation by increased DOC concentrations makes bacteria more competitive and they can finally outcompete phytoplankton for inorganic nutrients (Stets and Cotner, 2008; Jones, 1992). Results from a whole-lake experiment conducted in an oligotrophic clear-water lake, show a significant increase in bacterial production and a decrease in phytoplankton production as a consequence of the addition of uncoloured DOC during two consecutive years. This shift from a lake dominated by autotrophic phytoplankton towards the dominance of heterotrophic production can be attributed to the utilization of DOC itself by bacteria, because possible effects of light were excluded by using uncoloured DOC (Blomqvist

et al., 2001b). Also a study investigating a humic lake during four consecutive years found a stimulation of bacterial production at the expense of primary production during times when DOC inflow was high (Drakare *et al.*, 2002). Stets and Cotner (2008) conducted an experiment where they added DOC to an oligotrophic and a eutrophic lake. DOC additions to the oligotrophic lake stimulated bacterial production and biomass-specific P uptake, while phytoplankton production decreased. This shows again that DOC increases the ability of bacteria to outcompete phytoplankton for inorganic nutrients. In contrast, in the eutrophic lake the DOC addition had a weaker effect on bacterial and phytoplankton production, because inorganic nutrients were not a limiting resource (Stets and Cotner, 2008).

However, results from mesocosm experiments showed that bacterial production was positively correlated with DOC, while phytoplankton production was unaffected by DOC addition, suggesting that bacteria do not outcompete phytoplankton (Faithfull *et al.*, 2011b). Also a meta-analysis with lakes from a wide range of trophic status and locations conclude that bacteria and phytoplankton do not compete for the same nutrients and thus bacteria concentration does not influence phytoplankton growth (Faithfull *et al.*, 2011a). This is supported by a study from Peura *et al.* (2014), where the addition of colourless cane sugar as a DOC source to a lake did not decrease primary productivity (Peura *et al.*, 2014).

1.2.4 Higher trophic levels

Changes in phytoplankton or bacteria abundance due to DOC can have effects on higher trophic levels (Fig. 1g). It is possible that the increase in terrestrial DOC acts as a subsidy promoting bacterial growth and stimulating the microbial loop. The microbial loop describes the trophic pathway, where DOC is incorporated into bacterial biomass and thus introduces an additional energy and carbon source to the food chain. This energy can be passed to higher trophic levels (bottom-up-effect), provided that consumers can effectively graze bacterial production (Jones, 1992). The analysis of stable carbon isotopes in a whole-lake experiment revealed that added DOC is transferred through the whole food web to consumers (Peura *et al.*, 2014). Studies estimate that the biomass of consumers in higher trophic levels consists of 20-80% of allochthonous carbon (Berggren *et al.*, 2010).

However, studies about the subsidizing effect of DOC in higher tropic levels are ambiguous. After the addition of DOC to a clear-water lake Blomqvist *et al.* (2011) found an increase in the biomass of bacteria and heterotrophic flagellates, but no effect on top-consumers, suggesting that C was not passed effectively through the food chain (Blomqvist *et al.*, 2001b). Also Cole *et al.* (2006) found that only very little of the allochthonous DOC metabolized by bacteria is actually transported into higher trophic levels (Cole *et al.*, 2006). Therefore, it remains unclear if secondary productivity increases as a consequence of higher bacterial abundance due to DOC.

Phytoplankton abundance is also influenced by top-down effects such as grazing (Fig. 1g). When grazing pressure by herbivores is high, phytoplankton abundances can be low despite high growth rates. In some lakes the effect of grazing can be as significant as nutrient limitation (Saunders *et al.*, 2000).

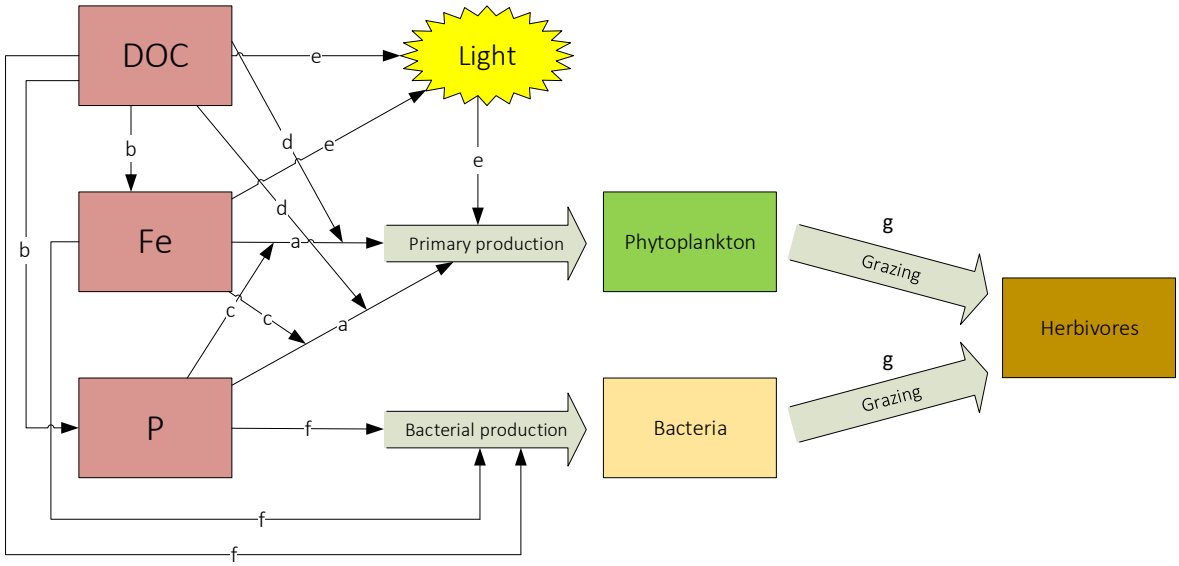


Figure 1: Different ways how DOC, Fe and P can affect primary productivity (PP). (a) Fe and P directly stimulate PP. (b) DOC carries associated P and Fe into lake. (c) P and Fe can precipitate with each other. (d) DOC affects P and Fe availability. (e) DOC and Fe decrease light penetration. (f) DOC subsidies give bacteria an advantage in competition with phytoplankton for P and Fe. (g) Herbivores can graze on phytoplankton and bacteria.

1.3 Lake Mälaren

Water from the mesotrophic Lake Mälaren was used for the experiments in this study. With a surface area of 1120 km^2, Lake Mälaren is the third largest lake in Sweden and its easternmost bay is located in central Stockholm (Fig. 2). It supplies drinking water to approximately 2 million people in the Stockholm area and therefore the water quality of the lake is of great importance for drinking water production. Moreover, the lake is an important resource for professional and recreational fishing. Regular monitoring of the lake has been going on for 50 years and has its origins in the 1960th, facing problems with excessive eutrophication. Despite a reduction of nutrient loading since the 1960th, the lake is still affected by eutrophication. The overall ecological status of Mälaren is moderate, but there are variations between the different parts of the lake. The lake can be considered as relatively shallow with an average depth of 12.8 m and a maximum depth of 64 m. It has a catchment area of 22600 km^2, representing about 5 % of Sweden. The catchment comprises forest, arable land and meadows as well as lakes. Substantial parts of the catchment area are located north and west of the lake (Fig. 2). Hence most of the catchment runoff enters the lake via rivers at the western site and some at the north-eastern site. The water generally drains from the west to the east and finally into the

Baltic Sea. The south-eastern part of Lake Mälaren is more nutrient-poor than other parts of Mälaren (Sonesten *et al.*, 2013).

In the western parts of Lake Mälaren the water colour is approximately three times higher than in the eastern parts. The high water colour in the western basin is caused by the high proportion of peatlands and coniferous forests in the drainage area, transporting terrestrial DOC and iron into the lake. While water colour in the western basin increased during the last 40 years due to increased terrestrial inputs of coloured substances (Fig. 3-4), the water colour in the eastern basin changed a lot less, indicating that the colour is lost while the water travels from west to east. The loss in water colour with increasing retention time is due to a loss of iron, DOC and a shift from coloured terrestrial DOC towards less coloured, autochthonous DOC (Köhler *et al.*, 2013). Iron disappears on the way from the western to the eastern parts of the lake because of flocculation and sedimentation (Weyhenmeyer *et al.*, 2014; Köhler *et al.*, 2013). DOC disappears because of photooxidation, bacterial degradation to CO_2 and flocculation followed by sedimentation. The DOC quality changes with water retention time, since allochthonous DOC is more easily flocculated and is thus selectively removed (Wachenfeldt and Tranvik, 2008). At the same time new autochthonous carbon is produced by phytoplankton, while the water travels through the lake. Since at the eastern side of Lake Mälaren, the light conditions are better due to lower water colour, more photosynthesis can take place and hence more autochthonous DOC can be produced. As a result of these processes, the water from the western basin is richer in nutrients, has a darker water colour and a higher share of allochthonous DOC. Water from the eastern basin is poorer in nutrients, has a lower water colour and a higher share of autochthonous DOC.

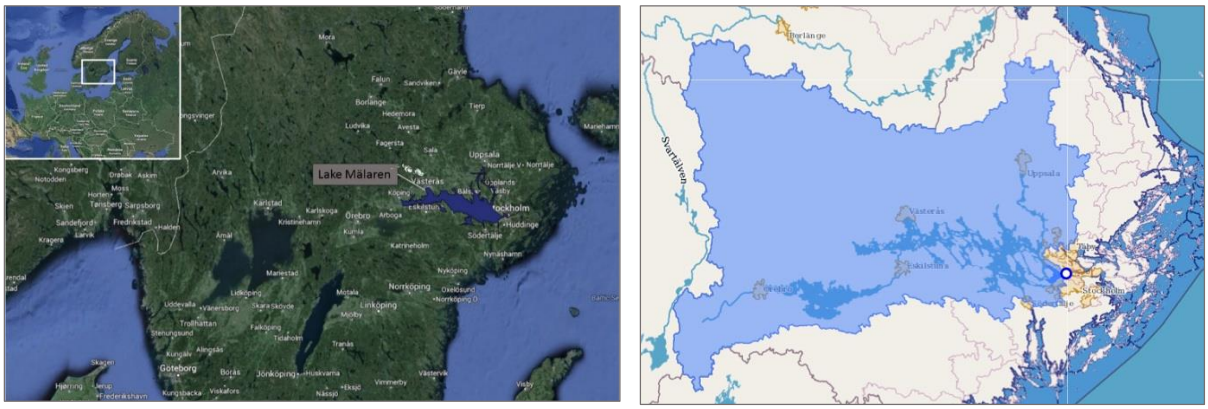


Figure 2: Geographical location (left) and catchment area (right) of Lake Mälaren. Source (left picture): https://www.google.de/maps/ Source (right picture): http://vattenwebb.smhi.se/

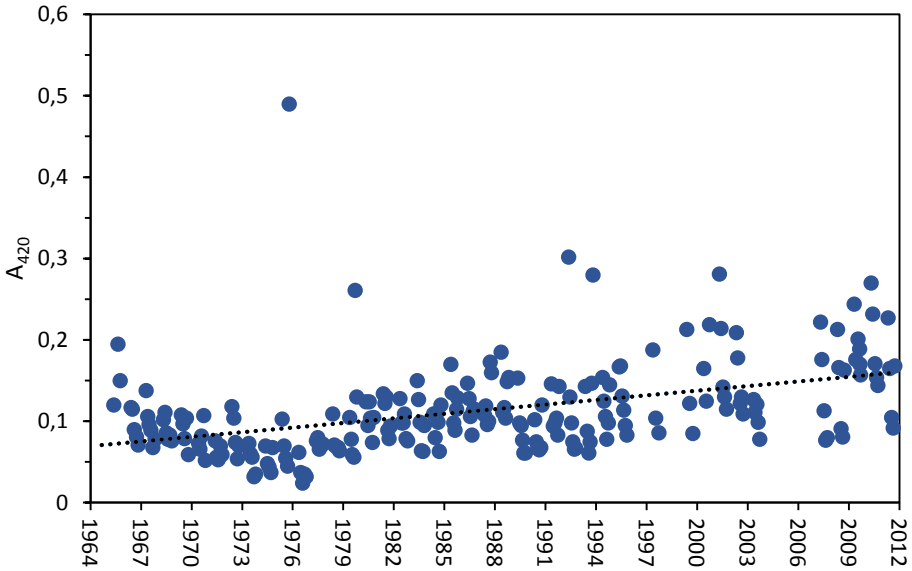


Figure 3: Water colour measured as absorbance at 420 nm in the western basin of Lake Mälaren (Galten). The water colour is significantly increasing by 0.00178 absorbance units per year (Spearman´s rank correlation, ρ=0.504, p>0.0001). Data source: Database for Swedish monitoring of lakes and watercourses.

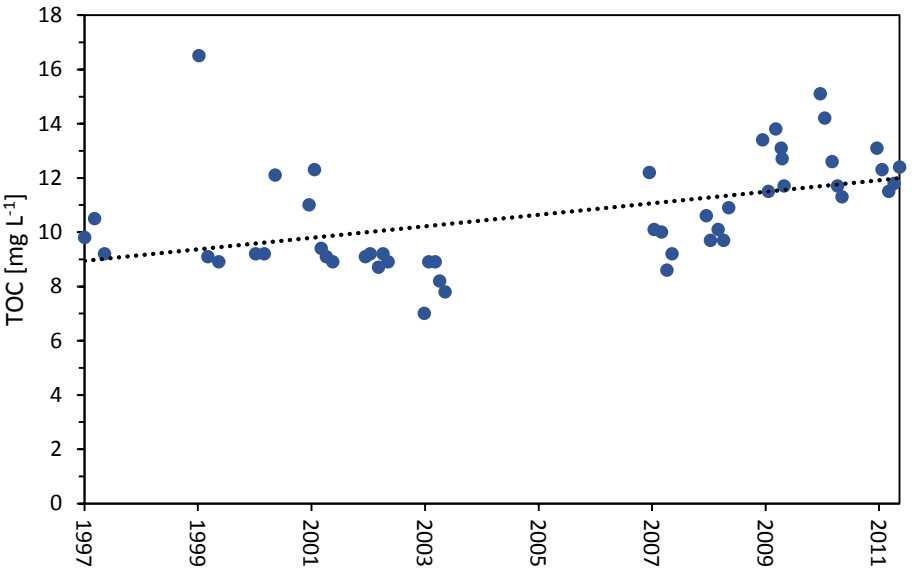


Figure 4: Total organic carbon concentration in the western basin of Lake Mälaren (Galten). TOC concentrations are significantly increasing by 0.214 mg L^{-1} y^{-1} (Spearman´s rank correlation, ρ=0.516, p>0.0001). Data source: Database for Swedish monitoring of lakes and watercourses.

1.4 Research questions

The main aim of this study is to investigate the effect of DOC on primary productivity under different P and Fe availability regimes. Considering the increased input of terrestrial organic carbon due to climate change this topic is of great environmental importance, especially for eutrophication. The effects of DOC on primary productivity due to light attenuation and competition by bacteria are rather well studied, while there is a lack of knowledge on how DOC interacts with nutrients, how DOC affects the availability of nutrients and which role the chemical composition of DOC plays. To focus on the interactions between DOC and nutrients, microcosm incubation experiments with natural phytoplankton communities were performed and effects of light and grazing were excluded. Lake Mälaren was chosen as a study site, because the future development of primary productivity is of great importance for the whole Stockholm area, since the lake serves as a drinking water reservoir. My main research questions were:

(1) How will changed DOC concentrations effect phytoplankton growth under different nutrient availability regimes?
(2) How do DOC, P and Fe interact to regulate primary productivity?
(3) How will an increase in DOC affect the supply of P and Fe to phytoplankton?
(4) Which effect does DOC quality has on nutrient availability and phytoplankton growth?
(5) Which factors constrain primary productivity in Lake Mälaren?

2 Material and Methods

To study the effect of DOC, P and Fe on planktonic primary productivity, microcosm incubation experiments with natural phytoplankton communities were conducted under controlled climatic conditions. The experiments were run with different DOC, Fe and P concentrations as well as water of different DOC qualities. In addition to main effects of DOC, P and Fe, interactions between DOC, Fe and P were expected. Interactions could be ascertained by combining the varied factors in all possible ways in a full factorial design. To measure the effects of the different treatments on phytoplankton, the specific growth rates were estimated from initial and final chlorophyll *a* measurements. Moreover, chemical conditions were measured at the beginning and at the end of the experiment to characterize the initial and final water and detect possible changes during the incubation period. For the determination of the optimal incubation length for the main experiments, a time-series experiment (pre-experiment) was conducted.

2.1 Experimental design: Pre-Experiment

To determine the optimal incubation length of the main experiments as well as the variation among replicates and the variation over time, a time-series experiment was conducted. This pre-experiment included a Fe, N and P addition treatment (FeNP) and a control treatment without nutrient addition (control). These treatments were chosen to observe the highest and lowest expected response in phytoplankton growth with a natural community from the northeast part of Lake Mälaren, Ekoln (Fig. 7). Both treatments were replicated 10 times. The experiment running for 11 days and chlorophyll samples were taken on days 0, 3, 5, 7, 9 and 11. The conditions during incubation and methods for chlorophyll *a* analysis are described in the sections 2.4 and 2.5.1. The time-series experiment showed that chlorophyll *a* concentration in the FeNP treatment reached its maximum on day 9. The increase in chlorophyll *a* was highest between day 3 and 5 and stagnated after day 7, suggesting that nutrients were depleted (Fig. 5). The variation between replicates of the FeNP treatment was highest on day 5 and decreased until day 11. In the control treatments chlorophyll *a* concentrations stayed rather constant for 1 week. From day 7 till 11 chlorophyll *a* increased, suggesting that processes are happening in the microcosm that may not mimic the natural situation and are just caused by long incubations in small volumes. For the main experiments a duration of 7.5 days was chosen, since it is long enough to ensure a strong response in chlorophyll *a*, but short enough to minimize the likelihood that the added nutrients become limiting. Moreover the short incubation time decreases the risk that the result are experimental artefacts caused by long incubation in small volumes and that other nutrients become depleted (Downing *et al.*, 1999; Frost *et al.*, 1988). For the main experiment 5 replicates per treatment were used. This number of replicates was chosen to have enough replicates to reduce the influence of outliers on statistical analysis. It was also the maximum number of bottles that could be fitted in the climate chamber.

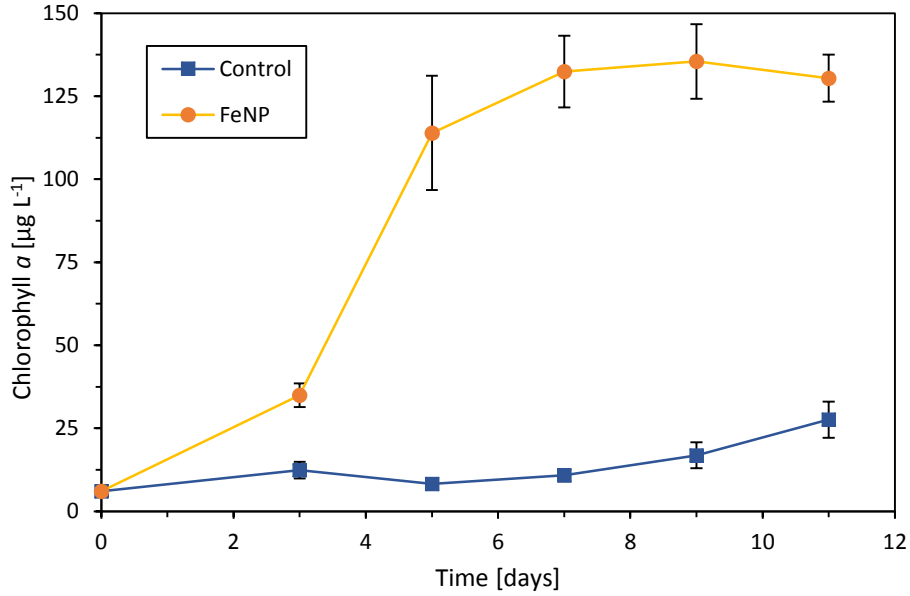


Figure 5: Development of chlorophyll *a* in the treatment with addition of Fe, N and P (FeNP) and the treatment without addition of nutrients (Control) during 11 days. The error bars show the standard deviation. N=10.

2.2 Experimental design: Main Experiments

The effects of DOC, P and Fe on phytoplankton were studied in incubation experiments, running for 7.5 days. A full factorial design was chosen, to be able to assess interactions between DOC, P and Fe (Fig. 6). The experiment consisted of two different Fe and P concentration levels and three DOC concentration levels, resulting in 12 different treatments. N was added to all treatments ensuring that N is not depleted during the experiment. To control that N does not act as a limiting nutrient, a treatment without the addition of N was established ($DOC_{ambient}$ without N). Furthermore, a treatment without algae inoculum served as control to observe the effects of phytoplankton on chemical parameters ($DOC_{ambient}$ without algae, light). To monitor whether light has a photodegrading effect on DOC, another control treatment without algae was included ($DOC_{ambient}$ without algae, dark), which was kept in the dark during the whole experiment. Each treatment was replicated five times. The experiment was conducted two times with exactly the same experimental setup, but with water from different areas of Lake Mälaren. The chosen sites differed in DOC quality, allowing to study the effects of DOC character on phytoplankton growth. For one experiment (experiment west) the water was taken from the western part, while the water for the other experiment originated from the eastern part of Lake Mälaren (experiment east). The DOC on the western site has a more terrestrial character, while the DOC from the eastern basin contains a higher proportion of in-lake produced DOC.

