The effect of tDOM and nutrients pulses with different intensity and frequency on bacterial production, respiration and nutrient limitation – A mesocosm study in lake Bolmen, Sweden

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#### Abstract

Lakes with increased water colour levels have been observed in many locations in the northern hemisphere, a phenomenon that is called brownification. The driving components of brownification are dissolved organic matter (DOM) and iron, which are affected by rainfall patterns as a large fraction originates from terrestrial sources. Brownification has many detrimental effects for the ecosystem, among which increased costs for drinking water production. Lake Bolmen, southwestern Sweden, is a drinking water source for nearly 600.000 citizens in Skåne. This study simulated three run-off scenarios with DOM and nutrients pulses of different intensity and frequency, and specifically looked at the effect on bacterial production, respiration and nutrient limitation. For this, a mesocosm experiment was executed on lake Bolmen during April and May 2023. The study found that different precipitation patterns do not affect bacterial production and respiration differently, but the addition of DOM and nutrients increased bacterial production and respiration. However, after a two-week recovery period the bacterial respiration dropped and bacterial production increased significantly. Moreover, bacteria were phosphorus limited at the start of the experiment but their response to P addition disappeared as soon as DOM and nutrients were added. A possible reason for the lack of differentiation between the treatment responses could be that lake Bolmen has already been suffering from brownification since the 1980's and the bacteria are therefore more tolerant to DOM additions.

Keywords Brownification · DOM · Bacterial production · Bacterial respiration · Nutrient limitation

#### Introduction

Anthropogenic activities greatly impact the world and cause global warming, with the average surface temperature in 2011 - 2020being 1.1°C above that of 1850 - 1900 2023). Globally (IPCC, increasing temperatures influence spatial precipitation patterns with increased rainfall in the higher regions of the world (Jeppesen et al., 2009), affecting the aquatic environments in those regions. Moreover, due to a higher level of water run-off in catchment areas and subsequent lower water retention times, increased concentrations of dissolved organic matter (DOM) and iron (Fe) have been observed in aquatic environments (Kritzberg & Ekström, 2012; Weyhenmeyer et al., 2014). Iron has also been shown to form complexes with dissolved organic carbon (DOC), which heightens water colour to a dark brown colour (Heikkinen & Ihme, 1995).

Brownification is the process of increasing water colour in aquatic systems due to higher concentrations of DOM in the water (Kritzberg et al., 2020). Iron, together with humic substances called coloured DOM (cDOM), is one of the major causes of darkening water (Maloney et al., 2005). Brownification has been mainly documented in water bodies in the boreal and temperate regions of the northern hemisphere (De Wit et al., 2016). It has detrimental consequences for aquatic environments which include: decreased light penetration resulting in increased benthic respiration which leads to anoxic and hypoxic zones (Brothers et al., 2014), more costly and less sustainable drinking water production (Keucken et al., 2017) and greenhouse emissions increased gas (Tranvik et al., 2009). Moreover, an internal loading process of phosphate due to hypoxic or anoxic areas can be triggered and could push the aquatic system towards a net-heterotrophic state, which could shift

lakes from being a carbon sink to a carbon source, releasing  $CO_2$  into the atmosphere (Brothers et al., 2014). With the internal loading process, the phosphate – iron bonds are broken down and iron is released (Brönmark & Hansson, 2018), which too can lead to increased brownification.

Increases in DOM concentrations depend on, among others: increased temperatures and precipitation levels due to climate change (De Wit et al., 2016; Kritzberg et al., 2020), reduced soil acidity due to recovery from atmospheric sulphur deposition (Evans et al., 2012) and changes in land-use (Kritzberg, 2017; Meyer-Jacob et al., 2015). Changing rainfall patterns influence the amount and loading patterns of organic matter and nutrients deposited into aquatic ecosystems (Klante et al., 2021). Water retention time (WRT) has a strong influence on the impact of DOM on aquatic systems, since in systems with a high WRT there is more time for DOM to be broken down by UV-light or lost through sedimentation (Soares et al., 2019), making water bodies with a low WRT more susceptible to brownification.

DOM originate can from both autochthonous (produced within the aquatic system) and allochthonous (produced outside of aquatic system) sources (Helms et al., 2008). Allochthonous terrestrial DOM (tDOM) is derived from terrestrial plant material, which then gets deposited into waterways through run-off through the soil. Catchment area can greatly influence tDOM transfer, e.g. wet soils have a reduced capacity of mineralization and thus deposit a greater amount of tDOM into aquatic environments (Solomon et al., 2015). DOM consists mainly of carbon, nitrogen and phosphorus (Voss et al., 2021) and is the main source of DOC in aquatic environments (Hessen & Tranvik, 1998). The carbon composition in DOC can vary greatly depending on the source (Spilling et al., 2022), which influences the bioavailability of the carbon and its readiness to be utilised by bacteria (Farjalla et al., 2002).

Bacteria play a major role in the carbon and nutrient cycles, they produce new biomass (bacterial production, BP), and respire organic carbon to inorganic carbon (bacterial respiration, BR) (Del Giorgio & Cole, 1998). Bacterial production is essential for the functioning of limnic ecosystems and is the basis of the food web (Figure 1)(Brönmark & Hansson, 2018).



Figure 1 Overview aquatic food web (Creed et al., 2018).