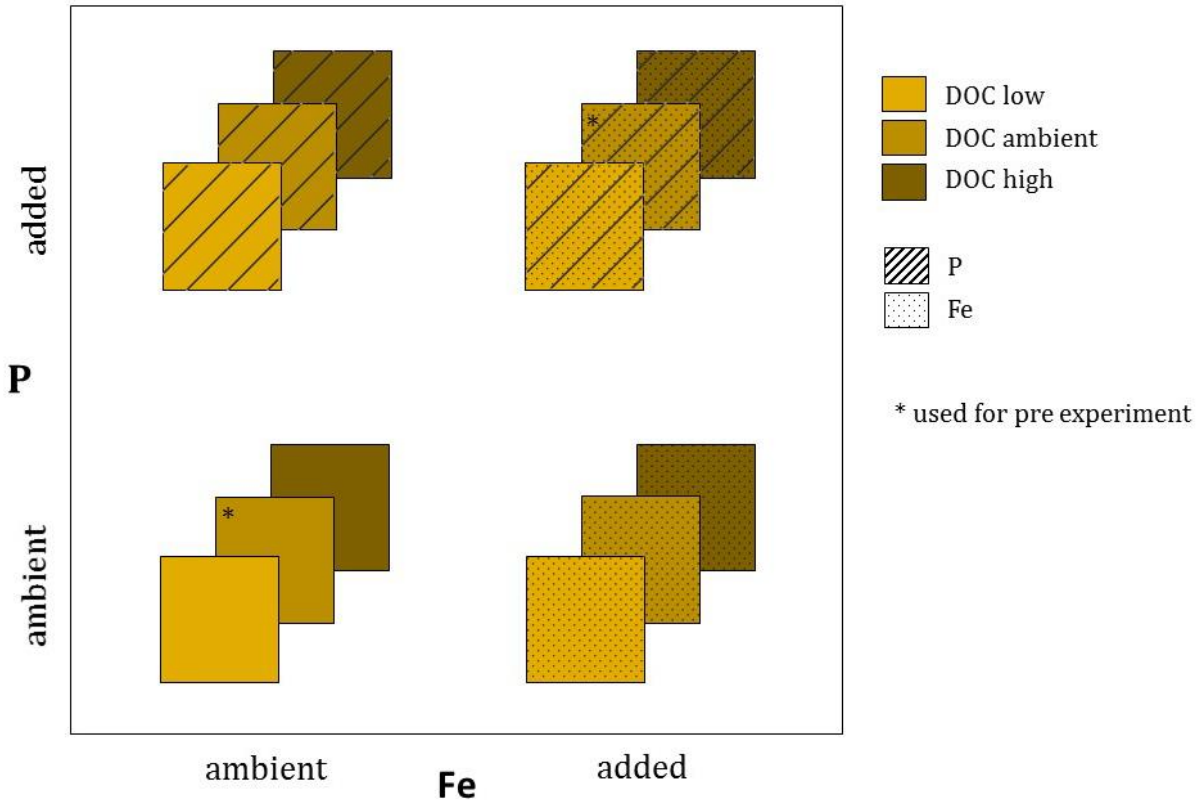


Figure 6: Full factorial experimental design with two levels of phosphorus (P) and iron (Fe) and three levels of dissolved organic carbon (DOC), resulting in 12 treatments in total. N=5 for all treatments.

2.3 Water sampling

To see the effects of contrasting DOC qualities, the experiments for this study were conducted with water from the western basin (Galten) and with water from the eastern basin (Görväln) (Fig. 7, Fig. 1 appendix). Compared to water from the eastern site, water from the western site is richer in nutrients, has a higher share of allochthonous DOC and thus a darker water colour. The water for the pre-experiment was taken from the north-eastern part (Ekoln). For the pre-experiment water sampling took place on the 28th of April 2014. The water was collected below the surface and the water temperature was 8.0 °C. For the main incubation experiments water was sampled from a boat on 9th June 2014 for experiment west and on 19th May 2014 for experiment east. The water was collected from 1 m depth with a Ruttner sampler and subsequently decanted into 25 l and 10 l polyethylene containers. The water temperature was 13.0 °C and 11.4 °C, respectively.

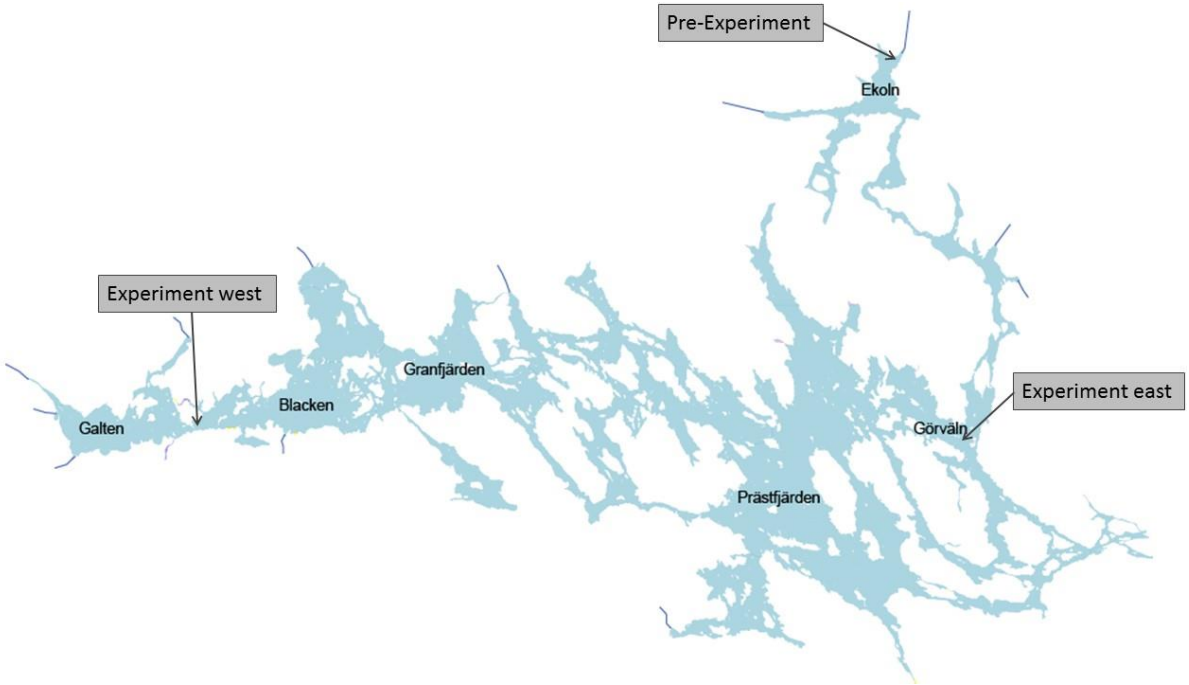


Figure 7: Sampling locations in Lake Mälaren for the pre-experiment, the experiment with water from the western basin (Experiment west) and the experiment with water from the eastern basin (Experiment east).

2.4 Cultivating conditions

The experiment was conducted using optically clear polystyrene BD Falcon Tissue Culture Flasks with a volume of 250 mL and a surface area of 75 cm^2. Since the water for creating different DOC concentrations has to be particle free, I decided to combine unfiltered and filtered water. The unfiltered water contained natural algae communities, while the filtered water was algae-free and could therefore be further processed to construct treatments with higher and lower DOC concentrations than the ambient one. Hence, each bottle was filled with 50 mL of unfiltered and 200 mL filtered lake water (Fig. 8). In contrast $DOC_{ambient}$ treatments without algae were solely filled with 250 mL filtered water. The filtered water was filtered through a glassmicrofiber filter (Whatman GF/F, 47 mm) to remove phytoplankton and other particles. To avoid contamination with substances originating from the GF/F filters, the filters were rinsed with 50 mL MilliQ water prior to sample filtration. The unfiltered water was passed through a net with 0.24 mm mesh size to remove macrozooplankton and thus minimize predation. Throughout the whole experiment, all equipment was rinsed with MilliQ water before usage to avoid contamination.

To create water containing higher or lower DOC concentrations than the ambient DOC concentration in the lake, nanofiltration was used. Nanofiltration is a pressure-driven filtration method, where a membrane with a pore size in the range of a few nanometers removes particles that cannot pass the membrane, such as natural organic matter (Hilal *et al.*, 2004). The nanofiltration process produces water that is concentrated in DOC, the retentate, and water that

is depleted in DOC, the permeate (Fig. 9). In my experiment GF/F filtered lake water was treated using the nanofiltration membrane T/RX-300 with a pore size of 500 Dalton. The retentate was used to create treatments with high DOC concentrations, while the permeate was used for treatments with low DOC concentrations. The lowest DOC concentration created for the experiments was 3.7±0.04 mg L^{-1} (mean±SD) and the highest 15.7±0.4 mg L^{-1}. Since these concentrations are within the natural range of Lake Mälaren, they can be considered as being realistic upper and lower experimental ranges (Sonesten *et al.*, 2013).

The nutrients P and Fe were added to the treatments in a full factorial design. N was added to all treatments except to the control treatment without N addition. P was added as K_2HPO_4, Fe as $FeCl_3$ and N as $NaNO_3$. The concentration of nutrients added was 50 µg P L^{-1}, 400 µg Fe L^{-1} and 449 µg N L^{-1}. These amounts were chosen to approximately double the Fe and P concentration compared to ambient concentrations at the experimental sites. Doubling the mean P and Fe values up to concentrations of 100 µg P L^{-1} and 800 µg Fe L^{-1} is within the natural variation observed in Lake Mälaren (Sonesten *et al.*, 2013). The amount of added N was selected to keep a constant 16:1 molar N:P ratio in the P addition treatments.

Two days after the collection of lake water, the experiments were started and they were running for 7.5 days. The experiment with water from the western basin lasted from 11th - 19th June 2014, the experiment with water from the eastern basin from 21th - 29th May 2014. The bottles were incubated in a climate chamber under stable conditions with a diurnal light cycle of 18 h light and 6 h dark (Fig. 8). Temperature was constantly monitored with a Tinytag Aquatic 2 Temperature Logger TG-4100 (Gemini Data Loggers, West Sussex, England) and was on average 15.7±1.0 °C (mean±SD). The climate chamber was equipped with broad spectrum day-light fluorescent tubes Grolux F36W/GRO-T8 (Sylvania, Erlangen, Germany) yielding a light intensity between 60 and 119 µE m^{-2} s^{-1}, depending on the position of the flask under the lamps. To ensure that all bottles got the same amount of light on average during the experiment, the light intensity at each position under the fluorescent tubes was determined with a light sensor QSL2101 (Biospherical Instruments, San Diego, USA). Based on these light measurements a rotation scheme with randomized starting positions was developed, where bottles were rotated every day resulting in an average light intensity of 88±1 µE m^{-2} s^{-1} for each bottle. Rotating bottles not only ensured the same average light intensity per bottle, but also reduced possible border effects. By putting the bottles in a flat position, the surface area for light absorption was maximized and the light extinction within the bottle was minimized. Therefore, I assume the water column was shallow enough to avoid an effect of DOC and Fe on the light regime that is strong enough to significantly affect phytoplankton growth.

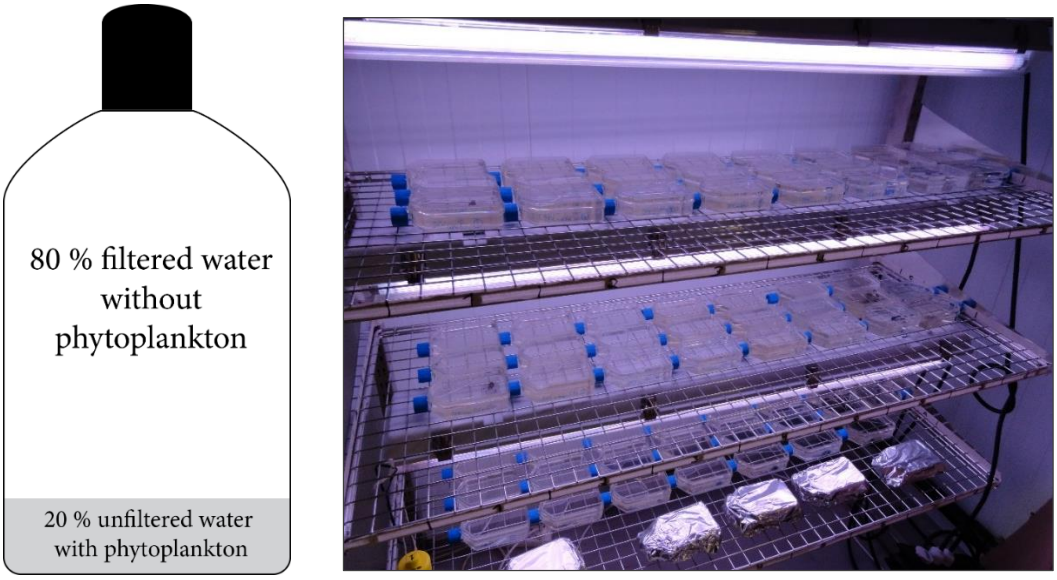
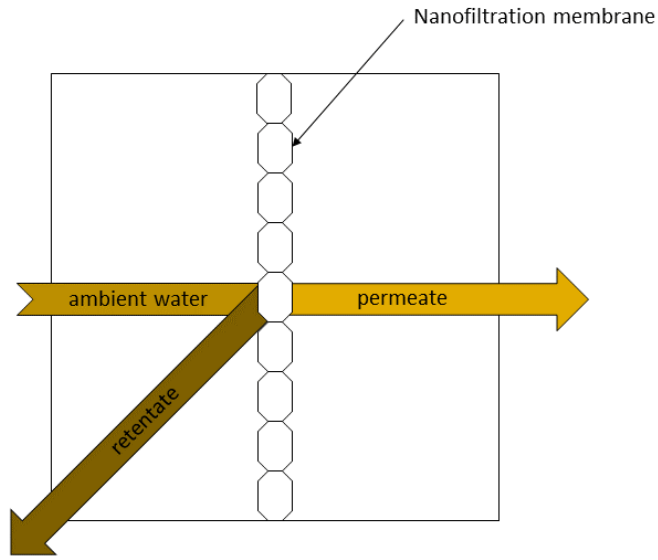


Figure 8: Bottles for treatments filled with 200 ml filtered water (without phytoplankton) and 50 ml unfiltered water (with phytoplankton) (left). Incubation was conducted in a climate chamber, equipped with fluorescent tubes and temperature sensors (right). Bottles incubated in the dark were covered with aluminium foil.



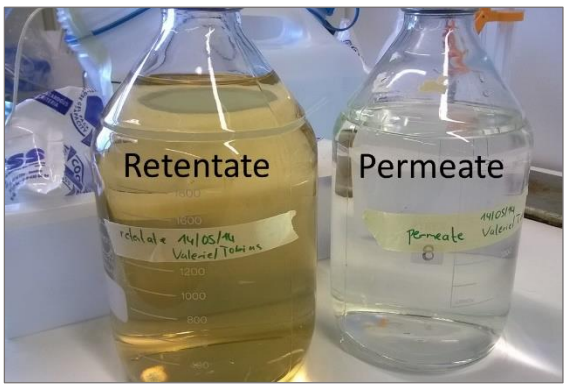


Figure 9: Nanofiltration. Filtering ambient water through the nanofiltration membrane creates water that is concentrated in DOC (retentate) and water that is depleted in DOC (permeate). Molecules that cannot pass the membrane stay in the retentate, while molecules that can pass the membrane end up in the permeate.

2.5 Biological and chemical analysis

To characterize the initial biological and chemical conditions, chlorophyll *a*, absorbance, pH, freshness index (ß:α), fluorescence index (FI), humification index (HIX) as well as DOC, total phosphorus, phosphate and total reactive iron (TRFe) concentration were measured at the beginning of the experiment. After the incubation, all replicates of all treatments were analysed for chlorophyll *a*, absorbance, DOC and TRFe. For one replicate per treatment pH, ß:α, FI and HIX were determined after the incubation at the end of the experiment. The samples for chemical analysis, except the ones for total phosphorus and phosphate, were stored at 5 °C for a maximum of one week before analysis. The samples for total phosphorus and phosphate were kept at -18 °C and were analysed within two weeks.

2.5.1 Phytoplankton growth

Phytoplankton growth was determined by measuring the chlorophyll *a* concentration in the water. For that purpose a defined sample volume (50 mL for pre-experiment, 100 mL for main experiment) was taken from each incubation bottle, filtered through a GF/F filter (25 mm in pre-experiment, 47 mm in main experiment) and stored at -18 °C until analysis. After extracting chlorophyll *a* with a defined volume 90 % acetone (5 mL in the pre-experiment, 10 mL in main experiment) (Merck, Darmstadt, Germany) for 24 h, it was measured fluorometrically with a TD-700 Fluorometer (Turner Designs, Sunnyvale, USA). By comparing the fluorescence measurements of the samples with the fluorescence measurement of a standard with known chlorophyll *a* concentration, the chlorophyll *a* concentrations of the samples were calculated. The specific growth rate of phytoplankton (μ [d^{-1}]) was calculated from the chlorophyll *a* concentration measured in the beginning ($Chl_{Initial}$) and in the end (Chl_{Final}) of the experiment:

$$\mu = \frac{\ln(Chl_{Final}) - \ln(Chl_{Initial})}{t},$$

where t is the duration of the experiment.

2.5.2 Total reactive iron

The concentration of total reactive iron (TRFe) was measured colourimetrically, using a modification of the TPTZ (2,4,6-Tris(2-pyridyl)-1,3,5-triazine) method described by Verschoor and Molot (2013). The main modifications were changes in the amount of added reagents (Fig. 2 appendix) and the time span between addition of the reductant and measurement of TRFe in the photometer (Fig. 3 appendix). Total reactive iron is defined as the "total amount of ferrous iron and reducible ferric iron that react with the reagents to form the chromogenic compound" (Verschoor and Molot, 2013). I assume that TRFe is the part of total Fe that is available to phytoplankton. To measure TRFe, 15 mL filtered sample water was mixed with

1 mL 10 % ascorbic acid solution (reductant) and incubated at room temperature for 21 h. The initial samples for experiment west were additionally measured after 1 h. After adding 1 mL sodium acetate buffer and 150 µl TPTZ reagent, absorbance at 595 nm was read immediately with an optical device photometer AvaLight-DHS-BAL (Avantes, Apeldoorn, Netherlands) in a 5 cm quartz cuvette. The photometer was zeroed with MilliQ water. The results from the absorbance measurement were calibrated against iron standards (0, 50, 100, 200, 300, 400, 600, 800 and 1200 µg L^{-1}), prepared from a 10 µg Fe mL^{-1} inorganic custom standard (Ultra Scientific, North Kingstown, USA) (Fig. 10).

The 10 % ascorbic acid reductant was produced by dissolving 25 g L(+)-ascorbic acid (AnalaR Normapur, VWR International BVBA, Leuven, Begium) in 250 mL MilliQ water. For the sodium acetate buffer 136 g sodium acetate anhydrous (Merck, Darmstadt, Germany) and 60 mL glacial acetic acid anhydrous (Merck, Darmstadt, Germany) were dissolved in 500 mL MilliQ water. To prepare the TPTZ reagent, 50 mL MilliQ water was mixed with 4 mL Suprapur hydrochloric acid 30 % (Merck, Darmstadt, Germany) before adding 75 mg TPTZ (CAS-No: 3682-35-7, Sigma-Alderich, Darmstadt, Germany). After dissolving TPTZ, the solution was bought up to a final volume of 250 mL.

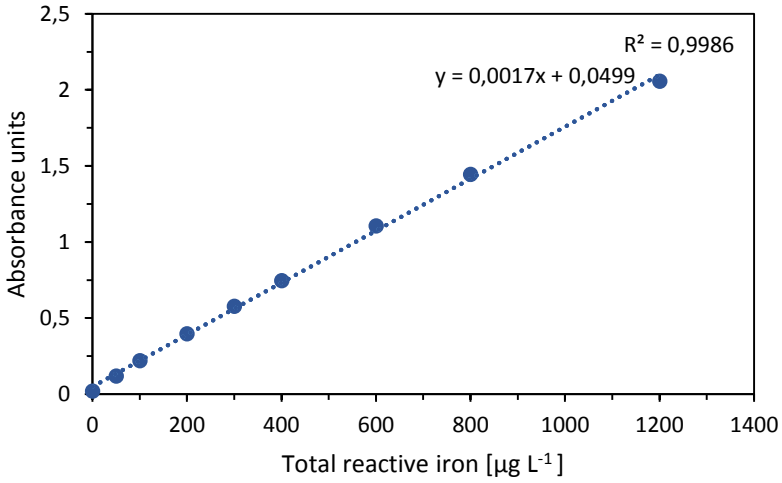


Figure 10: Calibration curve with total reactive iron standard concentrations of 0, 50, 100, 200, 300, 400, 600, 800 and 1200 µg L^{-1}.

2.5.3 Total phosphorus and phosphate

The concentration of phosphate was measured on unfiltered samples with a discrete photometric Analyzer Gallery Plus (Thermo Scientific, Waltham, USA), using the molybdate-blue method (Murphy and Riley, 1962). For the measurement of total phosphorus, unfiltered samples were oxidized with 5 % potassium persulphate for 60 minutes in an autoclave before they were analysed by the molybdate-blue method on a Technicon Autoanalyzer 3 (Bran & Leubbe, Norderstedt, Germany). The analysis was conducted by the Geochemical laboratory of the Department of Aquatic Sciences and Assessment, accredited by Swedac ISO/IEC17025.

2.5.4 Dissolved organic carbon

For the determination of DOC concentrations, GF/F filtered samples were measured on a Total Carbon Analyser TOC-V_{CPH} (Shimadzu, Kyoto, Japan) by combusting DOC at 680 °C in an oxygen rich environment and subsequent detection of CO_2 using a non-dispersive infrared sensor. Prior to analysis samples were acidified with 2 M hydrochloric acid (Bernd Kraft, Duisburg, Germany). The results from the DOC measurement were calibrated against potassium hydrogen phthalate standards (0, 2, 5, 10 and 20 mg C L^{-1}) and the instrument performance was controlled by an EDTA standard (10 mg C L^{-1}).

2.5.5 Absorbance, absorbance ratio and DOC specific absorbance

From GF/F filtered samples the absorbance at wavelengths ranging from 181 nm to 1100 nm was measured with an optical device photometer AvaLight-DHS-BAL (Avantes, Apeldoorn, Netherlands) in a 5 cm quartz cuvette. The instrument was zeroed with MilliQ water and absorbance data were analysed at the wavelength 254 nm (A_{254}), 365 nm (A365) and 420 nm (A420). The absorbance intensities at a particular wavelength were expressed in m^{-1}. Also the absorbance ratio at the wavelengths 254 nm and 365 nm (A_{254}/A_{365}) was calculated. A high absorbance ratio indicates low molecular weight of DOC (Ågren *et al.*, 2008). Compared to the more complex high molecular weight fractions, low molecular weight fractions are considered to be better substrates for bacteria (Tranvik and Jørgensen, 1995). A_{254} and A_{420} are measures for water colour, which is affected by DOC and colloidal Fe. While A_{420} is much more strongly controlled by colloidal Fe, A_{254} is mainly driven by DOC (Köhler *et al.*, 2013).

Based on the DOC and absorbance spectra measurements, specific absorbances per unit mass of organic carbon were calculated to characterize DOC. Specific metrics calculated were the specific UV absorbance at the wavelength 254 nm ($SUVA_{254}$), the specific visible absorbance at 420 nm (SVA_{420}) and the specific visible absorbance at 335 nm (SVA_{335}).