Increased input of allochthonous DOM supports the heterotrophic metabolism of the bacterial community (Ask et al., 2009). DOM is almost exclusively taken up by bacteria, where most of it is respired as CO<sub>2</sub> while a fraction is used for biomass production and so re-introduced into the classical food chain (Brönmark & Hansson, 2018). Bacteria need nitrogen and phosphorus, as well as other trace elements, in order to grow. In limnic ecosystems, phosphorus is generally the nutrient which limits bacterial growth, due to the phosphate-iron barrier where phosphate is bound to iron and precipitated and stored in the sediment (Brönmark & Hansson, 2018).

Aside from biogeological implications, there are also certain socio-economic consequences of brownification. It has been proven that the recreational value of lakes with brown water is reduced (Keeler et al., 2015), which is unfavourable to both society and the economy. Moreover, lakes provide several important ecosystem services, among which the supply of drinking water. This is also the case for lake Bolmen, a lake in southwestern Sweden (Figure 2), which serves as a drinking water reservoir for 17 municipalities in Skåne, the southernmost province of Sweden. Since been mid-1980's there the has а brownification trend ongoing in the lake (Borgström, 2020; Klante et al., 2021). Brownification can increase treatment costs for transforming water into drinking water (Klante et al., 2021). Furthermore, there is a potential for internal phosphorus loading in lake Bolmen. For now however, the

oxygenation of the lake is classified as good Vatteninformationsystem Sverige bv (VISS, 2023a) and the lake is classified as oligotrophic. Nitrogen concentrations in the lake have been at a relatively constant level over the last 50 years (Borgström, 2020), albeit at a mild level of eutrophication. Next to functioning as a drinking water reservoir, lake Bolmen holds a large cultural and economic value to the people living in the area as it serves as a source of income for many of them. Therefore, it is of significant value that the brownification trend ongoing in lake Bolmen is further investigated so as to determine the effect on society.

This study was part of a bigger project called AquaCosm+ run by the Swedish Infrastructure for Ecosystem Science (SITES). The goal of the experiment was to simulate three DOM run-off scenarios: (1) a wet season with small daily pulses of DOM and nutrients, (2) a "normal" scenario with intermediate and irregular pulses of DOM and nutrients and (3) extreme weather events with an extreme pulse of DOM and nutrients. Furthermore, in order



Figure 2 Lake Bolmen and its position in relation to southern Sweden. © Google Maps

to understand the growth dynamics of bacteria it is necessary to know which nutrient, nitrogen or phosphorus, is the limiting factor. The objectives of this study were to investigate how bacterial respiration production, and nutrient limitation is affected by DOM and nutrients additions of different frequency and intensity.

It was hypothesised that (1) bacterial production and respiration would increase in response to DOM and nutrient additions. Specifically, BP and BR would increase in the mesocosms with daily additions of DOM and nutrients. The gradual increase of available carbon during daily additions might lead the bacterial community to be more adapted to utilise carbon than if carbon is added less regularly or in more extreme doses. (2) the bacteria were limited by phosphorus at the start of the experiment, but that a lower or no degree of nutrient limitation would occur later on. This is due to the fact that over the course of the experiment, phosphorus and nitrogen have been added as part of the treatment.

### Material and methods

#### Study site

This experiment was performed at lake Bolmen (Figure 2), located in a hemiboreal climate (Peel et al., 2007). The lake is divided between three counties: Halland, Kronoberg and Jönkoping. It is part of the Lagan River basin and has a catchment area of 1650 km<sup>2</sup>, and the lake itself has a surface area of 173 km<sup>2</sup> (Borgström, 2020), making it the 12<sup>th</sup> largest lake in Sweden. Overall it is considered to be a shallow lake with an average depth of 6.2 m and deepest point of 38 m. Its main tributaries are Storån, Lillån and Lidhultsån, which together provide 71% of the water input. The water in the lake has a residence time of approximately 1.6 years (Klante, 2023). The catchment composition of Lake Bolmen consist mainly of industrial forest (64%), but also water (15%), wetlands (8%), agriculture (8%), fields (4%) and urban areas (1%). The soil in the catchment area consists mostly of till and peat. The lake is classified as an oligotrophic and dimictic lake and has a good ecological status (Klante, 2023; VISS, 2023a).



**Figure 3** The addition schedule for the three run-off scenarios. The x-axis represents the day of the experiment and the y-axis shows the percentage of total DOM and nutrient additions. The green circles under the x-axis signal the days on which bacterial production, respiration and nutrient limitation samples were taken. © Silke Langenheder

### Experimental setup

This experiment was part of the AquaCosm+ project executed by SITES. A mesocosm experiment was set up with a control (C, no addition of DOM and nutrients) and three treatments: daily (D, every day a small amount of DOM and nutrients were added), intermediate (I, the DOM and nutrients were added in different amounts in different time intervals) and extreme (E, all DOM and nutrients were added at one time)(Figure 3). In total the same amount of DOM and nutrients were added in all treatments (D, I and E). The control and treatments had four replicates and were equally distributed over the platform (Figure 4). The 16 mesocosms were filled with approximately 550 L of lake water, using a pump. Each mesocosm was equipped with three sensors; oxygen, chlorophyll, which PAR and sent continuous data over the course of the experiment. The mesocosms were cleaned daily by brushing the sensors.



Figure 4 Layout of platform with designated treatments in the mesocosms. © Silke Langenheder

The DOM used in this experiment was an extract made from peat according to Mustaffa et al. (2020). In order to make the peat extract, terrigenous dissolved organic matter (tDOM) was extracted from commercial peat (Hasselfors Naturtorv ®)

by alkaline extraction (Gall et al., 2017; Riedel et al., 2012). The solution was then filtered through steel-mesh sieves, the smallest 0.2  $\mu$ m, to remove particles (Gall et al., 2017). Each mesocosm got a total amount of 277.8 mL of peat extract. Phosphorus was added to the mesocosms as a KH<sub>2</sub>PO<sub>4</sub> solution and nitrogen as a NaNO<sub>3</sub> solution. The experiment ran during the springtime, from 25/04/2023 until 31/05/2023. Daily measurements were taken at 09h00 with a PAR sensor and YSI multiprobe.

## Mesocosm sampling

Sampling from the mesocosms was performed 6 times over the course of the experiment (Figure 3). During sampling, 3,5L of water was collected into a 5L container from each mesocosm with a Ruttner sampler. The water was then divided for the different measurements.

# *Bacterial production, bacterial respiration and water colour*

Bacterial production was analysed using the leucine incorporation method as described by Smith and Azam (1992). L-(4,5-3H) leucine (PerkinElmer, Boston, USA) was diluted to a 20% solution with cold leucine (PerkinElmer, Boston, USA). Samples and blanks were incubated for 1 hour at 20°C. DPM was measured in a liquid scintillation analyser (Tri-Carb R 2910 TR. PerkinElmer, Boston, USA), after which bacterial carbon production (ng C<sup>L-1</sup> h<sup>-1</sup>) was calculated (Kirchman, 1993).

Bacterial respiration was analysed with the Winkler method (Murray et al., 1968). Samples were incubated for 72 hours after which the concentration of oxygen was determined at wavelength a450 using a spectrophotometer (Labasque et al., 2004) (UV-VIS Spectrophotometer, Shimadzu, Japan). Carbon respiration was calculated in ng C L<sup>-1</sup> h<sup>-1</sup>. Bacterial growth efficiency (BGE) was calculated based on BP and BR data (BGE = BP / (BP + BR)) and represents the amount of new bacterial biomass produced per unit of organic C substrate assimilated (Del Giorgio & Cole, 1998).

Water colour (mg Pt  $L^{-1}$ ) was estimated using a spectrophotometer (UV-2600, Shimadzu, Japan) by multiplying absorbance 420 values with 500 (Pennanen et al., 1986).

## Nutrient limitation

For the nutrient limitation experiment, four 50 mL Falcon tubes per mesocosm were filled with 30 mL of the sampled water. One control (C) and three treatments were implemented to test by which nutrient the bacteria in the mesocosms were limited: nitrogen (N), phosphorus (P) and nitrogen + phosphorus (NP). Nutrients were added in excess according to the Redfield-ratio. Nitrogen was added to a final concentration of 16 µM (48 µL 10 mM NaNO<sub>3</sub>) and phosphorus to a concentration of 1  $\mu$ M (30 µL 1 mM KH<sub>2</sub>PO<sub>4</sub>). In the NP-treatment the concentrations were 16  $\mu$ M and 1  $\mu$ M for nitrogen and phosphorus, respectively. Since the 16 mesocosms were already replicates of the four run-off treatments, no additional replicates were done for nutrient limitation. To determine nutrient limitation, bacterial production was determined before and after a 72 hour incubation period.

## Statistical analysis

Statistical differences between treatments for bacterial production, respiration and nutrient limitation were assessed using twoway repeated measures ANOVAs and repeated measures mixed-effects models (REMLs), followed by a uncorrected Fisher's LSD. Statistical analysis was performed using GraphPad Prism version 10.0.3 (GraphPad Software. www.graphpad.com). An outlier test was executed and all outliers were removed from the dataset. Before performing the aforementioned statistical tests. the normality of the dataset was confirmed using a Shapiro-Wilk test. Graphs were created with R version 4.1.1 (R Core Team, 2021), specifically with the package ggplot2 (Wickham, 2016).

## Results

In this study it was investigated how bacterial utilization (Experiment 1) and nutrient limitation (Experiment 2) were affected by DOM and nutrient pulses of varying intensity and frequency.

## Environmental parameters

All environmental parameters (watercolour, chlorophyll, pH and dissolved oxygen) have a significant effect of treatment, time and the interaction between treatment and time (Two-way RM ANOVA, p < 0.01). Graphs for chlorophyll, pH and dissolved oxygen can be found in the supplementary information (Figures S1, S2 and S3).

The watercolour in the control and all treatments did not differ on the first sampling day (Figure 5). On the last sampling day, 30-May, there was no significant difference between the treatments, but all treatments differed significantly from the control (p < 0.0011). A large increase in water colour for the extreme treatment is visible on 03-May (p < 0.0001).



**Figure 5** Watercolour over time (average, SD) with sampling dates on the x-axis and watercolour in mg Pt per litre on the y-axis. C is control, D is daily pulses, I is intermediate pulses and E is the extreme pulse of DOM and nutrients (n = 4).

#### Experiment 1: Run-off

There was a significant effect of time on bacterial production (two-way RM ANOVA; p = 0.018) and a signification interaction between time and treatment (p <0.001). No significant effect of treatment alone was detected. Bacterial production in all treatments, except the control, increased over time in response to the DOM and nutrient additions (Figure 6a; p < 0.02; 25-Apr vs. 30-May). Specifically, a significant increase in bacterial production in all treatments, except the control, is observed after the recovery period between 15-May and 30-May (p < 0.05).

There is no effect of the treatments on bacterial respiration, however there is an effect of time (REML; p < 0.0001) and an interaction effect of treatment and time (p =0.003). A significant increase in BR was observed in the extreme treatment on 03-May (p < 0.01; 29-Apr vs. 03-May). However, there is a decrease in BR between 15-May and 30-May (Figure 6b), with a significant reduction in the intermediate treatment (p = 0.02), but statistics did not indicate a significant difference in the daily and extreme treatments.