The specific absorbances [L mg C^{-1} m^{-1}] were calculated:

$$specific\ absorbance\ = \frac{A/_d}{DOC} \cdot 100,$$

where A is the measured absorbance of the sample at the specific wavelength (254 nm for $SUVA_{254}$, 420 nm for SVA_{420} and 335 nm for SVA_{335}), d is the path length of the cuvette [cm] and DOC the DOC concentration [mg L^{-1}].

$SUVA_{254}$ was used as an indicator for the light–absorbing properties of DOC, since it is strongly correlated to aromaticity and aromatic rings are among the most important light-absorbing functional groups of DOC. A high $SUVA_{254}$ value indicates high aromaticity and thus a large light absorption (Erlandsson *et al.*, 2012; Ågren *et al.*, 2008).

2.5.6 Freshness index (ß:α), fluorescence index (FI) and humification index (HIX)

For the characterization of DOC quality, three-dimensional excitation-emission matrix (3DEEM) fluorescence spectra of filtered samples were recorded on an Aqualog instrument (Horiba Scientific, Kyoto, Japan). The emission intensity (EmI) from the fluorescence spectrometry data were used to estimate the DOC age, origin and degree of humification by calculating freshness index (ß:α), fluorescence index (FI) and humification index (HIX) (E. Lavonen, unpublished):

$$ß{:}\,\alpha = \frac{EmI_{380\ nm}}{MaxEmI_{420\ to\ 435\ nm}}\ for\ Ex_{\lambda} = 310\ nm$$

$$FI = \frac{EmI_{470\ nm}}{EmI_{520\ nm}}\ for\ Ex_{\lambda} = 370\ nm$$

$$HIX = \frac{\sum EmI_{435\ to\ 480\ nm}}{\sum EmI_{300\ to\ 345\ nm} + \sum EmI_{435\ to\ 480\ nm}}\ for\ Ex_{\lambda} = 254\ nm$$

where Ex_{λ} is the excitation at a specific wavelength.

The freshness index is related to the age of DOC and can be used as an indicator of the contribution of recently produced DOC. The ß component represents freshly derived DOC and is thus associated with high biological activity, such as the fast growth of algae. In contrast α represents older, more decomposed DOC. Hence, a high ß:α value denotes a high proportion of DOC with recent biological origin (Parlanti *et al.*, 2000).

The fluorescence index is connected to the source of DOC and allows distinguishing between the relative contributions of allochthonous versus autochthonous DOC. A high share of microbially derived fulvic acids is indicated by a high FI (~1.8). In contrast, a low FI (~1.2) designates a high contribution of terrestrially derived fulvic acids to the DOC pool (Cory and McKnight, 2005; McKnight *et al.*, 2001).

The extent of humification can be determined by the humification index, ranging from 0 to 1. As material becomes more humified, molecules become more condensed due to an increase in functional group content. This results in an increase in HIX with increasing degree of humification (Ohno, 2002; Zsolnay *et al.*, 1999).

2.5.7 pH

The measurement of pH value was conducted with an Orion 3 Star pH Portable (Thermo, Waltham, USA) on filtered water, which was constantly stirred during analysis. The reading of the pH value was taken when the meter came to equilibrium (within 10 minutes).

2.6 Statistics

Specific phytoplankton growth rates were analysed with full factorial ANOVA (experiments with water from eastern and western basin separately, and both experiments together), while chemical conditions were investigated with a principal components on correlation analysis (PCA) as well as independent paired t-tests. All analyses were performed using JMP version 10.0.

The full factorial ANOVA was employed to assess the effects of P and Fe addition and different DOC concentrations and all possible interactions between these factors on specific growth rate of phytoplankton, in each experiment separately. Moreover, a full factorial ANOVA excluding P addition treatments was conducted to detect effects of Fe and DOC that were otherwise shadowed by the effect of adding P. An ANOVA on both experiments together included the experiment as an additional factor and could explain the effect of the identity of the water (experiment) on phytoplankton growth. Since the residuals for specific growth rate meet the requirement of a normal distribution, no further transformation was required (Fig. 4 appendix).

The PCA was conducted with all available initial chemical parameters (except pH since it varied only marginally among treatments) to investigate the factors that caused variations in the chemical data and characterize chemical conditions at the beginning of the experiment. Moreover, a PCA on final chemical parameters was used to investigate differences in chemical parameters of all treatments in the end of the experiment.

PO_4 values that were below the detection limit of <1 were converted to 0.5 to be able to use them for statistical analysis.

To assess the effect of nanofiltration on DOC quantity, DOC quality, TRFe, total P and PO_4 concentration, paired two-sided t-tests on initial chemical conditions were performed (taking DOC_{low} and DOC_{high} treatments of the same nutrient concentration as pairs). Moreover, paired t-tests were used to investigate if the presence of P has an influence on initial TRFe concentrations (pairing PFe and Fe treatments, or P and no nutrient addition treatments, respectively) and vice versa (pairing PFe and P treatments, or Fe and no nutrient addition treatments, respectively).

To check whether a photodegradation effect of DOC is occurring, independent t-tests comparing final DOC quantity and quality data among treatments without algae kept in light and treatments without algae kept in dark were conducted. Furthermore, independent t-tests

comparing phytoplankton growth rates of treatments with N addition and treatments without N addition were performed to see if N acts as a limiting nutrient in the experiments. Before performing independent t-tests, the Welch´s test was used to check weather variances were equal. Since variances were equal in all cases, all independent t-tests were performed assuming equal variances.

The significance level for t-tests was set to 0.05. For the ANOVA results a significance level of 0.01 was chosen to avoid significances, which derive from the high number of replicates, but are biologically not important.

3 Results

3.1 Effects of DOC, P and Fe on phytoplankton growth

Chlorophyll *a* concentrations ranged from 3.8 to 80.3 µg L^{-1} in the experiment with water from the western basin and from 2.1 to 26.0 µg L^{-1} in the experiment with water from the eastern basin (Fig. 11). PFe treatments with ambient DOC concentrations resulted in the highest chlorophyll *a* concentrations, while in Fe treatments with low DOC concentrations the lowest chlorophyll *a* concentrations were measured. In both experiments, nutrient additions and alterations of DOC concentration significantly affected phytoplankton specific growth rates and can explain 99% of the variability (full factorial ANOVA, experiment west: $r^2_{adj.}=0.994$, $F_{11,48}=678$, $p<0.0001$; experiment east: $r^2_{adj.}=0.989$, $F_{11,48}=380$, $p<0.0001$) (Table 1-2). In both experiments P addition was the main source of variation (experiment west: 95.2 %; experiment east: 96.0 %) and had a strong positive effect on phytoplankton growth (parameter estimates see Table 1 appendix). DOC concentration also had a significant effect on growth rates (experiment west: 2.7 %; experiment east: 0.3 %). In both experiments, low DOC concentrations resulted in lower growth rates. In the experiment with water from the western basin high DOC concentrations had the strongest positive effect on phytoplankton growth, while in the eastern basin ambient DOC concentrations resulted in the highest growth rates. The addition of Fe alone was not significant, however, interaction effects between Fe and P significantly stimulated specific growth rates in both trials (experiment west: 0.5 %; experiment east: 0.7 %) (Fig. 12). Moreover, interaction effects between P and DOC explained variations in specific growth rates (experiment west: 0.7 %; experiment east: 1.5 %). In P addition treatments high DOC concentrations had a significantly negative effect, while low DOC concentrations a positive effect.

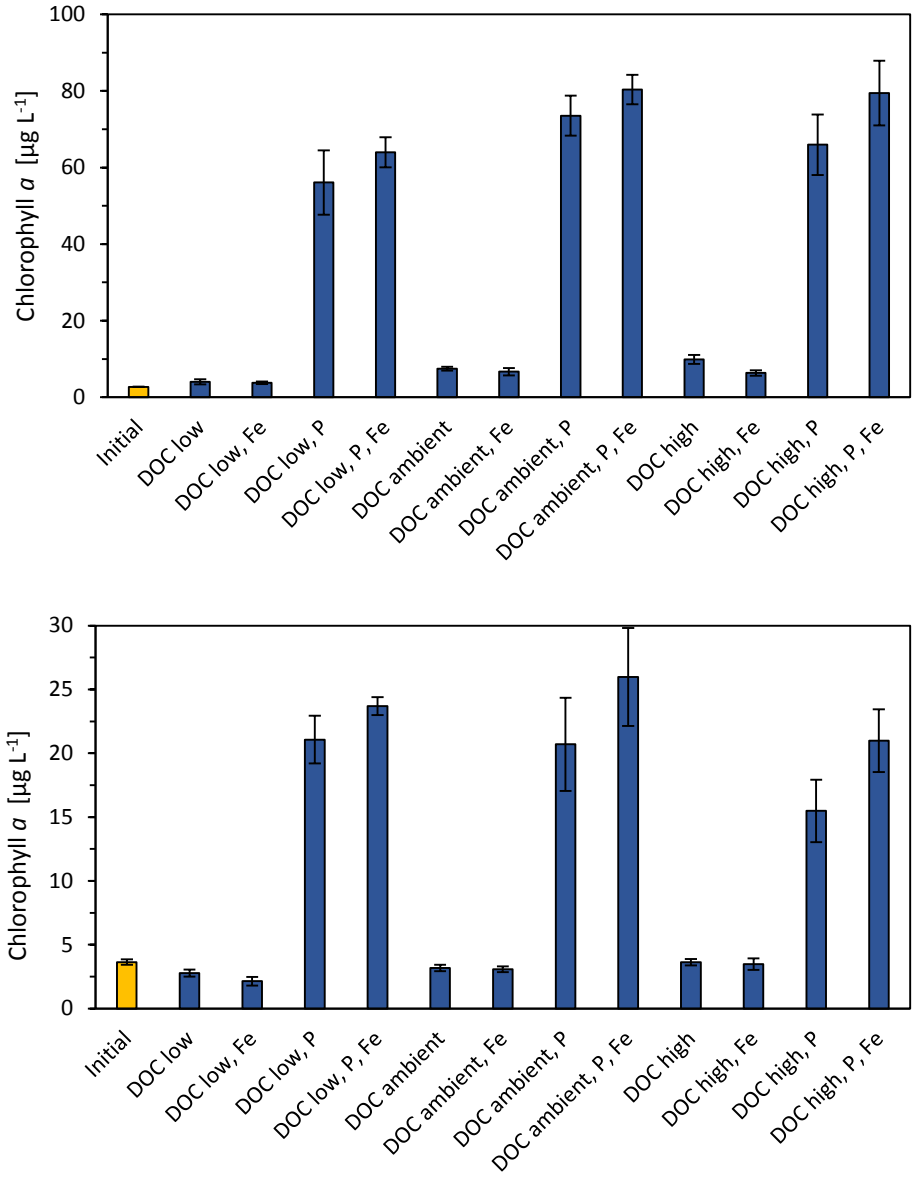


Figure 11: Initial (yellow) and final (blue) chlorophyll a concentrations from experiment with water from the western part (top) and eastern part (bottom) of Lake Mälaren. Error bars show the standard deviation. N=12 for initial and N=5 for final

Table 1: Results from full factorial ANOVA for specific phytoplankton growth rate in experiment with water from the western basin. "+" indicates that the nutrient significantly increased specific growth rate, while "–" indicates a significant decrease. For the DOC concentrations ">" indicates that growth rates were significantly different from each other, while a comma indicates that there was no significant difference. A significant difference was assumed when confidence intervals, calculated by 2•standard error, were not overlapping.

Source of variation	DF	SS	F	p	Effect direction
Model	11	1.66389	677.5	<0.0001	
Error	48	0.01072			
Effect					
Fe	1	0.00031	1.4	0.2468	
P	1	1.59397	7139.6	<0.0001	+
Fe*P	1	0.00800	35.8	<0.0001	+
DOC	2	0.04535	101.6	<0.0001	H,A>L
Fe*DOC	2	0.00123	2.8	0.0735	
P*DOC	2	0.01242	27.8	<0.0001	L>A>H
Fe*P*DOC	2	0.00261	5.8	0.0053	

DF: degrees of freedom, SS: sum of squares, MS: mean square, F: F ratio,
p: probability that $F>F_{crit.}$

Table 2: Results from full factorial ANOVA for specific phytoplankton growth rate in experiment with water from the eastern basin. "+" indicates that the nutrient significantly increased specific growth rate, while "–" indicates a significant decrease. For the DOC concentrations ">" indicates that growth rates were significantly different from each other, while a comma indicates that there was no significant difference. A significant difference was assumed when confidence intervals, calculated by 2•standard error, were not overlapping. "*" indicates that H is not significantly different from A and L.

Source of variation	DF	SS	F	p	Effect direction
Model	11	1.03861	379.7	<0.0001	
Error	48	0.01194			
Effect					
Fe	1	0.00072	2.9	0.095	
P	1	1.00833	4055.1	<0.0001	+
Fe*P	1	0.00748	30.1	<0.0001	+
DOC	2	0.00351	7.1	0.0021	A>L*
Fe*DOC	2	0.00216	4.3	0.0186	H,A>L
P*DOC	2	0.01621	32.6	<0.0001	L>A>H
Fe*P*DOC	2	0.00020	0.4	0.6762	

DF: degrees of freedom, SS: sum of squares, MS: mean square, F: F ratio,
p: probability that $F>F_{crit.}$

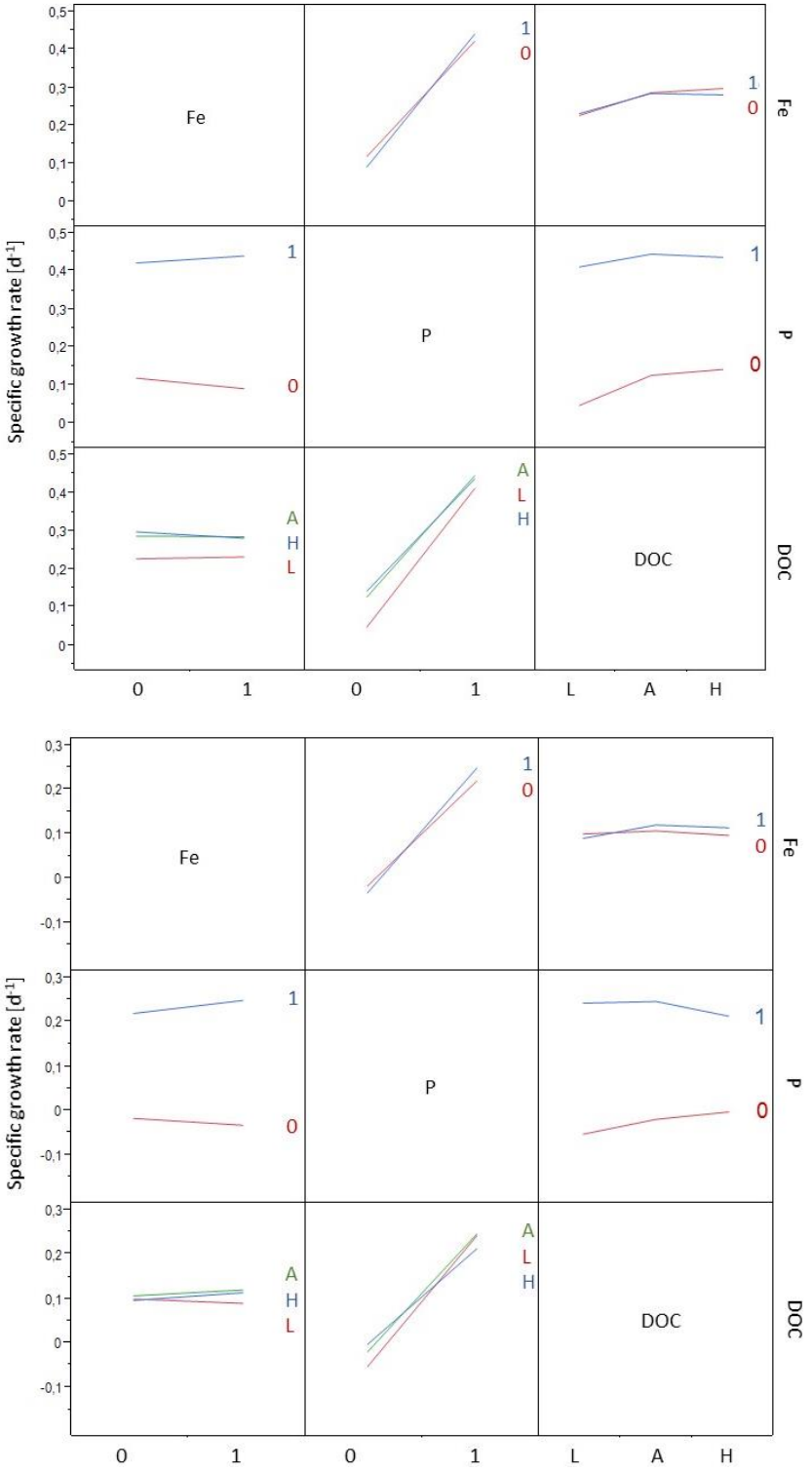


Figure 12: Interaction plots from full factorial ANOVA for specific phytoplankton growth rate in experiment with water from the western basin (top) and from the eastern basin (bottom), where 0=nutrient not added, 1=nutrient added, L=DOC_{low}, A=$DOC_{ambient}$, H=DOC_{high}. If lines in the interaction plots are parallel, there is no interaction. If lines are crossing, converging or diverging there is an interaction.

An ANOVA excluding P addition treatments (full factorial ANOVA, experiment west: $r^2_{adj.}$=0.89, $F_{5,24}$=47, p<0.0001; experiment east: $r^2_{adj.}$=0.721, $F_{5,24}$=16, p<0.0001) showed a significant negative effect of Fe addition on growth rates in both experiments (Table 3-4, parameter estimates see Table 2 appendix). Moreover low DOC concentrations negatively affected growth rates, while high DOC concentrations affected them positively. Furthermore, the interaction between DOC and Fe was significant in both trials. In the experiment with water from the western basin, the negative effect of adding Fe was stronger in high DOC treatments, while in the experiment with water from the eastern basin it was stronger in low DOC treatments (Fig. 13).

Table 3: Results of ANOVA on specific phytoplankton growth rate in the experiment with water from the western basin, excluding P addition treatments. "+" indicates that the nutrient significantly increased specific growth rate, while "–" indicates a significant decrease. For the DOC concentrations ">" indicates that growth rates were significantly different from each other, while a comma indicates that there was no significant difference. A significant difference was assumed when confidence intervals, calculated by 2·standard error, were not overlapping.

Source of variation	DF	SS	F	p	Effect direction
Model	5	0.06093	47.3	<0.0001	
Error	24	0.00618			
Effect					
Fe	1	0.00572	22.2	<0.0001	-
DOC	2	0.05158	100.1	<0.0001	H,A>L
Fe*DOC	2	0.00363	7.0	0.0039	L,A>H

DF: degrees of freedom, SS: sum of squares, MS: mean square, F: F ratio,
p: probability that $F>F_{crit.}$

Table 4: Results of ANOVA on specific phytoplankton growth rate in the experiment with water from the eastern basin, excluding P addition treatments.*H is not significantly different from A and L. "+" indicates that the nutrient significantly increased specific growth rate, while "–" indicates a significant decrease. For the DOC concentrations ">" indicates that growth rates were significantly different from each other, while a comma indicates that there was no significant difference. A significant difference was assumed when confidence intervals, calculated by 2·standard error, were not overlapping. "*" indicates that H is not significantly different from A and L.

Source of variation	DF	SS	F	p	Effect direction
Model	5	0.01644	16.0	<0.0001	
Error	24	0.00494			
Effect					
Fe	1	0.00178	8.6	0.0072	-
DOC	2	0.01310	31.8	<0.0001	H>A>L
Fe*DOC	2	0.00156	3.8	0.0373	A>L*

DF: degrees of freedom, SS: sum of squares, MS: mean square, F: F ratio,
p: probability that $F>F_{crit.}$

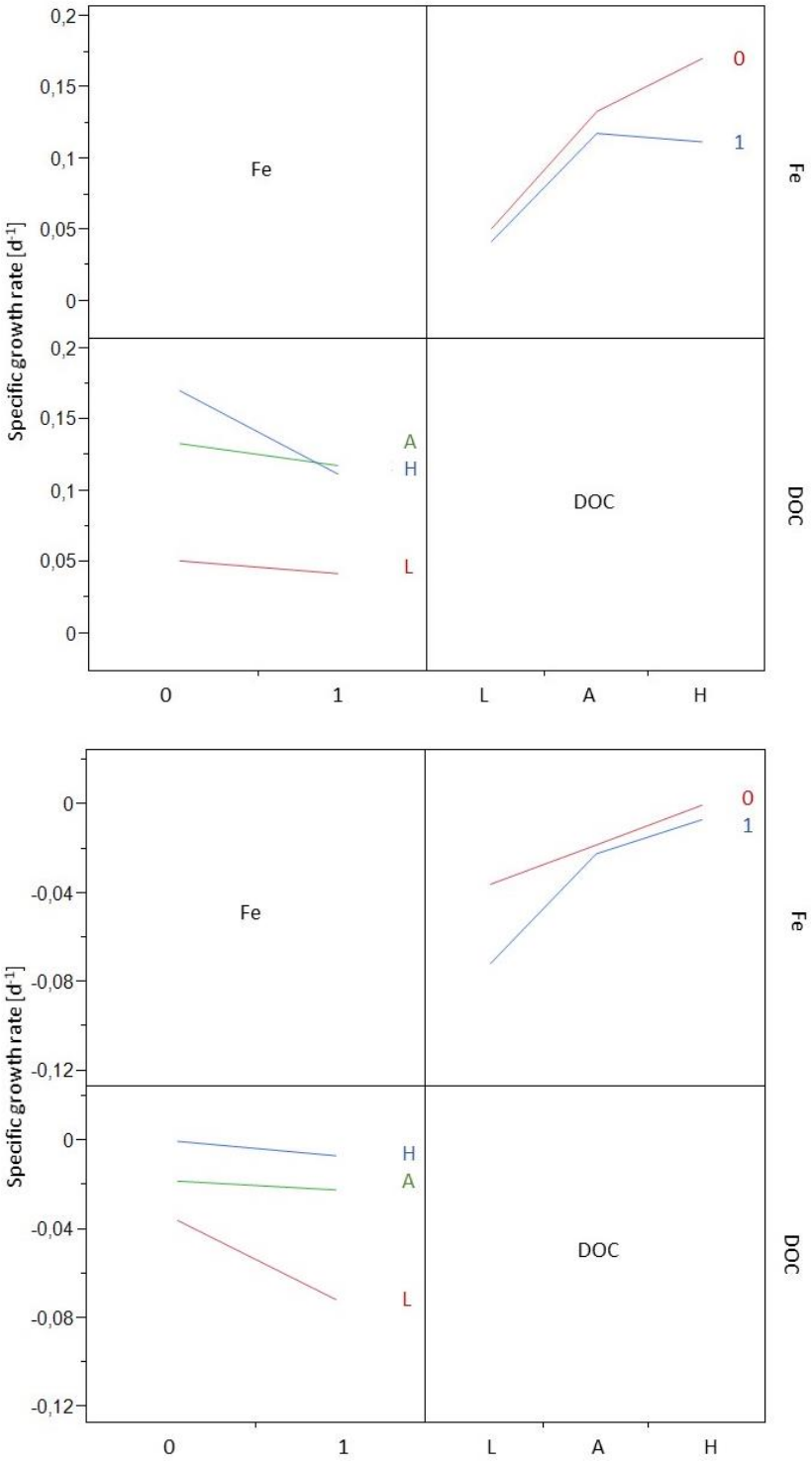


Figure 13: Interaction plots from full factorial ANOVA for specific phytoplankton growth rate in experiment with water from the western basin (top) and from the eastern basin (bottom), excluding P addition treatments, where 0=nutrient not added, 1=nutrient added, L=DOC_{low}, A=$DOC_{ambient}$, H=DOC_{high}. If lines in the interaction plots are parallel, there is no interaction. If lines are crossing, converging or diverging there is an interaction.