**Figure 6** (A) Bacterial production over time (median, whiskers extend to 1.5 \* inter-quartile range) with sampling dates on the x-axis and bacterial production in ng carbon per litre per hour on the y-axis. (B) Bacterial respiration over time (median, whiskers extend to 1.5 \* inter-quartile range) with sampling dates on the x-axis and bacterial respiration in ng carbon per litre per hour on the y-axis. Sampling dates are grouped in treatments, where C is control, D is daily pulses, I is intermediate pulses and E is the extreme pulse of DOM and nutrients (n = 4).

There is no effect of treatment on bacterial growth efficiency on any sampling day and neither is there an interaction effect between time and treatment. However, there is a significant effect of time on BGE (REML; p < 0.0001). During the recovery period between 15-May and 30-May, a significant increase in BGE is observed in all treatments except control (Figure 7; p < 0.03).



**Figure 7** Bacterial growth efficiency over time (median, whiskers extend to 1.5 \* inter-quartile range) with sampling dates on the x-axis and bacterial growth efficiency ratio on the y-axis. Sampling dates are grouped in treatments, where C is control, D is daily pulses, I is intermediate pulses and E is the extreme pulse of DOM and nutrients (n = 4).

#### **Experiment 2:** Nutrient limitation

On 25-Apr, before any additions of DOM and nutrients were made, there is phosphorus limitation of the bacteria as shown by the significant increase in bacterial production for all treatments in response to P addition (Figure 8a-d; REML; p < 0.01). No co-limitation between nitrogen and phosphorus was detected.

The control treatment remains phosphorus limited until 15-May (p < 0.005; Con vs. P), however after the recovery period there is no longer a significant increase in BP in response to the P addition. For both the daily and intermediate treatment the nutrient limitation ceased on the second sampling day, 29-Apr, after the first pulse(s) of DOM and nutrients, as there was no longer a significant difference between the control and P addition. The first nutrient pulse in the extreme treatment was on 02-May, and on the third sampling day, 03-May, no nutrient limitation (response to N or P addition) was observed in the extreme treatment either. On the last sampling day, 30-May, bacterial production was significantly lower in all treatments at the end of the incubation compared to the start of the incubation, resulting in negative  $\Delta BP$ values (Figure 8a-d; 0.0005 < p < 0.015).

### Discussion

DOM and nutrient additions caused an increase in bacterial production and bacterial respiration. However, there was no significant effect of the different run-off treatments on BP and BR. The hypothesis that daily additions of DOM and nutrients caused an increased carbon utilisation compared to the intermediate and extreme treatments was therefore not confirmed. The bacteria in the nutrient limitation experiment were phosphorus limited at the start of the experiment, which disappeared over time with the addition of DOM and This finding confirms nutrients. the hypothesis, namely that the bacteria were phosphorus, not nitrogen, limited and that a



**Figure 8** Difference in bacterial production before and after incubation with sampling dates on the x-axis and  $\Delta$ bacterial production in ng carbon per litre per hour on the y-axis. Sampling dates are grouped in treatments, where Con is control, N is nitrogen additions, P is phosphorus additions and NP is nitrogen + phosphorus additions. (A) Control treatment. (B) Daily pulses of DOM and nutrients. (C) Intermediate pulses of DOM and nutrients. (D) Extreme pulse of DOM and nutrients. Graph shows median values with whiskers extended to 1.5 \* inter-quartile range (n = 4). \* *Note that the y-axis in figure A differs from those in figures B,C and D*.

lower or no degree of nutrient limitation would be present later on during the experiment.

## Effect of DOM pulses on BP and BR

In the daily and intermediate treatments both BP and BR increased directly as a response to the DOM and nutrient However, in additions. the extreme treatment where the DOM and nutrient additions were given as a single large pulse there seemed to be a delay in the response of BP. The large pulse of DOM and nutrients was given to the extreme treatment on 02-May, which was immediately utilised for BR on 03-May, but not for BP, for which the increase was only measured on 07-May. Most likely, the bacteria will have used the big pulse of terrestrial DOM (tDOM) for respiration as it seems they prefer autochthonous carbon for production (Pérez & Sommaruga, 2006). Additionally, the BP might have increased earlier than 07-May, but since no sample was taken sooner this cannot be confirmed. Similar delays were not observed in the daily or intermediate treatment.

There is an opposite response of BP and BR during the recovery period between 15-May and 30-May. BP drastically increased during this period, whereas BR reduced severely. tDOM consists of different types of carbon with varving chemical characteristics, which leads to different levels of utilisation by bacteria (Spilling et al., 2022). Labile carbon, with simple chemical structures such as aliphatic compounds, is easy for bacteria to utilise whereas refractory carbon, with a more complex chemical structure like aromatic compounds, is harder for bacteria to breakdown and utilise (Tranvik et al., 2009). The labile carbon pool will be utilised first, and next the refractory carbon pool is broken down and utilised. Under the and influence of UV-light through photodegradation, refractory carbon is broken down into labile carbon (Soares et al., 2019). May 2023 was very sunny (SMHI, 2023), possibly leading to a high level of photodegradation. Therefore, there could be an increased amount of labile carbon available, resulting in a spike in BP. Moreover, chlorophyll concentrations and pH increased over time (Figures S1 and S2), which means more phytoplankton that release labile carbon (Bertilsson & Jones, 2003; Pérez & Sommaruga, 2006) which bacteria can utilise. Pérez and Sommaruga (2006) also suggest that bacteria utilise tDOM more for respiration, but autochthonous DOM more for production. Consequently, it could be that during the recovery period phytoplankton produced a lot of labile carbon, which boosted production but not respiration.