An ANOVA on both experiments together (full factorial ANOVA, $r^2_{adj.}$=0.99, $F_{23,69}$=646, p<0.0001) showed that the identity of the experiment explains a large proportion of the variation of growth rates between experiments (22.8 %) (Table 5). Among all treatments growth rates were higher in the experiment with water from the western part than in the experiment with water from the eastern part (parameter estimates see appendix Table 3). However, P addition was still the main source of variation (72.8 %), while DOC and interactions between Fe and P, P and DOC, P and experiment as well as DOC and experiment together explained 3.6 % of variation. The results from the ANOVA on both experiments together revealed a stronger effect of P addition on phytoplankton growth in the experiment with water from the western basin than in the experiment with water from the eastern basin (Fig. 14). Moreover the interaction between DOC and experiment was significant. Increasing the DOC concentration had a larger positive effect on phytoplankton at the western site than at the eastern site. The negative effect of low DOC concentrations on growth rates was stronger at the western site than at the eastern site.

Table 5: Results from full factorial ANOVA on both experiments together for specific phytoplankton growth rate. "+" indicates that the nutrient significantly increased specific growth rate, while "–" indicates a significant decrease. For the DOC concentrations ">" indicates that growth rates were significantly different from each other, while a comma indicates that there was no significant difference. A significant difference was assumed when confidence intervals, calculated by 2•standard error, were not overlapping. "*" indicates that A is not significantly different from L and H.

Source of variation	DF	SS	F	p	Effect direction
Model	23	3.50543	645.9	<0.0001	
Error	96	0.02265			
Effect					
Fe	1	0.00004	0.2	0.6682	
P	1	2.56892	10887.2	<0.0001	+
Fe*P	1	0.01548	65.6	<0.0001	+
DOC	2	0.03544	75.1	<0.0001	A,H>L
Fe*DOC	2	0.00036	0.8	0.4681	
P*DOC	2	0.02778	58.9	<0.0001	L>A>H
Fe*P*DOC	2	0.00161	3.4	0.0371	
Experiment	1	0.80293	3402.9	<0.0001	West>East
Fe*Experiment	1	0.00098	4.2	0.0438	
P*Experiment	1	0.03338	141.5	<0.0001	West>East
Fe*P*Experiment	1	0.00000	0.0	0.8925	
DOC*Experiment	2	0.01342	28.4	<0.0001	H,A>L
Fe*DOC*Experiment	2	0.00303	6.4	0.0024	L>H*
P*DOC*Experiment	2	0.00085	1.8	0.1721	
Fe*P*DOC*Experiment	2	0.00120	2.5	0.0842	

DF: degrees of freedom. SS: sum of squares. MS: mean square. F: F ratio. p: probability that $F>F_{crit.}$

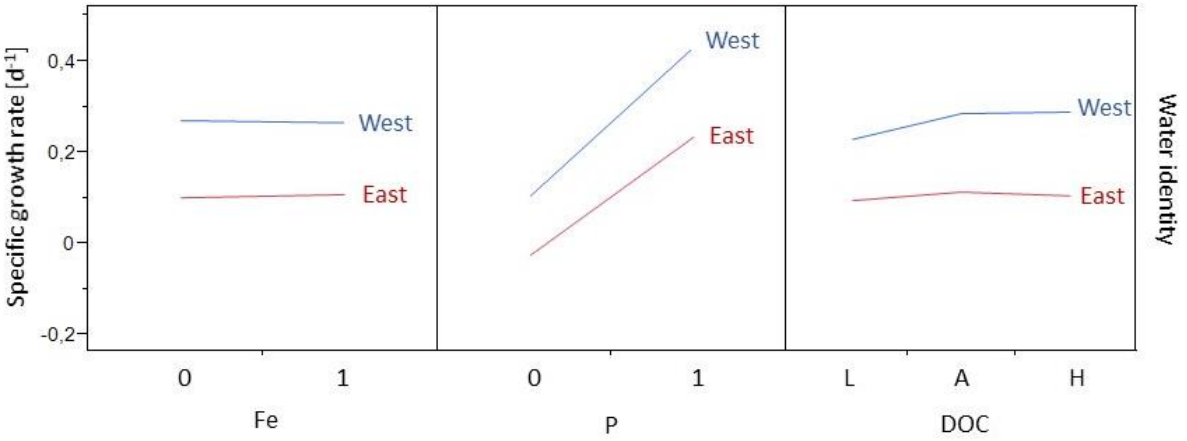


Figure 14: Interaction plots with Experiment as a factor from full factorial ANOVA on both experiments together for specific phytoplankton growth rate, where 0=nutrient not added, 1=nutrient added, L=DOC_{low}, A=$DOC_{ambient}$, H=DOC_{high}. If lines in the interaction plots are parallel, there is no interaction. If lines are crossing, converging or diverging there is an interaction.

3.2 Initial chemical conditions

3.2.1 Principal components analysis

In the beginning of the experiments the chemical characteristics were different in all 12 treatments, showing that a wide variety of chemical conditions was created by manipulating P, Fe and DOC (Fig. 15). The first PCA component accounts for 52 % of the total variance in chemical data in experiment west and 59.8 % in experiment east and is mainly influenced by DOC concentration and the fluorescence indices FI, HIX and ß:α. The second component explains 24.3 % of the total variation in experiment west, 23 % in experiment east respectively, and is mainly affected by PO_4, total P and Fe. Therefore, component 1 can be considered as a DOC quality and quantity axis, while component 2 is interpreted as a nutrient axis. However, in the experiment with water from the western basin, total P and Fe also largely contribute to the first axis, indicating that P and Fe concentrations are influenced when manipulating DOC concentration. The absorbances A_{420}, A_{254}, A_{254}/A_{365} and specific visible absorbances SVA_{420} and SVA_{335} contribute to both axis, since they are influenced by both, DOC and iron.

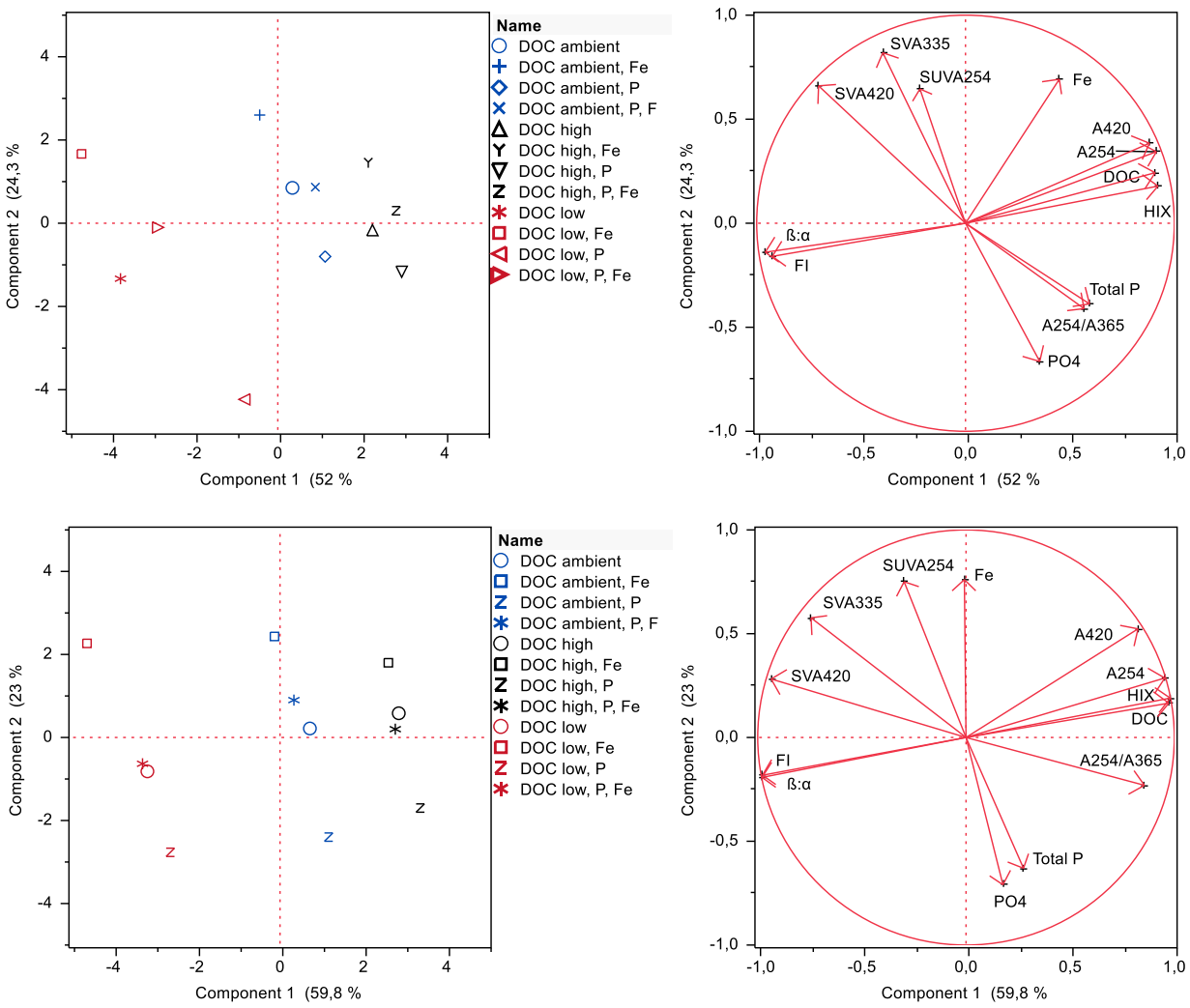


Figure 15: Principal component analysis of initial chemical conditions in the experiment with water from the western (top) and eastern (bottom) part of Lake Mälaren. Principal component 1 is the direction of greatest variability in the data and is plotted versus principal component 2, representing the next uncorrelated direction of greatest variability. Values in brackets represent percentages of variance explained by the principal components. Treatment ordinations are shown in the left figure, while chemical parameter responses are shown in the right figure. Note: Fluorescence indices for water from eastern part were only measured in the treatments without addition of nutrients since we assumed that adding nutrients does not affect DOC quality. An ANOVA on the DOC quality indices from experiment 2 showed that the addition of P and Fe has no significant effect on HIX, FI and ß:α, only the effect of DOC concentration was significant. Hence for the principal components analysis of experiment east, we assumed the indices of the nutrient addition treatments to be the same as the treatments without addition.

3.2.2 Comparison between treatments

The experiment was successful in creating a DOC gradient with concentrations of 3.7 ± 0.2, 9.0 ± 0.4 and 15.7 ± 0.4 µg L^{-1} in the experiment with water from the western basin, and concentrations of 3.7 ± 0.04, 8.4 ± 0.1 and 12.8 ± 0.3 µg L^{-1} in experiment east, respectively. Strongly connected to the DOC gradient, was a change in absorbance at the wavelength 254 nm and 420 nm (Table 6-7). DOC_{high} treatments had significantly higher absorbances than DOC_{low} treatments (Table 6). In the experiment with water from the eastern basin also the absorbance

ratio A_{254}/A_{365} was significantly influenced by the creation of the DOC gradient, with the highest ratio in DOC_{high} treatments. However, in the experiment with water from the western basin the absorbance ratio was unaffected by DOC concentration.

In addition to DOC quantity and absorbance, the three DOC concentrations also differed in DOC quality. The treatments with low DOC concentrations had a significantly lower humification index and higher fluorescence and freshness index (Table 6), indicating that DOC_{low} treatments contained a larger proportion of autochthonous DOC with recent biological origin and lower degree of humification. In contrast, the organic carbon in DOC_{high} treatments was older, more humified and with a higher share of terrestrial derived fulvic acids.

As desired, the total P concentrations were 49 ± 3 µg L^{-1} higher in P addition treatments compared to treatments where no P was added. Also PO_4 was on average 38 ± 10 µg L^{-1} higher in P treatments. In contrast to P addition treatments, where a large amount of total P was in form of PO_4, treatments without P addition contained no or very little PO_4 (Fig. 16). Treatments with higher DOC concentrations contained significantly more total P than corresponding treatments with low levels of DOC (Table 6). However, no significant difference in PO_4 concentration was found between treatments of different DOC concentrations.

As intended, the TRFe concentrations were 408 ± 64 µg L^{-1} higher in treatments where Fe was added compared to treatments without Fe addition. Connected with the addition of Fe was an increase in absorbance at 420 nm and 254 nm, since iron contributes to water colour.

Iron concentrations were significantly higher in DOC_{high} than in DOC_{low} treatments in the experiment with water from the western basin (Fig. 17), while there was no significant difference found in experiment with water from the eastern site (Table 6). In experiment west, the difference between iron concentrations measured 1 h and 21 h after the addition of ascorbic acid reductant was highest in DOC_{high} and lowest in DOC_{low} treatments (Fig. 18). The difference in iron concentrations measured at different times after the addition of the reductant can serve as an indicator for the form, in which Fe is present. A small difference in TRFe measured after 1 h and 21 h indicates a high proportion of readily available Fe and a low proportion of Fe, which is strongly bound to DOC and therefore just available after a long exposure time to the reductant. In contrast, a large share of strongly bound Fe is denoted by a large difference in TRFe measured after 1 h and 21 h. Hence, most iron contributing to the total iron pool in DOC_{low} treatments was readily available, while in treatments with high DOC levels there was a large proportion of Fe being bound to DOC.

The iron and phosphorus measurements give evidence, that Fe and P build a complex (Table 6-7). In the PCA, iron and phosphorus arrows show into opposing directions, indicating that they effect each other negatively (Fig. 15). Fe seems to reduce total phosphorus concentrations and especially binds to phosphate, suggesting the formation of a PO_4-Ferrihydrite complex (Fig. 16). In both experiments, PO_4 concentrations were significantly lower when adding Fe

(one sided, paired t-test, experiment east: t_5=2.19; p=0.04; experiment west: t_5=2.31, p=0.3). In experiment east also significantly lower total P concentrations were observed when adding Fe (one sided, paired t-test, t_5=2.74, p=0.02). The effect of adding Fe on total P concentrations was not significant in the experiment with water from the western basin (one sided, paired t-test, t_5=1.84, p=0.06), but a tendency towards lower total P concentrations when adding nutrients was perceived. The addition of P did not significantly affect TRFe concentrations (one-sided, paired t-test, experiment east: t_5=1.68, p=0.078; experiment west: t_5=0.03, p=0.48), however in the experiment with water from the eastern basin, TRFe concentrations tended to be lower when adding P.

Table 6: Initial chemical parameters of each treatments (N=1 per treatment) in the experiment with water from western basin of Lake Mälaren, including total reactive iron (TRFe) [µg L^{-1}], PO_4 [µg L^{-1}], P_{Total} [µg L^{-1}], dissolved organic carbon (DOC) [mg L^{-1}], absorbance at 420 nm (A_{420}), absorbance at 254 nm (A_{254}), absorbance ratio between 254 nm and 365 nm (A_{254}/A_{365}), specific UV absorbance at 254 nm ($SUVA_{254}$) [L mg C^{-1} m^{-1}], specific visible absorbance at 420 nm (SVA_{420}) [L mg C^{-1} m^{-1}], specific visible absorbance at 335 nm (SVA_{335}) [L mg C^{-1} m^{-1}], humification index (HIX), fluorescence index (FI), freshness index (ß:α) and pH.

Treatment	TRFe	PO_4	P_{Total}	DOC	A_{420}	A_{254}	A_{254}/A_{365}	$SUVA_{254}$	SVA_{420}	SVA_{335}	HIX	FI	ß:α	pH
DOC_{low}	72	1	8.5	3.5	1.48	12.25	3.99	3.45	0.417	1.282	0.776	1.503	0.707	7.5
$DOC_{low}Fe$	488	<1	9.3	3.6	1.79	14.46	3.67	3.99	0.494	1.608	0.796	1.526	0.725	7.5
$DOC_{low}P$	78	52	58.6	3.6	1.13	12.10	4.61	3.34	0.312	1.133	0.846	1.488	0.631	7.5
$DOC_{low}PFe$	477	20	54.2	3.9	1.69	14.57	3.80	3.74	0.432	1.476	0.832	1.515	0.657	7.4
$DOC_{ambient}$	281	2	20.6	8.8	3.55	36.48	4.48	4.13	0.403	1.439	0.903	1.439	0.529	7.5
$DOC_{ambient}Fe$	730	<1	20.1	8.6	3.81	38.44	4.33	4.45	0.441	1.599	0.879	1.455	0.575	7.4
$DOC_{ambient}P$	272	50	70	8.9	3.45	35.56	4.50	4.02	0.390	1.397	0.908	1.446	0.533	7.5
$DOC_{ambient}PFe$	728	28	67.3	9.5	3.88	38.38	4.26	4.04	0.409	1.473	0.904	1.456	0.539	7.4
DOC_{high}	454	2	30.6	15.7	5.31	53.69	4.36	3.43	0.339	1.241	0.902	1.449	0.518	7.4
$DOC_{high}Fe$	911	<1	31	15.7	5.86	54.97	4.10	3.51	0.374	1.346	0.900	1.439	0.532	7.4
$DOC_{high}P$	464	50	82.3	15.2	5.19	53.18	4.33	3.49	0.341	1.278	0.905	1.440	0.519	7.4
$DOC_{high}PFe$	917	32	79.2	16.2	5.90	55.16	4.07	3.40	0.363	1.318	0.897	1.447	0.528	7.4
$DOC_{ambient}$ without algae	290	2	17.6	9.5	3.43	36.86	4.58	3.89	0.363	1.329	0.908	1.447	0.534	7.5
$DOC_{ambient}$ without N	277	2	20.8	8.8	3.74	36.04	4.36	4.11	0.426	1.456	0.882	1.439	0.554	7.5

Table 7: Initial chemical parameters of each treatments (N=1 per treatment) in the experiment with water from eastern basin of Lake Mälaren, including total reactive iron (TRFe) [µg L^{-1}], PO_4 [µg L^{-1}], P_{Total} [µg L^{-1}], dissolved organic carbon (DOC) [mg L^{-1}], absorbance at 420 nm (A_{420}), absorbance at 254 nm (A_{254}), absorbance ratio between 254 nm and 365 nm (A_{254}/A_{365}), specific UV absorbance at 254 nm ($SUVA_{254}$) [L mg C^{-1} m^{-1}], specific visible absorbance at 420 nm (SVA_{420}) [L mg C^{-1} m^{-1}], specific visible absorbance at 335 nm (SVA_{335}) [L mg C^{-1} m^{-1}], humification index (HIX), fluorescence index (FI), freshness index (ß:α) and pH.

Treatment	TRFe	PO_4	P_{Total}	DOC	A_{420}	A_{254}	A_{254}/A_{365}	$SUVA_{254}$	SVA_{420}	SVA_{335}	HIX	FI	ß:α	pH
DOC_{low}	<1.3	<1	4.3	3.6	1.02	10.48	5.25	2.89	0.282	0.904	0.821	1.562	0.817	7.8
$DOC_{low}Fe$	352	<1	4.5	3.7	1.32	12.89	4.40	3.49	0.358	1.262				7.8
$DOC_{low}P$	<1.3	44	53.3	3.7	0.95	10.32	5.35	2.79	0.255	0.868	0.819	1.563	0.817	7.8
$DOC_{low}PFe$	230	31	48.4	3.7	0.98	12.15	5.19	3.27	0.264	1.060				7.8
$DOC_{ambient}$	<1.3	1	9	8.4	1.55	27.48	6.85	3.28	0.185	0.849	0.867	1.509	0.719	7.8
$DOC_{ambient}Fe$	434	<1	8.5	8.3	1.99	28.82	5.72	3.46	0.239	1.009				7.9
$DOC_{ambient}P$	<1.3	44	62.6	8.5	1.37	24.53	6.45	2.87	0.160	0.765				7.8
$DOC_{ambient}PFe$	410	35	58.8	8.3	1.89	29.08	6.06	3.52	0.228	0.990				7.8
DOC_{high}	<1.3	2	14.6	12.9	2.32	38.06	6.29	2.96	0.180	0.801	0.893	1.481	0.641	7.9
$DOC_{high}Fe$	446	1	13.2	12.9	2.21	39.12	6.01	3.03	0.171	0.862				7.8
$DOC_{high}P$	<1.3	45	63	13.0	1.86	37.13	6.60	2.85	0.143	0.751				7.9
$DOC_{high}PFe$	393	40	60.1	12.4	2.13	37.80	6.03	3.06	0.173	0.861				7.9
$DOC_{ambient}$ without algae	<1.3	<1	7	8.5	1.51	26.75	6.79	3.14	0.177	0.810				7.9
$DOC_{ambient}$ without N	<1.3	1	10.1	8.4	1.73	25.39	6.02	3.02	0.206	0.843				7.8

Table 8: Results from paired, two-sided t-tests on differences between DOC_{high} (H) and DOC_{low} (L) treatments in the chemical parameters total reactive iron (TRFe), PO_4, P_{Total}, dissolved organic carbon (DOC), absorbance at 420 nm (A_{420}), absorbance at 254 nm (A_{254}), absorbance ratio between 254 nm and 365 nm (A_{254}/A_{365}), specific UV absorbance at 254 nm ($SUVA_{254}$), specific visible absorbance at 420 nm (SVA_{420}), specific visible absorbance at 335 nm (SVA_{335}), fluorescence index (FI), freshness index (ß:α) and humification index (HIX). Effect direction is given for significant effects. Degrees of freedom were 3 in all tests.

	Experiment west			Experiment east		
	t	p>ltl	Effect direction	t	p>ltl	Effect direction
TRFe	-28.63	<0.0001	H>L	-1.62	0.2029	
PO_4	-0.87	0.4464		1.49	0.2325	
P_{total}	-30.44	<0.0001	H>L	-16.10	0.0005	H>L
DOC	-77.99	<0.0001	H>L	-58.03	<0.0001	H>L
A_{420}	-50.40	<0.0001	H>L	-10.78	0.0017	H>L
A_{254}	-187.06	<0.0001	H>L	-64.26	<0.0001	H>L
A_{254}/A_{365}	-1.19	0.3185		-7.26	0.0054	H>L
$SUVA_{254}$	1.20	0.316		1.08	0.36	
SVA_{335}	0.91	0.431		2.99	0.0581	L>H
SVA_{420}	1.89	0.155		5.67	0.0109	L>H
FI	7.31	0.0053	L>H	4.96	0.0157	L>H
ß:α	7.58	0.0048	L>H	13.00	0.001	L>H
HIX	-5.56	0.0115	H>L	-6.13	0.0087	H>L

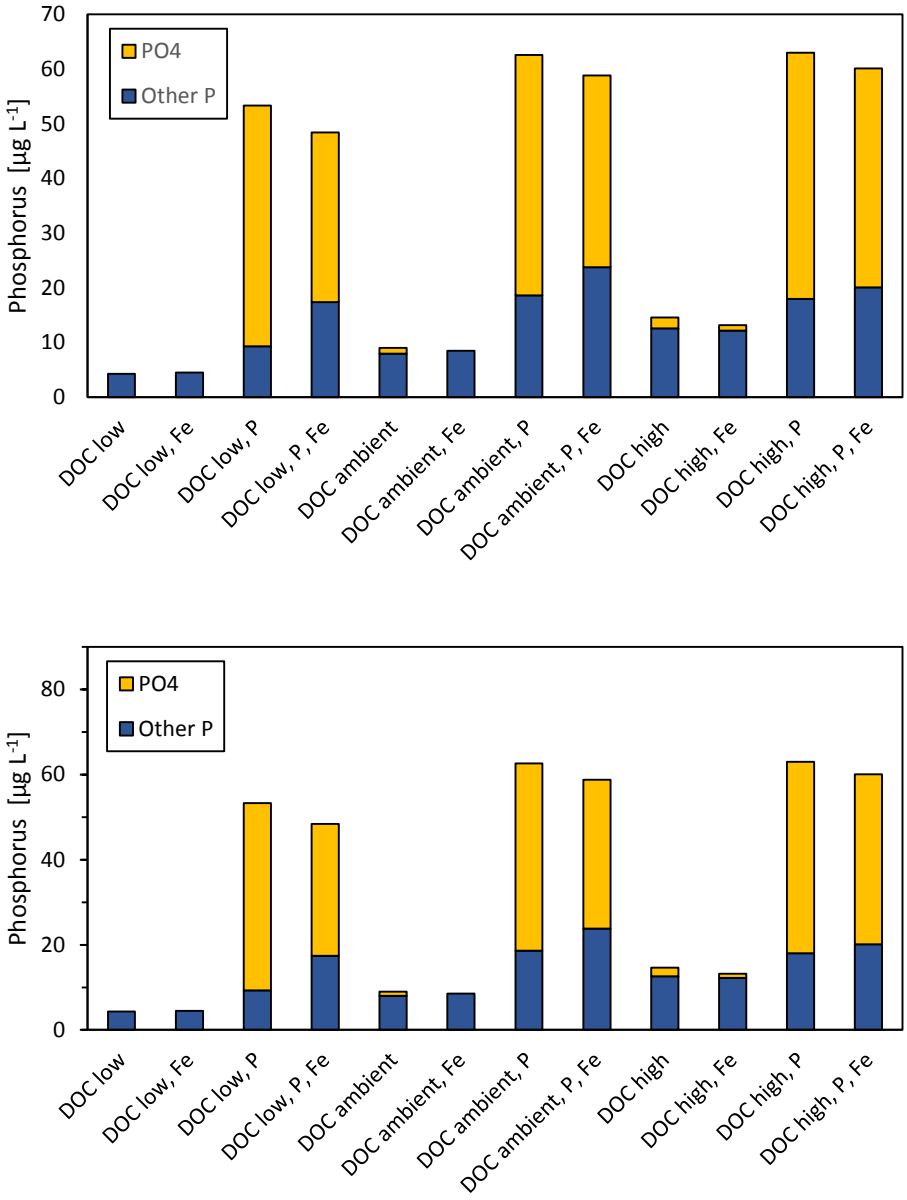


Figure 16: Phosphate (PO_4) and the difference between P_{total} and PO_4 (Other P) measured in the different treatments in the experiment with water from the western basin (top) and from the eastern basin (bottom). "Other P" is probably phosphate sorbed to precipitated ferrihydrite (PO_4-Ferrihydrite), as has been shown by modelling using the program VisualMinteq (Gustafsson 200x) (S. Köhler, unpublished results).

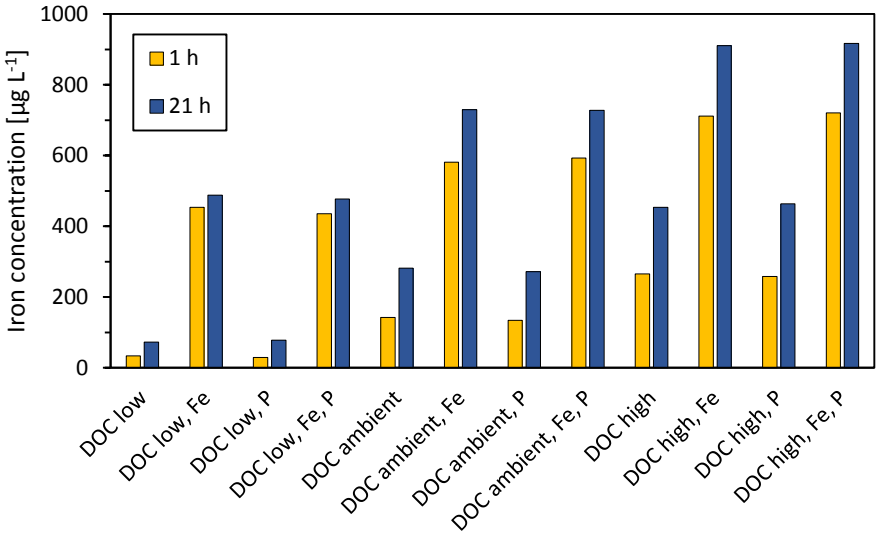


Figure 17: Iron concentrations in the beginning of the experiment with water from the western part of Lake Mälaren. Measured 1 h (yellow) and 21 h (blue) after the addition of ascorbic acid reductant.

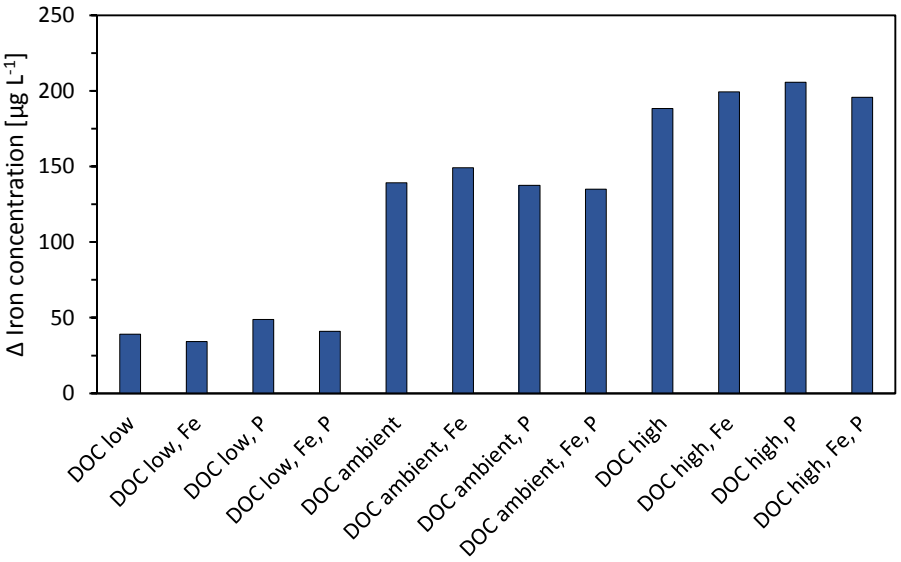


Figure 18: Difference between iron concentrations measured 1 h and 21 h after the addition of ascorbic acid reductant. Values show iron concentrations measured before starting the experiment with water from the western basin of Lake Mälaren.

3.2.3 Comparison between experiments

The water taken from the eastern and western basins of Lake Mälaren differed in nutrient content, absorbance and DOC quality, while the DOC concentrations were similar at both sites (Fig. 19, Table 6-7). Iron concentrations in the western basin were on average 298 µg L^{-1} higher than in the eastern basin. Also total P concentrations were 10.9 µg L^{-1} higher in the western basin, while there was no difference in PO_4 concentrations between the two experiments.

Compared to the eastern basin, DOC originating from the western site was characterized by a higher age, contribution of terrestrially derived DOC and degree of humification, as reflected by a higher HIX and lower FI and ß:α. The water from the western basin also showed a lower absorbance ratio A_{254}/A_{365} and higher specific absorbances $SUVA_{254}$, SVA_{420} and SVA_{335}, indicating that the DOC at the western site has a higher molecular weight and aromaticity. The absorbances at 420 nm and 254 nm were higher in the western that in the eastern basin, reflecting a darker water colour. The darker water colour at the western site is probably caused by higher Fe concentrations and a higher share of terrestrially derived DOC.

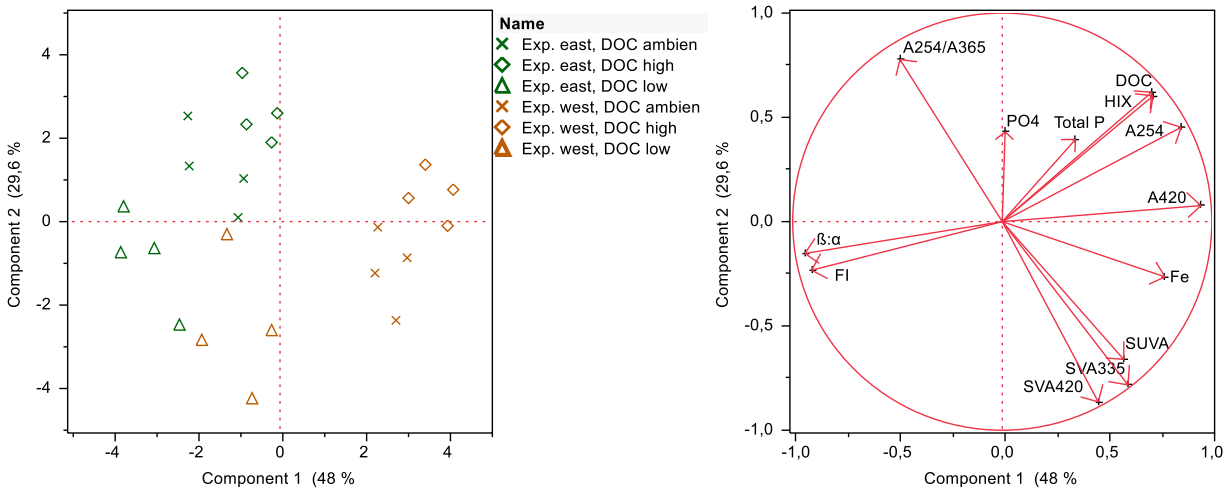


Figure 19: Principal component analysis of initial chemical conditions in both experiments together. Principal component 1 is the direction of greatest variability in the data and is plotted versus principal component 2, representing the next uncorrelated direction of greatest variability. Values in brackets represent percentages of variance explained by the principal components. Treatment ordinations are shown in the left figure, while chemical parameter responses are shown in the right figure.

3.3 Final chemical conditions

In the end of the experiments the chemical characteristics were still different between the treatments, while replicates of the same treatment showed similar chemical conditions, indicating that the variation within treatments was small (Fig. 20, raw data see Table 4-5 appendix). In the experiment with water from the eastern basin, the first component, identified by PCA, accounts for 54.5 % of the total variance in chemical data and is mainly influenced by DOC concentration and the fluorescence indices FI, HIX and ß:α. The second component explains 34.3 % of the total variation in experiment east and mainly explains variations in Fe. In the experiment with water from the western basin, 62.3 % of variation is explained by the first component and 20.7 % by the second component. DOC concentration as well as DOC quality indices contribute to the first and second component. Iron is mainly connected to the first component.

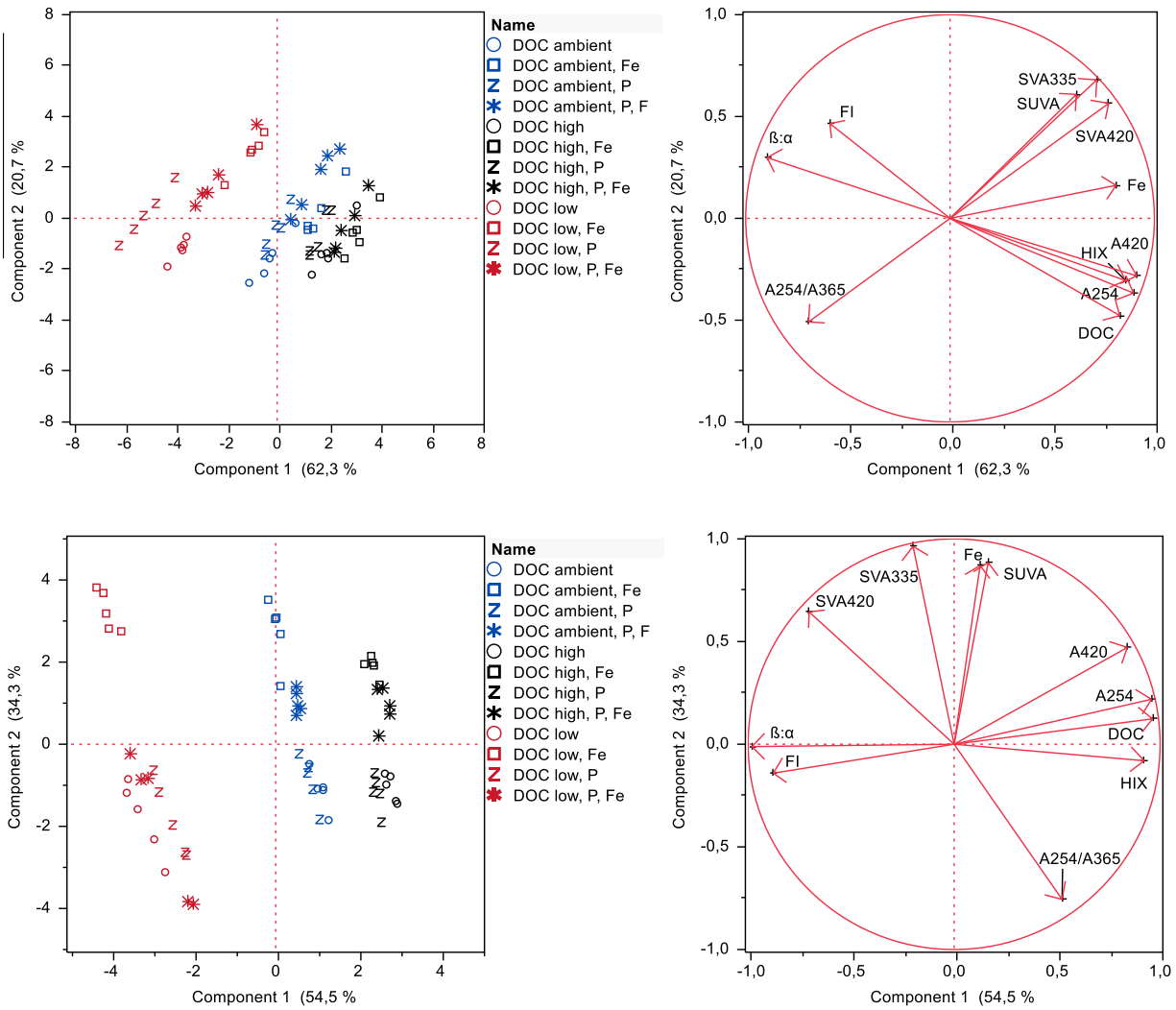


Figure 20: Principal component analysis of final chemical conditions in the experiment with water from the western (top) and eastern (bottom) part of Lake Mälaren. Principal component 1 is the direction of greatest variability in the data and is plotted versus principal component 2, representing the next uncorrelated direction of greatest variability. Values in brackets represent percentages of variance explained by the principal components. Treatment ordinations are shown in the left figure, while chemical parameter responses are shown in the right figure.

3.4 Photodegradation during incubation

DOC concentrations, absorbances (A_{420}, A_{254}) absorbance ratio (A_{254}/A_{365}), and specific absorbances ($SUVA_{254}$, SVA_{420}, SVA_{335}) showed no significant difference in treatment kept in light or kept in dark (two sided, independent t-test for A_{254}/A_{365}, eastern basin: t_8=1.67, p=0.13, western basin: t_8=1.21, p=0.26) (one sided, independent t-tests for DOC, absorbances and specific absorbances, eastern basin: t_8=-1.51-1.67, p=0.09-0.38; western basin: t_8=-1.67-1.73, p=0.06-0.94), suggesting that photodegradation did not occur during the incubation period of 7.5 days.

3.5 N as a limiting nutrient

In both experiments, growth rates in $DOC_{ambient}$ treatments without P and Fe addition were not significantly different from the treatments with N addition (two-sided, independent t-tests, experiment east: t_8=-0.63, p=0.54; experiment west: t_8=0.41, p=0.69), suggesting that N did not stimulate phytoplankton growth and hence was not acting as a primarily limiting nutrient.

4 Discussion

4.1 Effects of DOC, P and Fe on phytoplankton growth

Phytoplankton growth was mainly determined by P and partly by Fe and DOC. P was the primarily limiting nutrient, having the biggest overall effect on phytoplankton growth. Fe was a co-limiting element, stimulating phytoplankton growth in the presence of P. However, in the absence of P, Fe decreased primary productivity, probably due to the sorption of P to Fe. Increasing DOC concentration stimulated phytoplankton, but this effect was modified by P and Fe. Under ambient P conditions high DOC concentrations had a positive effect on growth rate, most likely due to stimulation by nutrients associated to DOC. Under P-rich conditions, higher DOC concentrations resulted in lower growth rates compared to ambient DOC concentrations, implying that increasing or high DOC concentrations may lead to a lower sensitivity of lakes towards P inputs. Moreover, DOC affects the accessibility of Fe to phytoplankton and the direction of this effect depends on DOC quality.

4.1.1 Effect of P addition on phytoplankton growth

At both sites of Lake Mälaren, the primary production was mainly regulated by P. Also Sonesten *et al.* (2013) found that P is the main limiting nutrient in Lake Mälaren during the time of the year, when water sampling for the experiments took place (Sonesten *et al.*, 2013). This result was not surprising, since P is commonly considered as the primary limiting nutrient controlling eutrophication in lakes (Correll, 1998; Conley *et al.*, 2009; Schindler, 1977; Kalff, 2002). Also a model for chlorophyll in northern temperate lakes concludes that in most cases phytoplankton abundance is driven by phosphorus, even though also other mechanism including DOC influence chlorophyll dynamics (Beisner *et al.*, 2003).

4.1.2 Effect of Fe addition on phytoplankton growth

Even though P was the main factor determining phytoplankton growth, also Fe showed a significant effect. When added together with P, Fe increased phytoplankton growth rates, while under ambient P conditions Fe showed no stimulating or even a negative effect. This indicates that P and Fe interact to limit primary productivity. To the best of my knowledge, this study is the first one showing concurrent limitation by P and Fe in Lake Mälaren. However, a co-limitation of P and Fe in freshwaters has been reported in other lakes. Several studies conducting nutrient enrichment bioassays, showed that phytoplankton growth rate was higher when enriched with P and Fe together, than with P or Fe alone (Twiss *et al.*, 2000; De-Wever *et al.*, 2008; Xu *et al.*, 2013; North *et al.*, 2007). Also Vrede and Tranvik (2006) conducted bioassay experiments with oligotrophic lake water and reported concurrent P and Fe limitation in seven out of nine lakes. By combining their experimental results with a large data set on

water chemistry of 659 Swedish lakes, they predicted, that Fe has a positive effect on phytoplankton growth in 88 % of these lakes, inferring that that P and Fe co-limitation is a widespread phenomenon (Vrede and Tranvik, 2006). The concurrent limitation by P and Fe observed in my study support the findings of all the previously mentioned studies and underline the conclusion of Sterner (2008) that co-limitation of multiple nutrients is expected and common (Sterner, 2008).

While in P addition treatments Fe stimulated phytoplankton growth, in ambient P treatments Fe had no or even a negative effect. This result was surprising, but could be explained by sorption of P to precipitated Fe. Ferrihydrite may sorb to PO_4 (Moore and Reddy, 1994), reducing the availability of P to phytoplankton. Since P is the primarily limiting nutrient, the reduced availability of P due to the addition of Fe leads to lower growth rates compared to ambient treatments. This negative effect of Fe was just visible in treatments without P addition, since in these treatments the available P was already very low. In P addition treatments the amount of P, which was precipitated by Fe, was too small and insignificant compared to the PO_4 that was still available, to have a negative effect on phytoplankton growth. The hypothesis that the complexation between Fe and P leads to the negative effect of Fe in the absence of P addition is supported by my chemical data, showing that total phosphorus as well as PO_4 concentrations are lower in the presence of Fe. Hence, under P-poor conditions, Fe seems to reduce the availability of P to phytoplankton. Since Fe shows a positive effect in P addition treatments, but a negative effect under ambient P conditions, I could infer that adding Fe to a eutrophic, P-rich lake stimulates primary productivity, while adding Fe to an oligotrophic lake decreases or does not affect algae growth. However, this result is not supported by other studies. Vrede and Tranvik (2006) found that the effect of Fe addition increased with decreasing P concentration (Vrede and Tranvik, 2006). A possible explanation for the different findings is that in the study of Vrede and Tranvik (2006) the added iron was kept in solution with the chelating agent EDTA. The addition of EDTA possibly prevented the formation of insoluble P-Fe complexes.