Another reason for the forementioned trend could be a change in the bacterial community composition that occurred during the recovery period which selected for bacteria which are able to utilise refractory carbon more efficiently, leading to a spike in BP and BGE. Studies have bacterial shown that community composition changes with the addition of terrestrial DOM (Jones et al., 2009; Judd et al., 2006). The significant increase in bacterial growth efficiency (BGE) from 15-May to 30-May shows that the bacteria have increased, possibly adapted, their ability to utilise carbon for biomass production instead of respiration. Alternatively, the bacteria could have been channelling the majority of energy into BP at the time of sampling, and BR spiked

before, as seen in the extreme treatment earlier during the experiment. However, since other samples were not taken during the recovery period, we might have missed these dynamics.

# *Effect of nutrient pulses on bacterial nutrient limitation*

Directly after the first pulse of DOM and nutrients, limitation in response to N and/or P could not be detected anymore. The negative response to nutrient addition in all treatments, including the control, on 30-May is surprising. An explanation could be that there were zooplankton present in the samples during the 3 day incubation period, leading to lower bacterial production after incubation due to grazing (Karlsson et al., 2003). It is known that there were zooplankton present, specifically filterfeeding Daphnia spp., in the samples during incubation on 07-May. Data on zooplankton levels in the mesocosms is so far unavailable, but zooplankton are efficient grazers and can drastically reduce bacterial biomass (Karlsson et al., 2003). One more explanation could be that during the recovery period the bacteria were no longer nitrogen or phosphorus limited, but carbon limited, based on the pool of available carbon as discussed before. No experiment was performed on carbon limitation, as it was assumed that this would not be the case as lake Bolmen already has a high concentration of carbon (Borgström, 2020) and carbon was added to the mesocosms during the run-off experiment.

## Considering other lakes and catchment areas

A possible reason for the lack of differentiation between the treatment responses could be that brownification has been ongoing in lake Bolmen since the 1980's (Klante et al., 2021). Therefore, the pulse variation in DOM simulated by the treatments might not prove to have any influence as the bacteria were already adapted to brownification. The bacterial community could have been adapted from the start to utilise tDOM, resulting in no differences between the treatments, as seen in the data. Due to this experiment being part of a bigger project, AquaCosm+ by SITES, the same experiment was executed in lake Erken near Uppsala in Sweden. Lake Erken is a clear water, meso-eutrophic lake (VISS, 2023b) and differs from lake Bolmen in many aspects, so treatment effects could be larger in this lake. In an overall study data from lake Bolmen will be compared with data from lake Erken, but this falls outside the scope of this study.

From a broader perspective, both the type of lake and the composition of the catchment area can affect the response to the DOM and nutrient pulses. Especially lakes with more agricultural land will most likely experience eutrophication, possibly leading to increased bacterial activity. Additionally, it would also mean there is less forest in the catchment area, which most likely lead to less brownification (Kritzberg et al., 2020).

## Further implications

There are certain parameters that, when would provide essential known, information about the processes observed in these experiments. Firstly, the bacterial community composition holds crucial information about the utilisation of carbon and nutrients added to the mesocosms (Jones et al., 2009). It is possible that during the experiment, or more precisely during recovery period, the bacterial the community composition shifted leading to

decrease in bacterial respiration the observed during the recovery period. Secondly, dissolved organic carbon concentrations together with a254 measurements, would give insight into the carbon composition and its utilisation potential (Goodman et al., 2011). Lastly, the continuing increase in watercolour during the recovery period (Figure 5) might not have originated from browning effects. The weather during the recovery period was sunny and with very little warm, precipitation (SMHI, 2023), leading to a high level of evaporation in the mesocosms. Thus the watercolour could be more concentrated with no necessary relation to biological processes occurring. Absorbance measurements would provide more framework to the change in watercolour over time.

criticisms Some and possible improvements to this study should be raised and discussed. Firstly, since the mesocosms were out in the lake, conditions were quite variable among individual mesocosms, leading to a large variation in the data. However, mesocosms do simulate experimental conditions most similar to actual lake conditions, which add more natural dynamics to the study. Secondly, this experiment did not include fish, which are an important part of the aquatic ecosystem as their presence influences the entire food web (Figure 1)(Brönmark & Hansson, 2018). For instance, the presence of fish leads to predation on zooplankton, which would decrease grazing pressure on bacteria. In this experiment zooplankton could continue to grow without any topdown restraint, which could have led to effects detrimental on the bacterial both in biomass community, and composition.

This experiment gives rise to several possibilities for further investigation and research. Firstly, it is evident that data on bacterial community composition would provide necessary insights into the effects of different precipitation patterns on bacterial processes in aquatic ecosystems. As well as to see the effect of nutrient limitation on bacterial community composition. Furthermore, it would be interesting to perform an experiment investigating the effects of photodegradation by UV-light on tDOM carbon composition and subsequently the bacterial community. Studies have shown that water retention time (WRT) plays an important role in the breakdown of tDOM as increased WRT provides a larger window of time for photodegradation of carbon, possibly reducing brownification (Soares et al., 2019).

## Conclusion

It is clear that brownification, and therefore increased carbon concentration in aquatic systems, lead to higher levels of bacterial production and respiration (Figure 6). In turn, this leads to an increased production of CO<sub>2</sub> which could shift lakes from carbon sinks to carbon sources (Brothers et al., 2014). It seems that despite the DOM and nutrient additions, the mesocosms were at the end still dominated by primary production. since dissolved oxygen concentrations did not vary a lot over the course of the experiment (Figure S3). Hypoxic or anoxic zones would not form in the mesocosms because they were continuously mixed by hand and wave action. However, when considering the entire lake, brownification can lead to reduced benthic primary production, which results in anoxic zones and phosphorus loading (Brothers et al., 2014). Increased phosphorus loading would stimulate bacterial growth if they are phosphorus limited.