4.1.3 Interactions between P and DOC

Besides the nutrients P and Fe, also DOC concentrations significantly affected phytoplankton growth rates. This study shows that the response of phytoplankton to DOC depends on phosphorus concentration. When no P was added to the lake water, growth rates were lowest in DOC_{low} treatments and increased with increasing DOC concentration. Under P-rich conditions low and high DOC concentrations had a negative effect, while phytoplankton growth was highest under ambient conditions. The negative effect of DOC in P addition treatments was higher in the experiment with water from the eastern basin than with water from the western basin. From these findings I conclude:

(I) Low DOC concentrations had a much stronger negative effect under ambient P than under P-rich conditions. I hypothesise that the negative effect of low DOC concentrations is due to

the lower amount of nutrients associated to DOC. The lower nutrient concentrations in DOC low treatments compared to DOC high treatments are caused by nanofiltration. During this process P and Fe are concentrated in the retentate and are thus lost from the water with low DOC concentrations (read more about this in discussion part 4.2.3). Under P-rich conditions P losses due to nanofiltration have no strong effect on growth rates, since there is still enough P left to stimulate algae growth. However, in a situation where the water is poor in P, the nutrients that are lost during nanofiltration can affect growth rates more strongly.

(II) Under ambient P concentrations, increasing the DOC concentration stimulated phytoplankton, probably due to the simultaneous increase in nutrients. The chemical measurements reveal that iron and phosphorus concentrations were higher in treatments with high DOC concentration than in those with low DOC concentrations. The nutrients associated to the DOC apparently stimulated algae growth. Under P-rich conditions the simultaneous increase in P with increasing DOC is negligible compared to the amount of added P. Therefore other mechanisms dominate in P-rich environments.

(III) Under the assumption that DOC could make PO_4 less available by complexing it (Drakare *et al.*, 2003) the stronger negative effect of high DOC concentrations under P-rich conditions at the eastern site compared to the western site points to the fact that P bound to autochthonous DOC is less accessible to phytoplankton than P bound to allochthonous DOC. Possibly autochthonous DOC binds stronger to P or it is able to bind more P. An alternative explanation for the weaker effect of high DOC concentrations at the western site is that the carrying capacity for nutrients of western-type DOC is already reached. Since the P and Fe concentrations at the western site of Lake Mälaren were high compared to the eastern site, it is likely that DOC is already P-saturated and cannot bind further P.

(IV) Moreover I infer that adding P has a stronger effect on growth rates in lakes with low DOC concentrations, while the effect of adding P is less strong in DOC rich lakes. This implies that humic lakes are more resistant towards high P inputs than clear-water lakes. Moreover one could conclude that the increase in DOC concentrations that is predicted for the future may result in lake ecosystems that are less sensitive to increases in P.

Relatively little is known about how interactions between DOC and P to influence primary productivity. However, there are some studies supporting parts of my findings. The stimulation of primary productivity by nutrients associated to DOC has also been observed by Finstad *et al.* (2013), who reports a positive correlation between DOC entering a boreal lake and the total amount of P and N, inferring that DOC carries associated nutrients into the lake. Although most of these nutrients are initially in organic form and thus not available for phytoplankton, mineralization makes fractions of this pool eventually available, stimulating primary production (Finstad *et al.*, 2013). Also Vahatalo et al. (2003) found that nutrients associated to DOC can be transformed into inorganic and bioavailable forms, which can stimulate primary productivity

(Vahatalo *et al.*, 2003). Also in an experimental lake in northern Manitoba the addition of organic moss-peat material initially enhanced primary productivity. This was probably due to the release of nitrogen and phosphorus from DOC. However, the initial increase was followed by a decrease in primary productivity, which was attributed to the binding of iron and phosphorus by DOC (Guilford *et al.*, 1987). Also a study by Drakare *et al.* (2003) gives evidence for a decrease in primary productivity due to complexation of P by DOC. They conducted whole-lake experiments in humic waters, where the fertilization with P resulted in a decrease of picophytoplankton, while large phytoplankton did not respond at all. The decrease and lack of response could most likely be explained by the binding of P to humic complexes (Drakare *et al.*, 2003).

4.1.4 Interactions between Fe and DOC

Under ambient P conditions, DOC and Fe interact to regulate phytoplankton growth. Adding iron has a different interaction with the DOC in the western basin than with the DOC in the eastern basin, as indicated by the opposing effect direction of the two experiments. Adding iron at the western site of Lake Mälaren has a stronger negative effect under high DOC concentrations, while at the eastern site a stronger negative effect was observed under low DOC concentrations. This shows that the iron-DOC interaction depends on DOC quality and that different DOC types affect Fe availability differently. It seems that one type of DOC makes Fe more available, while the other one makes it less available. Since Fe is not the main limiting nutrient and affects primary productivity in rather indirect ways, it is unclear which DOC type makes Fe more available. One possible explanation for the stronger negative effect of adding Fe to DOC high concentrations at the western site is that terrestrial DOC has a higher Fe-binding capacity than autochthonous DOC, thus making Fe less available for phytoplankton growth. The chemical measurements show that after the nanofiltration process there is more iron per DOC in the retentate (which had a higher share of allochthonous DOC than the permeate), indicating that allochthonous DOC has a higher Fe binding capacity. However, the explanation that terrestrial DOC binds stronger to Fe and thus makes it more available does not consider the fact, that the DOC-Fe effect was only observed in treatments without P addition, in which adding Fe did not stimulate algae growth, but instead had a negative effect on growth rates. As an alternative explanation terrestrial DOC could make Fe more susceptible to precipitation with P than autochthonous DOC. The P in the resulting PO_4-Ferrihydrite complexes could be less available for phytoplankton and consequently growth rates decrease. The facilitation of P-Fe-complexation by terrestrial DOC could indicate that terrestrial DOC binds so weakly to Fe that it can easily complex with P. Moreover the western type DOC could be already saturated with Fe, thus DOC has no capacity to bind Fe and Fe can precipitate with P. This would be supported by the iron measurements in the western basin after 1 h and 21 h, showing that the spiked iron

is exclusively in form of the fast reacting ferrihydrite. This suggests that the spiked iron was not bound by DOC, since DOC was saturated.

Due to climate change more allochthonous DOC will enter the lake. If simultaneously also the iron concentrations increase, my findings suggest that phytoplankton biomass would decrease, possibly due to complexation of Fe with P. If Fe concentrations do not increase with increasing terrestrial DOC inputs, algae growth could be stimulated. However, in eutrophic lakes I would not expect these effects, since in the P enriched treatments, no significant interaction was found between DOC and Fe. This was probably since the large stimulating effect of P overshadows smaller effects.

The literature about the effect of DOC on Fe in freshwaters is somewhat contradictory. Most studies support the hypothesis that DOC acts as a chelating agent, enhancing the availability of Fe to phytoplankton by keeping Fe in solution and preventing it from precipitation. Vrede and Tranvik (2006) found an increasing effect of Fe addition with decreasing DOC concentration. They conclude that the absence of organic chelators reduces the availability of Fe to phytoplankton and these suboptimal concentrations of bioavailable Fe finally cause Fe limitation. This shows that DOC increases the bioavailability of Fe (Vrede and Tranvik, 2006). Also Maloney et al (2005) concludes from a study about the role of iron and DOC in UVA attenuation, that DOC can bind to iron, enabling it to remain in solution (Maloney *et al.*, 2005). Moreover a study from Lake Superior, where an iron-dependent cyanobacterial bioreporter was used to determine the bioavailable iron forms, reports enhanced iron availability in the presence of DOC. They attribute this to increased amounts of organic ligands, maintaining Fe in the dissolved state. Furthermore their study reveals that most organically bound iron was highly available to phytoplankton (Hassler *et al.*, 2009).

However, there is also evidence that DOC could restrict the bioavailability of iron. Imai et al. (1999) found that the growth of the cyanobacterium *Microcystis aeruginosa* was inhibited by humic substances. They conclude that this growth inhibition was due to a lack of iron caused by complexation of iron with DOC (Imai *et al.*, 1999). The contradicting results in the literature could be explained by my findings, that different DOC qualities affect Fe availability differently. Therefore the outcomes of different studies may differ, depending on DOC type present in the water. My hypothesis that Fe bioavailability is influenced by DOC quality is strongly supported by recently published results from Sorichetti *et al.* (2014). They found high cyanobacteria densities in lakes with labile DOC (autochthonous DOC), while in lakes with DOC with refractory properties (allochthonous DOC) cyanobacteria densities were low. They conclude that labile DOC binds weakly to Fe, allowing cyanobacteria to scavenge Fe from DOC and overcoming Fe-limitation. In contrast refractory DOC probably has a higher binding capacity for Fe, limiting the access of Fe to cyanobacteria. The high-Fe binding capacity of allochthonous DOC has been attributed to its high humic acid content, while the low Fe-binding

capacity of autochthonous DOC is probably due to its low content of humic acids and high protein composition (Sorichetti *et al.*, 2014b). The findings of Sorichetti et al. (2014) together with the findings of this study point out that the chemical composition of DOC might be an important determinant for the availability of Fe to phytoplankton.

4.1.5 Differences between western and eastern site

Among all treatments phytoplankton growth rates were higher in water from the western site of Lake Mälaren than at the eastern site. One possible explanation for the higher response in the western basin is that terrestrial DOC stimulates phytoplankton growth more than autochthonous DOC. Alternatively, it is possible, that the differences in phytoplankton growth rates were caused by competition from bacteria, which were stimulated differently by the different quality of DOC at the eastern and western site. Studies have shown that autochthonous, labile DOC supports higher bacterial growth rates than humic, allochthonous DOC (Fonte *et al.*, 2013; Attermeyer *et al.*, 2014). Hence the higher proportion of autochthonous DOC at the eastern site may have stimulated bacteria growth, leading to increased competition with phytoplankton for inorganic nutrients and finally resulting in lower algae growth rates.

Another explanation for the higher growth rates in the western basin are differences in ambient nutrient concentrations. At the western site higher phosphorus and iron concentrations were observed, which might lead to higher growth rates. In addition to nutrients that were measured during the experiment also other nutrients such as nitrogen or silica might have been more abundant in western waters. According to monitoring data from Mälaren silica and nitrogen are more abundant in the western part of the lake than at the eastern part, indicating that the risk for nitrogen and silica limitation is higher in the eastern than in the western basin (Sonesten *et al.*, 2013). Besides the stimulation by nutrients or terrestrial DOC, also the phytoplankton community itself could explain the different responses. Because most rivers enter Mälaren at the western site, the phytoplankton community in the western basin might be more used to changing chemical conditions, resulting in a higher capacity to adapt to new chemical environments. This might lead to higher growth rates in a laboratory environments compared to the possibly less fit and less adapted community from the eastern site, which are used to stable conditions.

Besides the higher overall growth rates, the phytoplankton community from the western site also showed a stronger response to phosphorus addition than the eastern community, as shown by the significant interaction between P and experiment in the ANOVA on both experiments together. This may indicate that the phytoplankton community in the western basin is more strongly P-limited. Alternatively, differences in the physiological capacities to take up and store P could explain the differences between the two experiments. Since P-uptake rates, P-storage capacities and maximum specific growth rates differ among taxa (Andersen, 1997), the phytoplankton community in the western basin might be composed of species that are more

efficient in using the P, that was added in my experiments. Moreover, species respond differently to the single saturating addition of P. Some species have high initial P-uptake rates, high initial growth rates and a short lag phase, and are therefore superior competitions when the P pulse is of short duration. However, other species appear to be better adapted to long lasting P pulses, since they have higher long-term uptake rates, higher storage capacities, but a long lag phase (Spijkerman and Coesel, 1998). Hence the higher response to P addition observed at the western site of Lake Mälaren may be due to a phytoplankton community composed of taxa with a short lag phase and high initial P-uptake and growth rates.

4.2 Discussion of methods

4.2.1 Total reactive iron method

Total reactive iron was measured using a modified version of Verschoor and Molot (2013) (see Fig. 2-3 appendix). The measurements of TRFe 1 h and 21 h after adding the reductant (Fig. 16) reveal that there are fractions of Fe that are quickly available and others that are less accessible for the reductant. To identify these fractions and get further information on the chemical form, in which Fe was present in the experiments, a model by Sjöstedt *et al.* (2010) was used. In this model pH, DOC, total P and total Fe concentrations were used to calculate whether Fe is organically bound (Fe-DOC) or in form of ferrihydrite, using the program VisualMinteq (Gustafsson 200x) (Sjöstedt *et al.*, 2010). A correlation between the modelled chemical form of Fe and the TRFe concentrations measured after 1 h (Fe_{fast}) and the difference between TRFe measured after 21 h and 1 h (Fe_{slow}) for the initial samples in experiment west reveals that the fast reacting Fe is ferrihydrite (Fig. 21), while the slow reacting Fe is probably half ferrihydrite and half Fe bound to DOC (S. Köhler, unpublished results). The good fit between the modelled form of Fe and measured Fe_{fast} and Fe_{slow} allows drawing conclusions about the form in which Fe is present, simply based on measurements, where no modelling is required. Therefore I put forward a method to determine the amount of ferrihydrite relative to organically bound Fe by means of TRFe measurements 1 h and 21 h after addition of the reductant.

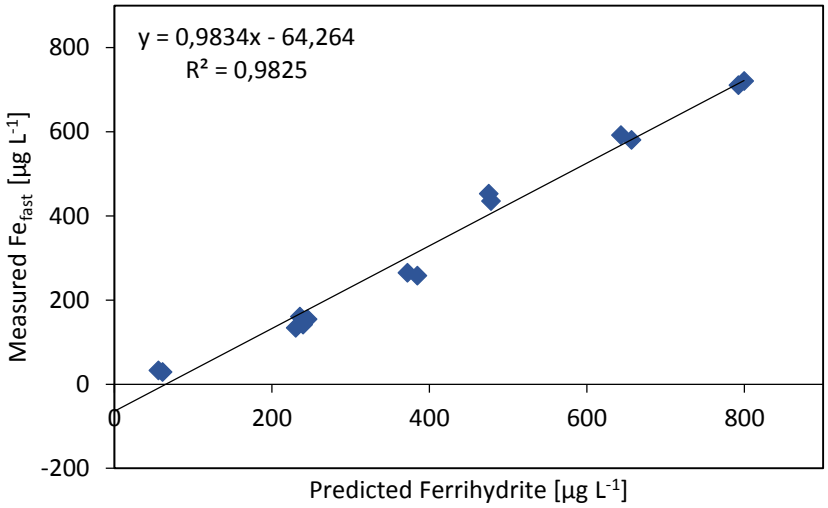


Figure 21: Plotting the ferrihydrite predicted by the model versus Fe_{fast} measured after 1 h gives a very good fit with an intercept close to zero and a slope close to 1. Hence all total reactive iron measured after 1 h incubation time is ferrihydrite (S. Köhler, unpublished results).

4.2.2 Chemical form of spiked iron

A comparison between treatments of the fast and slow reacting fractions of Fe show that the added iron is exclusively in form of fast reacting Fe (ferrihydrite), indicating that DOC did not bind to the spiked Fe. Probably the DOC was already saturated in Fe and could not bind further iron. Also Maloney *et al.* (2005) found a tipping point when adding Fe did not further increase water colour. Since DOC maintains Fe in solution and prevents it from precipitating, Fe mainly contributes to water colour when it is bound to DOC. Hence the tipping point when adding Fe did not further increase water colour indicates that DOC was saturated in Fe and cannot not bind further Fe. Instead of being bound to DOC, Fe then precipitates and does not further contribute to water colour (Maloney *et al.*, 2005).

4.2.3 Removal capacity of the nanofiltration membrane

As intended, the nanofiltration membrane removed more than 70 % of DOC. The nanofiltration process did not only change the DOC concentration, but also significantly effected iron concentration, total P concentration, absorbance at 420 nm and 254 nm, freshness index, humification index and fluorescence index, indicating that the membrane also removes nutrients and alters water colour and DOC quality. The DOC type in the retentate was older, more humified and with a higher contribution of terrestrial derived fulvic acids than the DOC type in the permeate, demonstrating that the membrane tends to remove allochthonous DOC, while autochthonous DOC can pass the membrane (Fig. 22). Moreover, nanofiltration removed total reactive iron and phosphorus. To specify which form of iron and phosphorus was removed, the model by Sjöstedt *et al.* (2010) (see above) was used. The model revealed that the fast reacting iron was ferrihydrite and the slow reacting iron was 50 % Fe bound to DOC and 50 %

ferrihydrite. Furthermore, the model calculated that the PO_4 measured in the experiments was not bound to ferrihydrite, while the difference between measured total P and PO_4 was PO_4 bound to ferrihydrite (S. Köhler, unpublished results). During nanofiltration fast reacting iron was reduced by more than 95 % and slow reacting iron by more than 80 %, indicating that ferrihydrite as well as iron bound to DOC is removed by the membrane. Ferrihydrite can exist as ferrihydrite >0.2 µm (large particulate ferrihydrite) or as ferrihydrite <0.2 µm (small colloidal ferrihydrite) (Neubauer *et al.*, 2013). Probably it is the fraction of ferrihydrite with large molecular size, which is retained by the membrane, while small ferrihydrite can pass. Moreover P is removed during nanofiltration. While PO_4 that is not bound to ferrihydrite can pass the nanofiltration membrane, more than 70 % of PO_4 that is bound to ferrihydrite is removed during the nanofiltration process. Probably nanofiltration removes the PO_4 that is bound to large ferrihydrite, but PO_4 bound to small ferrihydrite is retained. As indicated by lower absorbances in the permeate, nanofiltration reduces the water colour. The reduction in water colour can be attributed to the removal of iron and terrestrial, more coloured DOC.

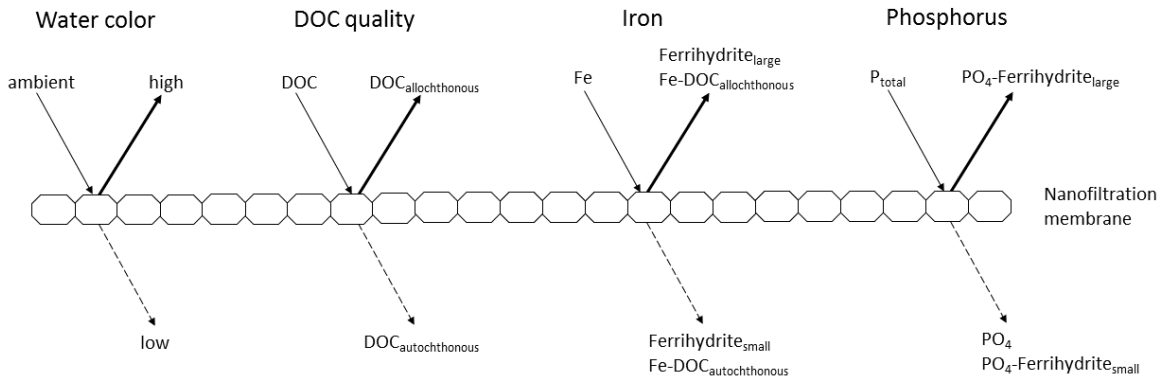


Figure 22: Removal processes by nanofiltration membrane. Ordinary arrows represent ambient water, bold arrows represent retentate and dashed arrows represent permeate. Nanofiltration changes iron concentrations (Fe), phosphorus concentrations (P), dissolved organic carbon (DOC) quality and water colour. The fractions that can more easily pass the membrane are autochthonous DOC ($DOC_{autochthonous}$), ferrihydrite <0.2 µm ($Ferrihydrite_{small}$), iron bound to autochthonous DOC ($Fe\text{-}DOC_{autochthonous}$), phosphate not bound to ferrihydrite (PO_4) and phosphate bound to small ferrihydrite ($PO_4\text{-}Ferrihydrite_{small}$). The molecules that are mostly retained by the membrane are allochthonous DOC ($DOC_{allochthonous}$), ferrihydrite >0.2 µm ($Ferrihydrite_{large}$), iron bound to allochthonous DOC ($Fe\text{-}DOC_{allochthonous}$) and phosphate bound to large ferrihydrite ($PO_4\text{-}Ferrihydrite_{large}$).

4.2.4 Alternatives to nanofiltration for creating a DOC gradient

Nanofiltration was chosen as a method for manipulating DOC, since I consider the resulting DOC gradient as being most realistic and close to natural conditions, compared to other options, mainly because the natural DOC type is kept. However, using nanofiltration to create a DOC gradient introduces some experimental limitations. As mentioned before, nanofiltration changes the type of DOC towards more terrestrial, humified, older carbon in DOC_{high} treatments. Since the change of DOC character possibly influences phytoplankton growth, the effect of DOC

concentration and DOC character are difficult to disentangle. One possible option to manipulate DOC concentration independent from DOC type, is the use of artificial DOC (Stets and Cotner, 2008; Peura *et al.*, 2014; Blomqvist *et al.*, 2001a; Andersson *et al.*, 2013, Blomqvist *et al.*, 2001b) or dried DOC, isolated from other humic lakes (Hessen *et al.*, 2004; Andersson *et al.*, 2013). The downside of these methods is that the added DOC is not the natural type of DOC occurring in the corresponding aquatic system. Since DOC is a complex and diverse substance group with varying functions, it is likely that the system reacts different to different types of DOC. Hence it is important to keep the natural type of DOC to get a realistic and nature-like response. Another experimental limitation of adding DOC instead of using nanofiltration is that the creation of DOC concentrations lower than the ambient one is not possible.

Nanofiltration did not only change DOC, but also nutrient concentrations. Since P and Fe concentrations co-varied with DOC during nanofiltration, DOC_{high} treatments contained more Fe and P than the DOC_{low} treatments. It is possible that the higher amounts of nutrients in DOC_{high} treatments affected phytoplankton growth. This nutrient effect is not distinguishable from the pure DOC effect in my experimental design, since nutrients and DOC co-vary. One possible option to disentangle both effects would be to compensate for the nutrients lost during nanofiltration. However, adding nutrients to DOC_{low} and $DOC_{ambient}$ treatments to reach the same nutrient levels as in DOC_{high} also causes some experimental problems. Since we would add nutrients to $DOC_{ambient}$ treatments, these would not reflect the natural conditions found in the lake any more. Furthermore, it is likely that added nutrients are more available to phytoplankton than the nutrients that cannot pass the nanofiltration membrane and thus remain in the water used to construct DOC_{high} treatments. The nutrients that cannot pass the membrane are probably strongly bound to DOC or in form of insoluble PO_4-Ferrihydrite complexes and are only to a small extent accessible for phytoplankton. Thus, compensating for these nutrients with easily accessible nutrients entails the risk that nutrients in DOC_{low} treatments stimulate phytoplankton more than the nutrients in DOC_{high} treatments. Hence compensating for nutrients lost during nanofiltration, might also distort the effect of DOC.