The results from this study show that increased precipitation and tDOM transport will increase bacterial activity in lake Bolmen, however the pattern, intensity and frequency of pulses, will not necessarily result in different responses of this. It is hard to make general conclusions from this data since for instance clear water lakes will probably be affected in other ways. Lakes with hypoxic or anoxic areas might experience a boost in bacterial activity due to the bacteria being phosphorus limited, but in lakes with sufficient oxygenation this may not be a problem. All in all, more detailed information is necessary to make more precise statements about the effect of precipitation patterns, especially about the bacterial community composition. Every lake is different and the effects of brownification with possible remedies should be tailor made to each one of them.

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**Figure S1** Chlorophyll concentrations over time (average, SD) with sampling dates on the x-axis and chlorophyll in RFU on the y-axis. C is control, D is daily pulses, I is intermediate pulses and E is the extreme pulse of DOM and nutrients (n = 4).

#### Supplementary information



**Figure S2** pH over time (average, SD) with sampling dates on the x-axis and pH on the y-axis. C is control, D is daily pulses, I is intermediate pulses and E is the extreme pulse of DOM and nutrients (n = 4).



**Figure S3** Dissolved oxygen concentration over time (average, SD) with sampling dates on the x-axis and dissolved oxygen in mg  $O_2$  per litre on the y-axis. C is control, D is daily pulses, I is intermediate pulses and E the is extreme pulse of DOM and nutrients (n = 4).

#### References

- Ask, J., Karlsson, J., Persson, L., Ask, P., Byström, P., & Jansson, M. (2009). Terrestrial organic matter and light penetration: Effects on bacterial and primary production in lakes. *Limnology and Oceanography*, 54(6), 2034-2040. <u>https://doi.org/10.4319/lo.2009.54.6.2034</u>
- Bertilsson, S., & Jones, J. B. (2003). Supply of dissolved organic matter to aquatic ecosystems: Autochthonous sources. Academic Press.
- Borgström, A. (2020). Lake Bolmen: Past, present and future.
- Brönmark, C., & Hansson, L. A. (2018). *The Biology* of Lakes and Ponds (3 ed.). Oxford University Press.
- Brothers, S., Köhler, J., Attermeyer, K., Grossart, H. P., Mehner, T., Meyer, N., Scharnweber, K., & Hilt, S. (2014). A feedback loop links brownification and anoxia in a temperate, shallow lake. *Limnology and Oceanography*,

59(4),

1388-1398. https://doi.org/10.4319/lo.2014.59.4.1388

- Creed, I. F., Bergstrom, A. K., Trick, C. G., Grimm, N. B., Hessen, D. O., Karlsson, J., Kidd, K. A., Kritzberg, E., McKnight, D. M., Freeman, E. C., Senar, O. E., Andersson, A., Ask, J., Berggren, M., Cherif, M., Giesler, R., Hotchkiss, E. R., Kortelainen, P., Palta, M. M., . . . Weyhenmeyer, G. A. (2018). Global changedriven effects on dissolved organic matter composition: Implications for food webs of northern lakes. Glob Chang Biol, 24(8), 3692-3714. https://doi.org/10.1111/gcb.14129
- De Wit, H. A., Valinia, S., Weyhenmeyer, G. A., Futter, M. N., Kortelainen, P., Austnes, K., Hessen, D. O., Räike, A., Laudon, H., & Vuorenmaa, J. (2016). Current Browning of Surface Waters Will Be Further Promoted by Wetter Climate. Environmental Science & amp; Technology Letters, 430-435. 3(12),https://doi.org/10.1021/acs.estlett.6b00396
- Del Giorgio, P. A., & Cole, J. J. (1998). BACTERIAL GROWTH EFFICIENCY IN NATURAL AQUATIC SYSTEMS. Annual Review of Ecology and Systematics, 29(1), 503-541. https://doi.org/10.1146/annurev.ecolsys.29.1.5

<u>03</u>

- Evans, C. D., Jones, T. G., Burden, A., Ostle, N., Zieliński, P., Cooper, M. D. A., Peacock, M., Clark, J. M., Oulehle, F., Cooper, D., & Freeman, C. (2012). Acidity controls on dissolved organic carbon mobility in organic soils. Global Change Biology, 18(11), 3317https://doi.org/10.1111/j.1365-3331. 2486.2012.02794.x
- Farjalla, V. F., Faria, B. M., & Esteves, F. A. (2002). The relationship between DOC and planktonic bacteria in tropical coastal lagoons. Archiv für Hydrobiologie, 156(1),22. https://doi.org/10.1127/0003-9136/2002/0156-0097
- Gall, A., Uebel, U., Ebensen, U., Hillebrand, H., Meier, S., Singer, G., Wacker, A., & Striebel, M. (2017). Planktotrons: A novel indoor mesocosm facility for aquatic biodiversity and food web research. Limnology and Oceanography: Methods, 15(7), 663-677. https://doi.org/10.1002/lom3.10196
- Goodman, K. J., Baker, M. A., & Wurtsbaugh, W. A. (2011). Lakes as buffers of stream dissolved organic matter (DOM) variability: Temporal patterns of DOM characteristics in mountain stream-lake systems. Journal of Geophysical Research. 116. https://doi.org/10.1029/2011ig001709
- Heikkinen, K., & Ihme, R. (1995). Retention of organic Fe-P-colloids from peat mining water in an overland flow wetland treatment system in

northern Finland. Archiv für Hydrobiologie, 134(4), 13.

- Helms, J. R., Stubbins, A., Ritchie, J. D., Minor, E. C., Kieber, D. J., & Mopper, K. (2008). Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnology and Oceanography, 955-969. 53(3), https://doi.org/10.4319/lo.2008.53.3.0955
- Hessen, D. O., & Tranvik, L. J. (1998). Aquatic Humic Substances: Ecology and Biogeochemistry. Springer-Verlag. https://books.google.se/books?hl=nl&lr=&id= bmkaYfrkQ4UC&oi=fnd&pg=PA1&dq=Hess en,+D.+O.,+%26+Tranvik,+L.+J.+(1998).+Aq uatic+humic+substances%E2%80%AF:+ecolo gy+and+biogeochemistry.+Springer+eBooks. &ots=EcMBjeMOB&sig=FuzjpEIhqsmR2sMJEq3HbNRFh hU&redir esc=y#v=onepage&g&f=false
- IPCC. (2023). Section 2 Current Status and Trends. Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero 35-115. https://doi.org/ (eds.)], 10.59327/IPCC/AR6-9789291691647
- Jeppesen, E., Kronvang, B., Meerhoff, M., Søndergaard, M., Hansen, K. M., Andersen, H. E., Lauridsen, T. L., Liboriussen, L., Beklioglu, M., Özen, A., & Olesen, J. E. (2009). Climate Change Effects on Runoff, Catchment Phosphorus Loading and Lake Ecological State, and Potential Adaptations. Journal of Environmental Quality, 38(5), 1930-1941. https://doi.org/10.2134/jeq2008.0113
- Jones, S. E., Newton, R. J., & McMahon, K. D. (2009). Evidence for structuring of bacterial community composition by organic carbon source in temperate lakes. Environmental Microbiology, 11(9), 2463-2472. https://doi.org/10.1111/j.1462-2920.2009.01977.x
- Judd, K. E., Crump, B. C., & Kling, G. W. (2006). Variation in dissolved organic matter controls bacterial production and community composition. Ecology, 87(8), 11. https://doi.org/10.1890/0012-9658(2006)87
- Karlsson, J., Jonsson, A., Meili, M., & Jansson, M. (2003). Control of zooplankton dependence on allochthonous organic carbon in humic and lakes in northern clear-water Sweden. Limnology and Oceanography, 48(1), 269-276. https://doi.org/10.4319/lo.2003.48.1.0269
- Keeler, B. L., Wood, S. A., Polasky, S., Kling, C., Filstrup, C. T., & Downing, J. A. (2015). Recreational demand for clean water: evidence from geotagged photographs by visitors to

lakes. Frontiers in Ecology and the Environment, 13(2), 76-81. https://doi.org/10.1890/140124

- Keucken, A., Heinicke, G., Persson, K., & Köhler, S. (2017). Combined Coagulation and Ultrafiltration Process to Counteract Increasing NOM in Brown Surface Water. *Water*, 9(9), 697. <u>https://doi.org/10.3390/w9090697</u>
- Kirchman, D. L. (1993). Handbook of methods in aquatic microbial ecology (P. F. Kemp, E. B. Sherr, B. F. Sherr, & J. J. Cole, Eds.). Lewis Publishers.
- Klante, C. (2023). *Hydrophysical processes* governing brownification: A case study of lake Bolmen, Sweden. Lund University. Lund, Sweden.
- Klante, C., Larson, M., & Persson, K. M. (2021). Brownification in Lake Bolmen, Sweden, and its relationship to natural and human-induced changes. *Journal of Hydrology: Regional Studies*, 36. https://doi.org/10.1016/j.ejrh.2021.100863
- Kritzberg, E. S. (2017). Centennial-long trends of lake browning show major effect of afforestation. *Limnology and Oceanography Letters*, 2(4), 105-112. <u>https://doi.org/10.1002/lol2.10041</u>
- Kritzberg, E. S., & Ekström, S. M. (2012). Increasing iron concentrations in surface waters – a factor behind brownification? *Biogeosciences*, 9(4), 1465-1478. <u>https://doi.org/10.5194/bg-9-1465-</u> 2012
- Kritzberg, E. S., Hasselquist, E. M., Skerlep, M., Lofgren, S., Olsson, O., Stadmark, J., Valinia, S., Hansson, L. A., & Laudon, H. (2020). Browning of freshwaters: Consequences to ecosystem services, underlying drivers, and potential mitigation measures. *Ambio*, 49(2), 375-390. <u>https://doi.org/10.1007/s13280-019-01227-5</u>
- Labasque, T., Chaumery, C., Aminot, A., & Kergoat, G. (2004). Spectrophotometric Winkler determination of dissolved oxygen: reexamination of critical factors and reliability. *Marine Chemistry*, 88(1-2), 53-60. https://doi.org/10.1016/j.marchem.2004.03.004
- Maloney, K. O., Morris, D. P., Moses, C. O., & Osburn, C. L. (2005). The Role of Iron and Dissolved Organic Carbon in the Absorption of Ultraviolet Radiation in Humic Lake Water. *Biogeochemistry*, 75(3), 393-407. <u>https://doi.org/10.1007/s10533-005-1675-3</u>
- Meyer-Jacob, C., Tolu, J., Bigler, C., Yang, H., & Bindler, R. (2015). Early land use and centennial scale changes in lake-water organic carbon prior to contemporary monitoring. *Proceedings of the National Academy of Sciences*, *112*(21), 6579-6584. <u>https://doi.org/10.1073/pnas.1501505112</u>