Besides the problem of disentangling nutrient and DOC effects, the process of co-varying nutrients with DOC during nanofiltration can be seen as a positive side effect, since it mimics the natural situation. Several studies have shown, that nutrient concentrations in freshwaters increase with increasing terrestrial DOC input, since nutrients are closely associated with DOC (Finstad *et al.*, 2013; Dillon and Molot, 2005; Hessen *et al.*, 2010; Larsen *et al.*, 2011; Hessen *et al.*, 2009; Thrane *et al.*, 2014). Hence the increase of Fe and P when increasing DOC concentrations during nanofiltration resemble trends observed in nature.

4.2.5 Experimental scale

When conducting experiments it is important to consider the experimental scale, both in time and space. To investigate the effects of increasing DOC concentrations on phytoplankton

growth with special focus on the availability of nutrients, I choose a laboratory microcosm experiment. Experiments on a microscale allow for replication and therefore create ideal datasets for statistical analysis. They are a good tool to understand a single feature of a complex system, because the system is simplified. However, due to the small size and short duration of microscale experiments they might exclude or distort important features and might therefore not represent ecological phenomena. They can lead to a conclusion that cannot directly be extrapolated to natural systems (Carpenter, 1996; Schindler, 1998). To overcome these limitations, large-scale, mesocosm experiments or whole-lake manipulations are alternatives. They add more complexity to the system and are closer to nature. However, large-scale experiments are difficult to replicate and hence one must be careful about inferring from one single lake to other lakes, since the initial conditions might differ (Sterner, 2008). Furthermore small effects might be overshadowed by bigger effects in large-scale experiments. There are also external conditions or disturbances that cannot be controlled, which might lead to changes in the system, that are not a direct response of the treatment. Hence in large-scale experiments it is more difficult to understand the cause-effect relationship. Since the aim of this study was to investigate the cause-effect relationship between DOC, Fe and P and phytoplankton growth, I considered a microscale experiment to be most suitable. To focus on the effect of DOC and nutrients in a best possible way, other factors such as light, bacteria or grazing were excluded from the manipulation. However, I fully acknowledge, that these factors are important in understanding the overall response of primary productivity and that studies at ecosystem scale should be taken into account to be able to make accurate predictions and take good management decisions.

4.2.6 Effect of grazing

The experiment was constructed to exclude grazing pressure on phytoplankton. However, in the end of the incubation period in some treatments copepods were present occasionally. Possibly the copepods were small enough to pass the 0.24 mm net in the beginning of the experiment, but grew large during incubation. The copepods could represent an unintended grazing pressure, affecting phytoplankton biomass and causing variation between replicates. Nevertheless, since variation among replicates was still small, I conclude that grazing did not substantially affect chlorophyll *a* concentrations.

4.3 Limitations of this study and suggestions for further research

4.3.1 Possible N-limitation

It has been shown that also nitrogen can be an important limiting nutrient in freshwater ecosystems and cause eutrophication in lakes (Bergstrom and Jansson, 2006; Bergström *et al.*, 2013; Elser *et al.*, 2009; Hogan *et al.*, 2014; Mischler *et al.*, 2014). The comparison between

the treatments with and without N addition suggests that N was not acting as a main limiting nutrient in my experiments. This is supported by Sonesten *et al.* (2013), who found that nitrogen limitation in Lake Mälaren is not likely to occur during the season when the water sampling for the trials took place (Sonesten *et al.*, 2013). However, since in this study no Fe and P was added to the control treatment without N addition, the result solely shows that N was not the main limiting nutrient, but possibly N was co-limiting. For example, N can mediate the ability of primary producers to access P and is thus act as a co-limiting nutrient together with P (Perini and Bracken, 2014; Harpole *et al.*, 2011; Sterner, 2008). Moreover N can be co-limited by Fe. Results from enrichment experiments revealed that Fe facilitates the uptake of N (Romero *et al.*, 2013), since both, nitrate up-take and nitrogen fixation are catalysed by enzymes with Fe as a co-factor, namely nitrogenase (Kupper *et al.*, 2008) and nitrate reductase (North *et al.*, 2007). By reducing the N limitation when Fe was added, the phytoplankton community becomes more strongly P limited. Changes in P concentrations would then result in a larger response in growth rate. In case the main role of Fe was to stimulate N-uptake in my experiments, the observed P-Fe-co-limitation in this study is not an independent nutrient co-limitation of P and Fe, but instead a biochemically dependent co-limitation of P, N and Fe (Saito *et al.*, 2008). To check whether N acts as a co-limiting nutrient, it would be advisable to establish an additional control treatment, where P and Fe, but no N was added ($DOC_{ambient}$, P, Fe, without N). If the chlorophyll *a* concentrations in the PFeN treatment would be higher than in PFe treatments, N is a co-limiting nutrient. To determine, whether N is co-limiting with P or with Fe, further treatments without N addition and with the addition of either Fe or P should be established. Moreover, it would be interesting to conduct the experiments in a primarily N limited system. This could be done with water from Galten taken between July and September, since the likelihood for N-limitation is highest in the western part of Lake Mälaren and in late summer (Sonesten *et al.*, 2013).

4.3.2 Effect of DOC on Fe availability

Since the phytoplankton communities in this study were mainly phosphorus limited, it would be important to conduct the experiments in primarily iron-limited systems. This would allow getting a better understanding of the interactions between Fe and DOC and answering the question whether DOC makes Fe more or less available to phytoplankton.

4.3.3 Disentangling effects of DOC quality and phytoplankton community

Since for each experiment the ambient phytoplankton communities were used and different phytoplankton communities might perform differently, it is not possible to conclude if differences between experiment west and east are due to differences in DOC quality, phytoplankton community performance or other factors (e.g. nutrients that were not measured such as silica). This limitation could be overcome by a reciprocal transplant design. In addition

to incubating phytoplankton communities in the water where they are originating from, the phytoplankton community originating from the western basin would be incubated in filtered water originating from the eastern basin, and the other way around. By using this experimental setup, the effects of DOC quality and phytoplankton community performance could be disentangled. However, using such a design in this study was impossible, because of logistic reasons.

4.3.4 Shifts in community composition due to P, Fe and DOC

It has been shown that different algal groups are differently stimulated by nutrient addition. Therefore it would be interesting to analyse if there are differences in community composition between the treatments. Twiss *et al.* (2000) found that mainly pico- and nanoplankton was favoured by iron addition (Twiss *et al.*, 2000). This is supported a study of De Wever *et al.* (2008), where they observed enhanced growth of prokaryotic picoplankton in Fe treatments, while NP stimulated green algae and partly diatoms (De-Wever *et al.*, 2008). Especially cyanobacteria respond strongly to Fe additions, since they have higher iron requirements than eukaryotic algae. Therefore Fe could be an important explanation for the ability of cyanobacteria to dominate phytoplankton communities and the development of algal blooms (Hyenstrand *et al.*, 2001; Molot *et al.*, 2010; Sorichetti *et al.*, 2014b; Molot *et al.*, 2014; Sorichetti *et al.*, 2014a). Besides nutrients, also DOC concentration has been shown to influence phytoplankton community composition. As a result of the addition of uncoloured DOC to an oligotrophic clearwater lake a shift from obligate autotrophic phytoplankton towards mixo- and heterotrophic flagellates was found (Blomqvist *et al.*, 2001b). This is supported by field and whole-lake studies, concluding that mixotrophic flagellates are able to outcompete obligate autotrophic phytoplankton under DOC-rich conditions (Bergström *et al.*, 2003). Also an analysis of lakes along a DOC gradient reports the dominance of large autotrophic phytoplankton in clearwater lakes, while picophytoplankton dominated the community at intermediate DOC concentrations and flagellates in humic lakes (Blomqvist *et al.*, 2001a; Drakare *et al.*, 2003; Blomqvist *et al.*, 2001b). In a time series analysis over 14 years of nutrient-poor lakes a decrease of chlorophytes coherent with increasing water colour was found (Bloch and Weyhenmeyer, 2012). Based on results from these studies, I would hypothesise that also in my experiments different components of the phytoplankton community respond differently to different nutrients. In this study, I preserved phytoplankton samples of all treatments with Lugol´s solution, but due to time limitations it was so far not possible to analyse them for community composition.

4.3.5 Influence of competition by bacteria on phytoplankton

It is possible that bacteria influenced chlorophyll *a* concentrations in my experiment. Several studies have shown that DOC subsidises bacteria (Tranvik, 1988; Roiha *et al.*, 2012; Jones,

1992) and bacteria might outcompete phytoplankton for nutrients (Drakare *et al.*, 2002; Stets and Cotner, 2008; Blomqvist *et al.*, 2001b). However, other studies found no effect of bacteria on phytoplankton abundance due to increases in DOC (Faithfull *et al.*, 2011a; Peura *et al.*, 2014; Faithfull *et al.*, 2011b). To see if bacteria were supported by DOC in this study and if bacteria are negatively correlated to chlorophyll a concentrations, it would be necessary to count bacteria cells. For the experiment with water from the western basin bacteria samples have been preserved, but due to time constraints they have not been analysed so far. I would hypothesise that there are more bacteria in the DOC_{high} treatments than in the DOC_{low} treatments, since additional carbon seems to promote bacteria growth. However, since the lowest growth rates were observed in waters with low DOC concentrations with potentially low bacteria abundance, I assume that in this study competition by bacteria was not an important factor influencing primary productivity.

4.3.6 Including effect of DOC on light attenuation

This experiment focused on the effects of DOC on nutrient availability and P and Fe as stimulators for algae growth, while excluding the effect of DOC and Fe on light attenuation. However, several studies have shown that the effect of DOC on light extinction is of great importance for phytoplankton and might decrease primary productivity in lakes (Einem and Granéli, 2010; Finstad *et al.*, 2013; Andersson *et al.*, 2013; Karlsson *et al.*, 2009; Thrane *et al.*, 2014). Including the light effect in the experiments would be an important step in making more realistic predictions about the overall effect of DOC on lake productivity.

4.3.7 Number of replicates

For similar experiments conducted in the future, I would suggest to reduce the number of replicates from five to four replicates per treatment, since the within treatment variation was very small compared to the among treatment variation. Reducing the number of replicates would allow adding more treatments. For instance treatments could be added to test the effect of other nutrients (e.g. nitrogen or silica), have a larger gradient in DOC concentration or test waters of different eutrophication status or DOC qualities.

5 Conclusions

This study shows that P, Fe and DOC as well as interactions between them regulate primary productivity in Lake Mälaren (Fig. 23). P was the primarily limiting nutrient, having the largest overall effect on phytoplankton growth. Fe was a co-limiting nutrient, further stimulating primary productivity in the presence of P. The results show that DOC per se had a stimulating effect on phytoplankton growth rate, but P and Fe modify this effect. Under ambient P concentrations, Fe and DOC interact to regulate PP. It turned out that the bioavailability of Fe is affected by DOC and the direction of this effect depends on DOC type. This indicates that different chemical composition of DOC influences Fe bioavailability differently. Adding iron to water, where DOC was mainly of allochthonous origin showed a negative effect on phytoplankton growth rates, while adding iron to waters with autochthonous DOC sources showed no effect. Assuming the scenario that P concentrations remain unchanged and terrestrial DOC inputs increase in the future, phytoplankton growth would increase in the absence of Fe addition, but decrease if Fe concentrations increase.

Under P-rich conditions, increasing DOC concentrations result in a lower response of phytoplankton to P addition compared to ambient DOC concentrations. Hence DOC-rich systems, such as brown humic lakes, may be more resistant towards P inputs when compared to low-DOC systems, such as clear-water lakes. I also infer that with increasing DOC concentrations lakes could become less sensitive towards P-input and eutrophication in general. To conclude, the study reveals that the effect of increasing DOC largely depends on DOC quality and nutrient regime.

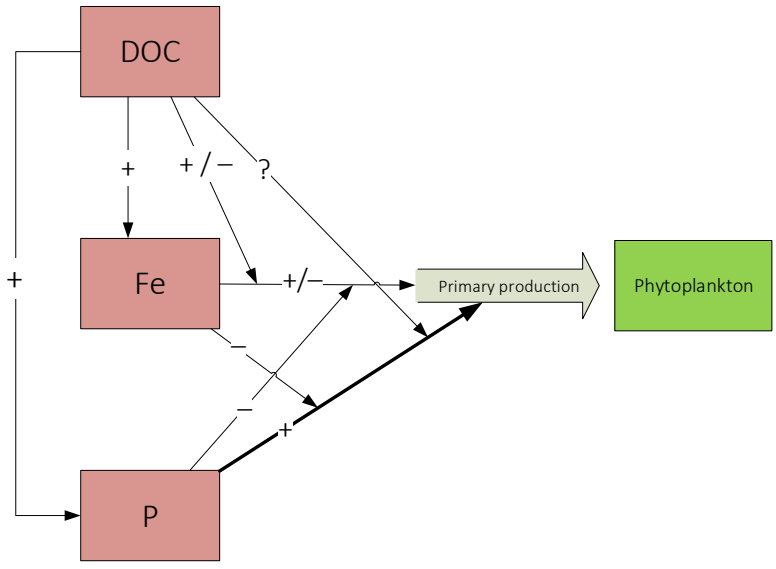


Figure 23: Ways how DOC, P and Fe affect primary productivity (PP) in Lake Mälaren, based on the results of this study. P increases PP and was the main limiting nutrient. Fe increases PP when P was added, but has no or a negative effect on PP when no P was added. Fe and P precipitate with each other, reducing available Fe and P. Fe and P are associated to DOC. DOC makes Fe more or less available, depending on DOC quality. The effect of DOC on P availability remains unclear.

6 Popular summary

In a world without water, there would not only be no oceans, lakes and rivers, but also no life because humans, animals and plants need water to survive. Freshwater is the most important essential resource for every human society, as we need it for drinking water supply, irrigation to produce crops, fish production, industry and numerous other areas. However, only a very tiny fraction of the world´s total water volume is freshwater, which is readily accessible as surface water. This readily accessible water is stored in lakes, rivers or wetlands. Since it is such an essential, but limited resource, it is of special importance to maintain a good water quality for us and for future generations.

Phytoplankton in lakes are a key factor in determining water quality. Phytoplankton are microscopic plants that float freely in the water body. One single phytoplankton individual is often too small to be seen with the naked eye, but when present in high numbers they are visible as a green discoloration of the water. Even though phytoplankton are an important food source for other organisms and largely determine fish production, their excessive growth poses many threats. For example algae blooms can cause fish kills, the loss of biodiversity as well as problems for drinking water production and health, due to poisonous substances produced by some phytoplankton species. So what determines the amount of phytoplankton in a lake? Mainly it is nutrients, which phytoplankton need to survive and grow. Especially phosphorous is often a key limiting factor for freshwater ecosystems and causes the excessive growth of phytoplankton when overly supplied. There are also other essential plant nutrients, such as iron, that may affect the growth of phytoplankton. Recently, researchers discovered that also dissolved organic carbon determines the growth of phytoplankton in a lake. Dissolved organic carbon is the fraction of natural organic matter that is smaller than 0.45 micrometres. Either it is produced in the lake or it is imported from terrestrial soils and wetlands into the lake. In contrast to inorganic phosphorus and iron, dissolved organic carbon cannot be taken up by phytoplankton, but it can influence the growth of phytoplankton in many indirect ways. For example, dissolved organic carbon can increase phytoplankton growth by supplying inorganic key nutrients associated with it or by making them more available to phytoplankton. On the other hand dissolved organic carbon can decrease phytoplankton growth by forming complexes with iron and phosphorus and thus making them unavailable for phytoplankton. Apparently, dissolved organic carbon can both, stimulate and suppress the growth of phytoplankton in a lake.

Due to human impacts, such as climate change, altered land use and reversed acidification, more and more organic carbon is entering lakes. During the past 40 years the concentration of dissolved organic carbon has been continuously increasing in thousands of lakes in the Northern hemisphere and it is predicted to further increase in the future. Given these changes in dissolved organic carbon loads to lakes, it is of great importance to understand how phytoplankton will

respond. When more dissolved organic carbon is entering the lake, will this suppress the growth of phytoplankton, or will it stimulate them, causing more algae blooms? What happens when not only dissolved organic carbon concentrations increase, but also phosphorus and iron inputs? Are lakes with dissolved organic carbon concentrations more or less sensitive to phosphorus and iron inputs? And also: Do iron and phosphorus interact with dissolved organic carbon to regulate pyhtoplankton growth? To come a little bit closer to an answer to all these questions, I conducted laboratory experiments in a climate chamber with water from Lake Mälaren, the third largest lake in Sweden. The most interesting result from these experiments was that it depends on the amount of iron and phosphorus in the water, if dissolved organic carbon stimulates or suppresses phytoplankton growth. Apparently iron, phosphorus and dissolved organic carbon interact to regulate phytoplankton growth. In waters with high phosphorus concentrations, increasing inputs of dissolved organic carbon stimulated phytoplankton growth much less than in waters with low phosphorus concentrations. This means that lakes, that already have high levels of phosphorus, are not so sensitive to increasing inputs of organic carbon compared to those lakes with low phosphorus concentrations. It also connotes, that phosphorous stimulates the growth of phytoplankton to a lesser extent in lakes with higher levels of dissolved organic carbon. Hence the increasing concentrations of dissolved organic carbon in the future could make lakes less sensitive to phosphorus inputs from human discharges.

Overall, the conducted experiments also showed that higher dissolved organic carbon inputs stimulate the growth of phytoplankton, mainly because dissolved organic carbon transports nutrients into the lake. This implies that the amount of phytoplankton could increase in the future. However, we have to be careful with this statement, since it is just based on the result of laboratory experiments. Outside in the field also other factors play a role. For example dissolved organic carbon attenuates a lot of light. Since phytoplankton need light to grow, higher dissolved organic carbon concentrations can suppress phytoplankton growth. Clearly, dissolved organic carbon can influence phytoplankton in many different ways. This makes it quite difficult to predict what will happen to phytoplankton in lakes in the future. The net response of phytoplankton to dissolved organic carbon depends on many factors, such as nutrient content, temperature, location and lake shape. Therefore each individual lake might react differently to the higher dissolved organic carbon inputs. To be able to make more precise predictions about the net response of phytoplankton to dissolved organic carbon, more research is needed.

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9 Appendix

Figure 1: Sampling sites in Lake Mälaren in the western basin (left picture) and eastern basin (right picture).

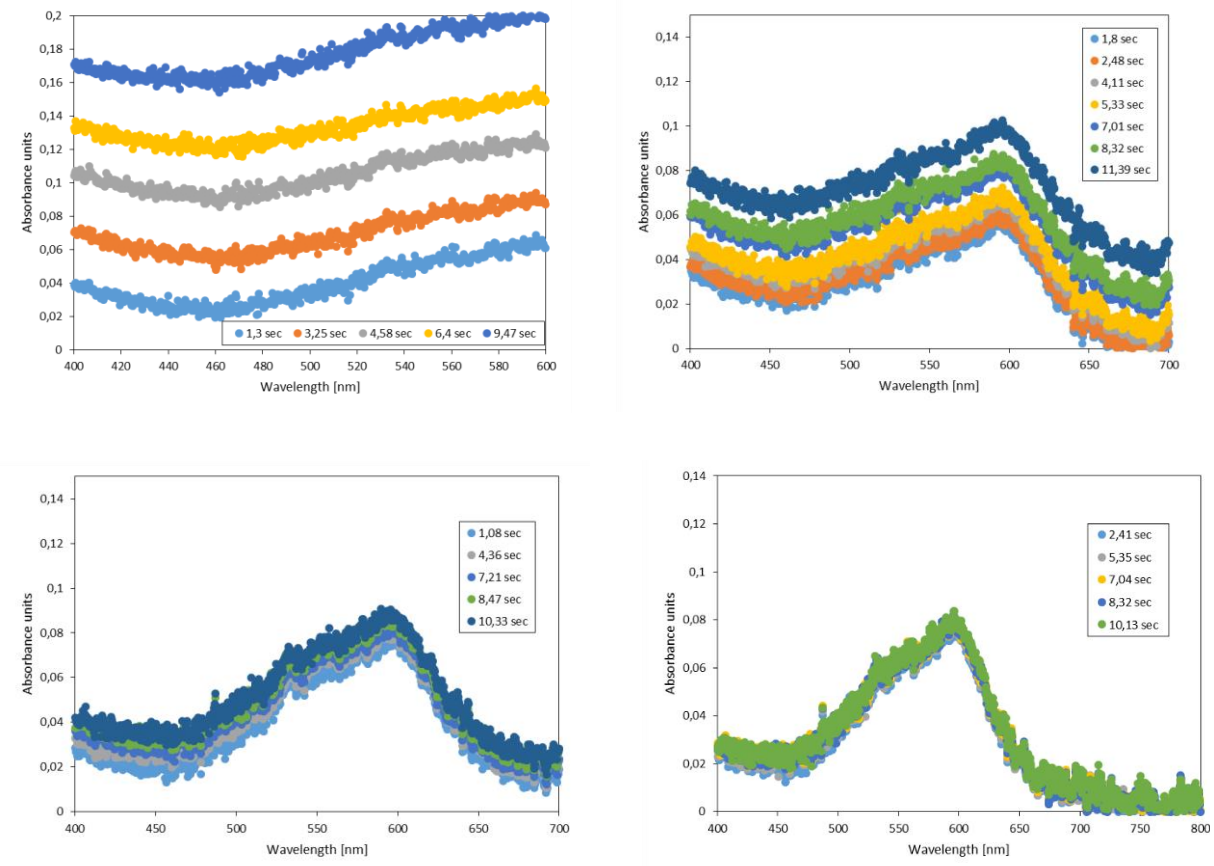

Figure 2: The absorbance spectra of various TPTZ concentrations (0.5 mL top left, 0.35 mL top right, 0.25 mL bottom left, 0.15 mL bottom right) measured at different times after the addition of TPTZ. It turned out that the measured absorbances (used as an indicator for total reactive iron concentrations) increases over time, due to flocculation processes. The more TPTZ was added, the higher was the increase in absorbance over time. To avoid a time dependent effect on the measured absorbance, a concentration of 0.1 mL TPTZ was chosen to measure total reactive iron. Moreover, iron was measured as quick as possible after the addition of TPTZ.