- Murray, C. N., Riley, J. P., & Wilson, T. R. S. (1968). The solubility of oxygen in winkler reagents used for the determination of dissolved oxygen. *Deep Sea Research and Oceanographic Abstracts*, 15(2), 2. <u>https://doi.org/https://doi.org/10.1016/0011-</u> 7471(68)90046-6
- Mustaffa, N. I. H., Kallajoki, L., Biederbick, J., Binder, F. I., Schlenker, A., & Striebel, M. (2020). Coastal Ocean Darkening Effects via Terrigenous DOM Addition on Plankton: An Indoor Mesocosm Experiment. *Frontiers in Marine* Science, 7. https://doi.org/10.3389/fmars.2020.547829
- Peel, M. C., Finlayson, B. L., & McMahon, T. A. (2007). Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences*, 11(5), 1633-1644. <u>https://doi.org/10.5194/hess-11-1633-2007</u>
- Pennanen, V. K., Kortelainen, P., & Mannio, J. (1986). Comparative study on the estimation of humic matter in natural waters. *Natural Board* of Waters, 10.
- Pérez, M. T., & Sommaruga, R. (2006). Differential effect of algal- and soil-derived dissolved organic matter on alpine lake bacterial community composition and activity. *Limnology and Oceanography*, 51(6), 2527-2537.

https://doi.org/10.4319/lo.2006.51.6.2527

- R Core Team. (2021). R: A Language and Environment for Statistical Computing. In R Foundation for Statistical Computing. https://www.R-project.org/
- Riedel, T., Biester, H., & Dittmar, T. (2012). Molecular Fractionation of Dissolved Organic Matter with Metal Salts. *Environmental Science* & amp; Technology, 46(8), 4419-4426. <u>https://doi.org/10.1021/es203901u</u>
- SMHI. (2023, 21-08-2023). Maj 2023 Torrt i nästan hela landet. Retrieved 29-11-2023 from https://www.smhi.se/klimat/klimatet-da-ochnu/manadens-vader-och-vattensverige/manadens-vader-i-sverige/maj-2023torrt-i-nastan-hela-landet-1.194941
- Smith, D. C., & Azam, F. (1992). A simple, economical method for measuring bacterial protein synthesis rates in sea water using 3Hleucine. *Marine Microbial Food Webs*, 6(2), 8.
- Soares, A. R. A., Lapierre, J.-F., Selvam, B. P., Lindström, G., & Berggren, M. (2019). Controls on Dissolved Organic Carbon Bioreactivity in River Systems. *Scientific Reports*, 9(1). <u>https://doi.org/10.1038/s41598-019-50552-y</u>
- Solomon, C. T., Jones, S. E., Weidel, B. C., Buffam,
  I., Fork, M. L., Karlsson, J., Larsen, S., Lennon,
  J. T., Read, J. S., Sadro, S., & Saros, J. E.
  (2015). Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter
  to Lakes: Current Knowledge and Future

Challenges. *Ecosystems*, 18(3), 376-389. https://doi.org/10.1007/s10021-015-9848-y

- Spilling, K., Asmala, E., Haavisto, N., Haraguchi, L., Kraft, K., Lehto, A.-M., Lewandowska, A. M., Norkko, J., Piiparinen, J., Seppälä, J., Vanharanta, M., Vehmaa, A., Ylöstalo, P., & Tamminen, T. (2022). Brownification affects phytoplankton community composition but not primary productivity in eutrophic coastal waters: A mesocosm experiment in the Baltic Sea. Science of the Total Environment, 841. https://doi.org/http://dx.doi.org/10.1016/j.scitot env.2022.156510
- Tranvik, L. J., Downing, J. A., Cotner, J. B., Loiselle, S. A., Striegl, R. G., Ballatore, T. J., Dillon, P., Finlay, K., Fortino, K., Knoll, L. B., Kortelainen, P. L., Kutser, T., Larsen, S., Laurion, I., Leech, D. M., McCallister, S. L., McKnight, D. M., Melack, J. M., Overholt, E., . . Weyhenmeyer, G. A. (2009). Lakes and reservoirs as regulators of carbon cycling and climate. *Limnology and Oceanography*, *54*(6part2), 2298-2314. <u>https://doi.org/10.4319/lo.2009.54.6\_part\_2.22</u> <u>98</u>
- VISS. (2023a). Bolmen. https://viss.lansstyrelsen.se/Waters.aspx?water MSCD=WA29456646
- VISS. (2023b). Erken. Retrieved 28-11-2023 from https://viss.lansstyrelsen.se/Waters.aspx?water MSCD=WA47593094
- Voss, M., Asmala, E., Bartl, I., Carstensen, J., Conley, D. J., Dippner, J. W., Humborg, C., Lukkari, K., Petkuviene, J., Reader, H., Stedmon, C., Vybernaite-Lubiene, I., Wannicke, N., & Zilius, M. (2021). Origin and fate of dissolved organic matter in four shallow Baltic Sea estuaries. *Biogeochemistry*, 154(2), 385-403. <u>https://doi.org/10.1007/s10533-020-00703-5</u>
- Weyhenmeyer, G. A., Prairie, Y. T., & Tranvik, L. J. (2014). Browning of Boreal Freshwaters Coupled to Carbon-Iron Interactions along the Aquatic Continuum. *PLoS One*, 9(2), e88104. https://doi.org/10.1371/journal.pone.0088104
- Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. https://ggplot2.tidyverse.org