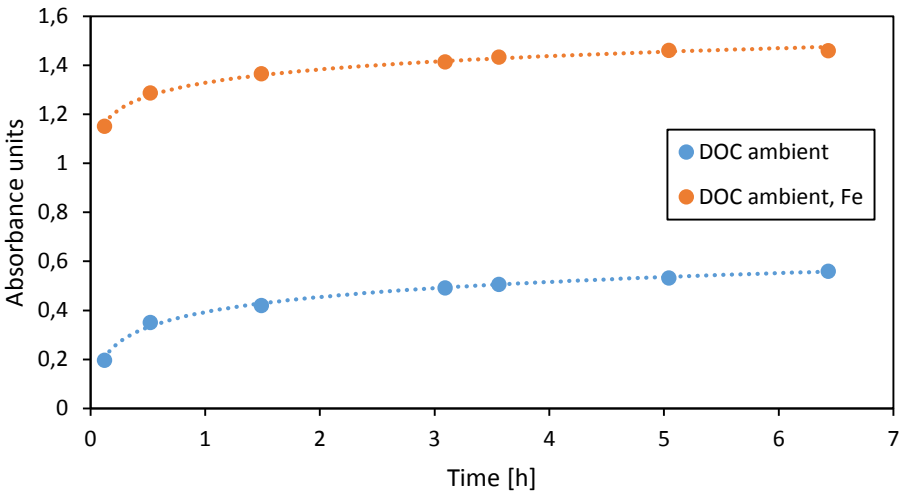

Figure 3: Absorbance at 595 nm (used as an indicator for total reactive iron concentrations) measured at different times after the addition of ascorbic acid reductant for samples of ambient DOC concentration, with and without iron addition. The trend line is logarithmic. The measurements show that absorbance increased over time, first very quickly and later slowly levelling off. This indicates that parts of the iron fraction are readily available, while others are not so easily accessible for the reductant. To get an estimate on how much iron is readily available and how much is not so easily accessible, iron was measured after 1 h and 21 h for initial samples from the western basin. For all other samples the time point 21 h was selected, since also the iron, which is more difficult to access, could be important for phytoplankton growth.

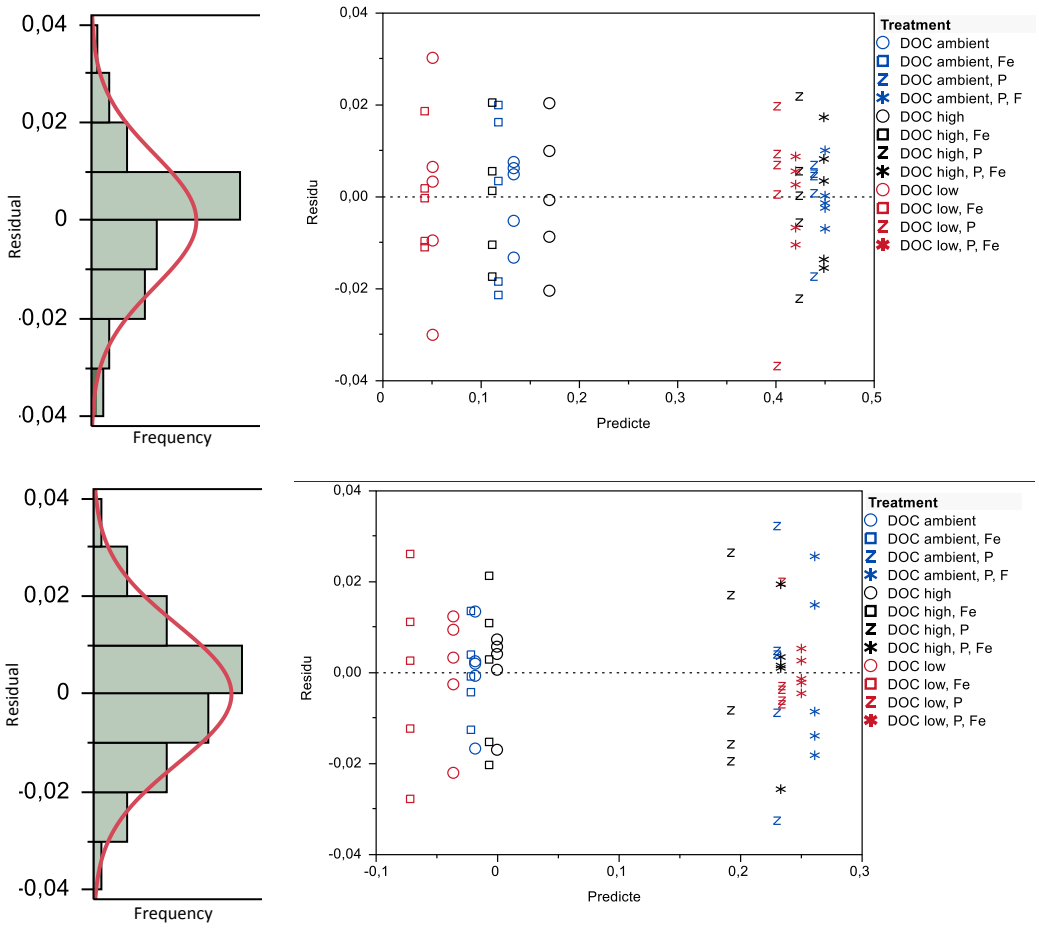

Figure 4: Residual plots for specific phytoplankton growth rate from ANOVA for experiment with water from the western basin (top) and water from the eastern basin (bottom) of Lake Mälaren. The frequency analysis of residuals (left) demonstrates that the distribution of residuals does not differ signifcantly from a fitted normal distribution (red line) (Shapiro-Wilk´s test, experiment east: W=0.989, p=0.86; experiment west: W=0.981, p=0.49). In the residual by predicted plot (right) residuals are approximately equally scattered around zero within all treatments, demonstrating that the variances within treatments are similar.

Table 1: Parameter estimates from full factorial ANOVA for specific phytoplankton growth rate in experiment with water from the western and eastern site.

	Experiment west			Experiment east		
Effect	Estimate	t	p	Estimate	t	p
Intercept	0.2673	138.59	<0.0001	0.1037	50.96	<0.0001
Fe	-0.0023	-1.17	0.2468	0.0035	1.7	0.095
P	0.1630	84.5	<0.0001	0.1296	63.68	<0.0001
Fe*P	0.0115	5.99	<0.0001	0.0112	5.49	<0.0001
DOC low	-0.0388	-14.23	<0.0001	-0.0097	-3.37	0.0015
DOC ambient	0.0176	6.47	<0.0001	0.0090	3.13	0.003
DOC high	0.0212	7.77	<0.0001	0.0007	0.24	0.8078
Fe*DOC low	0.0047	1.72	0.0911	-0.0084	-2.92	0.0053
Fe*DOC ambient	0.0014	0.52	0.6063	0.0032	1.12	0.268
Fe*DOC high	-0.0061	-2.24	0.0296	0.0052	1.8	0.0786
P*DOC low	0.0191	7.01	<0.0001	0.0182	6.33	<0.0001
P*DOC ambient	-0.0035	-1.3	0.201	0.0034	1.17	0.2488
P*DOC high	-0.0156	-5.71	<0.0001	-0.0216	-7.5	<0.0001

Table 2: Parameter estimates from full factorial ANOVA excluding P addition treatments for specific phytoplankton growth rate in experiment with water from the western and eastern site.

	Experiment west				Experiment east	
Effect	Estimate	t	p	Estimate	t	p
Intercept	0.1043	35.61	<0.0001	-0.0259	-9.89	<0.0001
Fe	-0.0138	-4.71	<0.0001	-0.0077	-2.94	0.0072
DOC low	-0.0579	-13.98	<0.0001	-0.0279	-7.54	<0.0001
DOC ambient	0.0212	5.11	<0.0001	0.0056	1.52	0.1413
DOC high	0.0368	8.87	<0.0001	0.0223	6.02	<0.0001
Fe*DOC low	0.0093	2.25	0.0338	-0.0102	-2.74	0.0113
Fe*DOC ambient	0.0061	1.48	0.1527	0.0057	1.54	0.1362
Fe*DOC high	-0.0154	-3.73	0.001	0.0045	1.2	0.2412

Table 3: Parameter estimates from full factorial ANOVA on both experiments together for specific phytoplankton growth rate.

Effect	Estimate	t	p
Intercept	0.1855	132.31	<0.0001
Fe	0.0006	0.43	0.6682
P	0.1463	104.34	<0.0001
Fe*P	0.0114	8.1	<0.0001
DOC low	-0.0243	-12.24	<0.0001
DOC ambient	0.0133	6.72	<0.0001
DOC high	0.0109	5.52	<0.0001
Fe*DOC low	-0.0018	-0.93	0.3535
Fe*DOC ambient	0.0023	1.17	0.2448
Fe*DOC high	-0.0005	-0.24	0.8125
P*DOC low	0.0187	9.42	<0.0001
P*DOC ambient	-0.0001	-0.04	0.9647
P*DOC high	-0.0186	-9.37	<0.0001
Fe*P*DOC low	-0.0014	-0.72	0.4722
Fe*P*DOC ambient	-0.0036	-1.81	0.073
Fe*P*DOC high	0.0050	2.53	0.0129
Water west	0.0818	58.33	<0.0001
Fe*Water west	-0.0029	-2.04	0.0438
P*Water west	0.0167	11.89	<0.0001
Fe*P*Water west	0.0002	0.14	0.8925
DOC low*Water west	-0.0146	-7.34	<0.0001
DOC ambient*Water west	0.0043	2.18	0.0318
DOC high*Water west	0.0102	5.16	<0.0001
Fe*DOC low*Water west	0.0066	3.3	0.0013
Fe*DOC ambient*Water west	-0.0009	-0.46	0.6489
Fe*DOC high*Water west	-0.0056	-2.85	0.0054
P*DOC low*Water west	0.0004	0.22	0.8247
P*DOC ambient*Water west	-0.0034	-1.74	0.0852
P*DOC high*Water west	0.0030	1.52	0.1325
Fe*P*DOC low*Water west	-0.0032	-1.61	0.1105
Fe*P*DOC ambient*Water west	-0.0011	-0.56	0.5772
Fe*P*DOC high*Water west	0.0043	2.17	0.0325

Table 4: Final biological and chemical data from experiment with water from eastern site of Lake Mälaren. Values are given as mean values ± standard deviation. N=5 for specific phytoplankton growth rate [d^{-1}], chlorophyll *a* [µg L^{-1}], total reactive iron (TRFe) [µg L^{-1}], dissolved organic carbon (DOC) [mg L^{-1}], absorbance at 420 nm (A_{420}), absorbance at 254 nm (A_{254}), absorbance ratio between 254 nm and 365 nm (A_{254}/A_{365}), specific UV absorbance at 254 nm ($SUVA_{254}$) [L mg C^{-1} m^{-1}], specific visible absorbance at 420 nm (SVA_{420}) [L mg C^{-1} m^{-1}] and specific visible absorbance at 335 nm (SVA_{335}) [L mg C^{-1} m^{-1}]. N=1 for the humification index (HIX), fluorescence index (FI), freshness index (ß:α) and pH.

Treatment	Growth rate	Chlorophyll *a*	TRFe	DOC	A_{420}	A_{254}	A_{254}/A_{365}	$SUVA_{254}$	SVA_{420}	SVA_{335}	HIX	FI	ß:α	pH
DOC_{low}	-0.036 ± 0.014	2.79 ± 0.27	0 ± 0	3.6 ± 0.1	0.82 ± 0.16	9.46 ± 0.14	5.89 ± 0.68	2.63 ± 0.06	0.227 ± 0.043	0.723 ± 0.055	0.752	1.588	0.873	7.9
DOC_{low}Fe	-0.072 ± 0.021	2.14 ± 0.33	258 ± 16	3.5 ± 0.1	1.17 ± 0.12	10.97 ± 0.28	4.56 ± 0.28	3.17 ± 0.06	0.338 ± 0.030	1.067 ± 0.031	0.765	1.613	0.871	7.9
DOC_{low}P	0.234 ± 0.011	21.07 ± 1.87	0 ± 0	3.6 ± 0.1	0.82 ± 0.13	9.53 ± 0.17	5.92 ± 0.61	2.64 ± 0.04	0.227 ± 0.037	0.731 ± 0.054	0.804	1.593	0.820	8.0
DOC_{low}PFe	0.250 ± 0.004	23.69 ± 0.70	7 ± 8	3.4 ± 0.1	0.78 ± 0.24	9.07 ± 0.24	6.00 ± 1.19	2.65 ± 0.06	0.229 ± 0.074	0.730 ± 0.099	0.815	1.629	0.822	8.0
$DOC_{ambient}$	-0.018 ± 0.011	3.18 ± 0.25	0 ± 0	8.1 ± 0.3	1.44 ± 0.21	23.49 ± 0.59	6.44 ± 0.39	2.90 ± 0.06	0.178 ± 0.025	0.767 ± 0.022	0.869	1.512	0.721	8.0
$DOC_{ambient}$Fe	-0.022 ± 0.010	3.08 ± 0.23	368 ± 27	7.9 ± 0.3	1.95 ± 0.24	25.53 ± 0.92	5.46 ± 0.28	3.22 ± 0.07	0.245 ± 0.029	0.963 ± 0.046	0.824	1.537	0.766	8.0
$DOC_{ambient}$P	0.230 ± 0.024	20.71 ± 3.65	0 ± 0	8.3 ± 0.4	1.59 ± 0.16	23.79 ± 0.65	6.17 ± 0.28	2.86 ± 0.06	0.192 ± 0.023	0.784 ± 0.034	0.871	1.542	0.710	7.8
$DOC_{ambient}$PFe	0.261 ± 0.019	25.98 ± 3.83	196 ± 14	8.0 ± 0.2	1.76 ± 0.09	24.54 ± 0.56	5.75 ± 0.08	3.05 ± 0.06	0.219 ± 0.009	0.879 ± 0.019	0.863	1.549	0.717	8.1
DOC_{high}	< 0.001 ± 0.010	3.63 ± 0.26	<1 ± 1	12.6 ± 0.5	2.09 ± 0.16	35.36 ± 0.95	6.47 ± 0.21	2.81 ± 0.03	0.166 ± 0.015	0.747 ± 0.016	0.881	1.511	0.665	7.9
DOC_{high}Fe	-0.007 ± 0.017	3.48 ± 0.45	372 ± 15	12.4 ± 0.4	2.57 ± 0.10	37.24 ± 0.90	5.75 ± 0.09	3.00 ± 0.06	0.207 ± 0.011	0.871 ± 0.019	0.856	1.524	0.688	8.0
DOC_{high}P	0.192 ± 0.021	15.48 ± 2.44	2 ± 4	13.0 ± 0.6	2.15 ± 0.14	36.03 ± 0.58	6.45 ± 0.18	2.78 ± 0.11	0.166 ± 0.011	0.742 ± 0.029	0.881	1.559	0.667	8.0
DOC_{high}PFe	0.233 ± 0.016	20.99 ± 2.47	249 ± 17	12.4 ± 0.5	2.40 ± 0.21	36.68 ± 1.38	6.01 ± 0.17	2.95 ± 0.05	0.193 ± 0.017	0.830 ± 0.025	0.876	1.523	0.670	8.0
$DOC_{ambient}$ without algae light	0.286 ± 0.127	0.92 ± 0.62	0 ± 0	8.1 ± 0.3	1.53 ± 0.15	23.60 ± 0.61	6.34 ± 0.27	2.90 ± 0.05	0.188 ± 0.017	0.776 ± 0.022	0.871	1.524	0.707	7.9
$DOC_{ambient}$ without algae dark	-0.016 ± 0.017	0.07 ± 0.01	0 ± 0	8.2 ± 0.1	1.67 ± 0.15	23.87 ± 0.17	6.08 ± 0.21	2.92 ± 0.02	0.204 ± 0.018	0.796 ± 0.020	0.837	1.480	0.747	8.0
$DOC_{ambient}$ without N	-0.014 ± 0.011	3.28 ± 0.28	0 ± 0	8.2 ± 0.2	1.47 ± 0.26	23.75 ± 0.49	6.49 ± 0.56	2.88 ± 0.04	0.178 ± 0.031	0.762 ± 0.041	0.869	1.530	0.712	7.9

Table 5: Final biological and chemical data from experiment with water from western site of Lake Mälaren. Values are given as mean values ± standard deviation. N=5 for specific phytoplankton growth rate [d^{-1}], chlorophyll *a* [µg L^{-1}], total reactive iron (TRFe) [µg L^{-1}], dissolved organic carbon (DOC) [mg L^{-1}], absorbance at 420 nm (A_{420}), absorbance at 254 nm (A_{254}), absorbance ratio between 254 nm and 365 nm (A_{254}/A_{365}), specific UV absorbance at 254 nm ($SUVA_{254}$) [L mg C^{-1} m^{-1}], specific visible absorbance at 420 nm (SVA_{420}) [L mg C^{-1} m^{-1}] and specific visible absorbance at 335 nm (SVA_{335}) [L mg C^{-1} m^{-1}]. N=1 for the humification index (HIX), fluorescence index (FI), freshness index (ß:α) and pH.

Treatment	Growth rate	Chlorophyll *a*	TRFe	DOC	A_{420}	A_{254}	A_{254}/A_{365}	$SUVA_{254}$	SVA_{420}	SVA_{335}	HIX	FI	ß:α	pH
DOC_{low}	0.051 ± 0.022	4.05 ± 0.67	74 ± 17	3.8 ± 0.2	0.83 ± 0.06	11.50 ± 0.49	5.21 ± 0.23	3.07 ± 0.07	0.222 ± 0.018	0.975 ± 0.034	0.850	1.542	0.633	7.5
DOC_{low}Fe	0.042 ± 0.012	3.76 ± 0.34	478 ± 33	3.7 ± 0.1	1.25 ± 0.13	13.58 ± 0.57	4.29 ± 0.23	3.71 ± 0.13	0.341 ± 0.037	1.378 ± 0.087	0.827	1.564	0.639	7.5
DOC_{low}P	0.401 ± 0.022	56.08 ± 8.41	58 ± 14	3.9 ± 0.2	0.93 ± 0.23	11.48 ± 0.62	4.95 ± 0.51	2.93 ± 0.04	0.236 ± 0.056	0.983 ± 0.075	0.757	1.623	0.741	7.6
DOC_{low}PFe	0.420 ± 0.008	63.99 ± 3.90	412 ± 39	3.9 ± 0.2	1.07 ± 0.26	13.27 ± 0.64	4.57 ± 0.42	3.42 ± 0.14	0.277 ± 0.080	1.234 ± 0.107	0.844	1.640	0.638	7.6
$DOC_{ambient}$	0.133 ± 0.009	7.45 ± 0.49	271 ± 19	10.3 ± 0.6	2.81 ± 0.24	35.05 ± 1.36	4.96 ± 0.18	3.40 ± 0.22	0.273 ± 0.036	1.125 ± 0.098	0.898	1.489	0.541	7.4
$DOC_{ambient}$Fe	0.118 ± 0.019	6.68 ± 0.95	718 ± 15	10.0 ± 0.7	3.34 ± 0.20	37.77 ± 0.79	4.59 ± 0.17	3.78 ± 0.25	0.336 ± 0.039	1.315 ± 0.111	0.905	1.499	0.536	7.5
$DOC_{ambient}$P	0.438 ± 0.010	73.53 ± 5.24	256 ± 22	9.8 ± 0.9	2.86 ± 0.16	34.20 ± 1.12	4.71 ± 0.14	3.50 ± 0.22	0.293 ± 0.030	1.206 ± 0.089	0.906	1.560	0.535	7.6
$DOC_{ambient}$PFe	0.450 ± 0.006	80.35 ± 3.86	639 ± 31	9.2 ± 0.8	3.27 ± 0.18	35.87 ± 1.09	4.42 ± 0.14	3.91 ± 0.33	0.357 ± 0.045	1.415 ± 0.141	0.904	1.573	0.540	7.6
DOC_{high}	0.170 ± 0.016	9.88 ± 1.18	440 ± 22	15.0 ± 1.1	4.86 ± 0.33	52.12 ± 0.49	4.51 ± 0.17	3.49 ± 0.26	0.327 ± 0.045	1.240 ± 0.109	0.911	1.488	0.526	7.5
DOC_{high}Fe	0.112 ± 0.015	6.37 ± 0.71	883 ± 44	15.4 ± 1.2	5.41 ± 0.18	53.17 ± 0.83	4.17 ± 0.09	3.47 ± 0.28	0.352 ± 0.030	1.313 ± 0.106	0.907	1.489	0.525	7.5
DOC_{high}P	0.423 ± 0.016	65.94 ± 7.90	421 ± 21	15.0 ± 1.4	4.82 ± 0.18	51.36 ± 1.51	4.41 ± 0.07	3.46 ± 0.30	0.324 ± 0.023	1.255 ± 0.108	0.909	1.567	0.534	7.6
DOC_{high}PFe	0.448 ± 0.014	79.44 ± 8.41	867 ± 21	15.8 ± 1.4	5.28 ± 0.13	54.27 ± 0.98	4.23 ± 0.10	3.45 ± 0.32	0.336 ± 0.036	1.305 ± 0.128	0.907	1.553	0.524	7.6
$DOC_{ambient}$ without algae light	0.106 ± 0.122	0.35 ± 0.32	310 ± 32	9.4 ± 0.6	3.23 ± 0.19	35.33 ± 1.30	4.63 ± 0.09	3.75 ± 0.12	0.343 ± 0.019	1.283 ± 0.057	0.904	1.493	0.532	7.5
$DOC_{ambient}$ without algae dark	-0.072 ± 0.097	0.08 ± 0.08	298 ± 20	10.0 ± 0.6	3.39 ± 0.19	35.74 ± 0.63	4.56 ± 0.09	3.57 ± 0.20	0.338 ± 0.025	1.222 ± 0.074	0.886	1.434	0.551	7.5
$DOC_{ambient}$ without N	0.130 ± 0.015	7.30 ± 0.78	263 ± 19	9.3 ± 0.6	3.11 ± 0.50	34.40 ± 0.97	4.69 ± 0.24	3.70 ± 0.19	0.334 ± 0.051	1.259 ± 0.083	0.908	1.504	0.530	7.5