PhD thesis

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Sources and fate of dissolved organic matter in lakes

Light penetration and the importance of UV-mediated bacterial mineralisation

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Light penetration and importance of UV-mediated bacterial mineralisation

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Cover photo: Lake Tvorup in Thy National Park, Denmark, August 2015. Photo: Mikkel Madsen-Østerbye.

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Preface

This Ph.D. thesis represents four years of work ranging from September 2014 to December 2018, of which one year was dedicated to teaching and course activities. The thesis was prepared at the Freshwater Biology Laboratory (FBL) at the University of Copenhagen, Denmark, and was funded by the Villum Kann Rasmussen Foundation. Furthermore, this Ph.D. followed the 4 + 4 scheme, meaning that the Ph.D. was four years long and divided into two parts. The first part involved an examination which was held in December 2016. This exam represented the ending of my master, and I received my master’s degree, Cand.scient. The second part of the Ph.D. ends with this thesis. The motivation behind the Ph.D. project was to study the decline in the abundance and colonisation of the pristine lake isoetid vegetation of small rosette species threatened by increased brownification over the past 30-50 years. The thesis includes four manuscripts (two published and two submitted for publication) of which I am the first author on three of them. The emphasis of the manuscripts was on the input, concentrations and in particular on the degradation of Coloured Dissolved Organic Matter (CDOM); the compound resulting in the brownish colour of the waters. Hence, this thesis encompasses studies investigating the effect of vegetation types on CDOM input, but also the interaction between light attenuation parameters in the water. It is my hope that the work of this thesis will add to the current knowledge of CDOM dynamics in aquatic systems as well as the management of brownification.

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# Table of Content

PREFACE .......................................................................................................................... II

ACKNOWLEDGEMENTS .................................................................................................. III

TABLE OF CONTENT ...................................................................................................... 1

ABSTRACT ....................................................................................................................... 2

DANSKE RESUMÉ .......................................................................................................... 3

THESIS INTRODUCTION ................................................................................................. 4

THESIS AIMS ................................................................................................................... 9

WORK INCLUDED IN THIS CURRENT THESIS ............................................................ 10

CONCLUSION AND IMPLICATIONS ............................................................................. 14

LITERATURE CITED IN THE INTRODUCTION ............................................................... 16

CHAPTER 1: COUPLED UV-EXPOSURE AND MICROBIAL DECOMPOSITION IMPROVES MEASURES OF ORGANIC MATTER DEGRADATION AND LIGHT MODELS IN HUMIC LAKE .................................................................................................................. 19

CHAPTER 2: HIGH REMOVAL OF DISSOLVED ORGANIC MATTER UNDER COMBINED PHOTOCHEMICAL AND MICROBIAL DEGRADATION AS A RESPONSE TO UV LIGHT INTENSITIES ................................................................................................................................. 30

CHAPTER 3: CATCHMENT TRACERS REVEAL DISCHARGE, RECHARGE AND SOURCES OF GROUNDWATER-BORNE POLLUTANTS IN NOVEL LAKE MODELLING APPROACH .................................................................................................................... 47

CHAPTER 4: LIGHT CLIMATE AND SUBMERGED PLANTS IN A LARGE RE-ESTABLISHED LAKE ON AGRICULTURAL LAND ................................................................................................................................. 62

SUPPORTING INFORMATION TO CHAPTER 1 AND 3 .................................................................................................................. 82
Abstract

Increasing input of terrestrially derived coloured dissolved organic matter (CDOM) to aquatic ecosystems has been observed throughout the Northern Hemisphere over the past decades. The accompanying increase in water colour has been termed browning or brownification. The impact of browning on lake ecosystems has been profound with documented consequences for light climate, food webs and biodiversity. Particularly submerged macrophytes are likely to suffer from reduced light availability, which influences the trophic structure and ecological quality of the ecosystem. Despite the negative implications of browning, few studies have addressed the degradation of CDOM in lake waters. In this thesis, I present new insights on CDOM transformation and light climate dynamics in lakes. By applying a new experimental setup mimicking the natural processes in surface waters, I show that CDOM degradation occurs at a constant rate resulting in a much higher CDOM removal than previously found. In closed laboratory systems, imitation of the seasonal solar irradiation resulted in the removal of 90% of the CDOM within a year in the humic Lake Tvorup. This finding suggests that CDOM degradation alone could improve the light climate; however, input of new CDOM impeded this development and played a fundamental role in controlling the actual light climate. Furthermore, I showed that a change in catchment vegetation from coniferous forest to heathland could result in a four-fold improvement of the light climate, expanding the macrophyte colonisation area from 3% to 35% in Lake Tvorup.

Monitoring of the light climate over four years in the re-established Lake Fil showed a high complexity in the temporal dynamics of the light attenuation parameters. A decrease in CDOM- or particle-induced light attenuation could result in an increase in phytoplankton biomass and associated light attenuation. Despite unclear waters, low light penetration and low depth colonisation of macrophytes the open lake shores and extreme shallow waters supported a high species richness of macrophytes.

In conclusion, I showed that UV-mediated bacterial mineralisation of CDOM and DOC is linear with time when only a small proportion of the water is exposed to UV light. I also showed that in situ UV-mediated bacterial removal of CDOM is substantial, while continuous input of CDOM from the catchment will mask this removal. Finally, I showed that changes in land use from coniferous forest to heather could be a powerful management tool to protect, or even restore, the pristine isoetid vegetation of brownified Lobelia lakes.
Dansk resumé


Et andet studium af lysforholdene gennem 4 år i den genopprettede Filsø viste en høj kompleksitet imellem de forskellige lyssvækkelsesparametre. Et fald i CDOM mængden eller lyssvækkelsen fra ophvirvlede partikler fra søbunden medførte bedre lysforhold for planktonalgerne og dermed en stigning i deres biomasses lysvækkelse. På trods af uklart vand og en general høj lyssvækkelse samt en relativ lav koloniseringsdybde af vandplanter på blot 0,6 m, giver Filsøs placering, lysåbne græssede søbredder og relativt lav vanddybde grundlag for en høj artsrigdom af vandplanter - blandt de allerstørste i landet.

Med dette projekt har jeg vist at UV-induceret bakteriel nedbrydning af CDOM og DOC er lineær over tid, når kun en lille andel af vandet udsættes for UV-lyst. Jeg viste også at in situ UV-induceret bakteriel nedbrydning af CDOM er væsentligt større end tidligere antaget, men at et kontinuerligt input af nyt CDOM mindsker denne effekt. Til sidst viste jeg, at ændringer i arealanvendelsen fra nåleskov til lynghede kan være et værktøj til at beskytte eller genoprette den sjældne isoetid-vegetation i brunfarvede lobelie søer.
Aquatic macrophytes play a fundamental role in ecosystem functioning, and their development depends on a variety of parameters (Hilt et al. 2017; Kirk 1994). The most fundamental of these parameters is the light availability. However, in the last decades increasing deterioration of the light climate has taken place in many lakes in the Northern Hemisphere due to increasing inputs of terrestrial coloured dissolved organic matter (CDOM, (Solomon et al. 2015)). This Ph.D.-thesis deals with the dynamics of CDOM and the probability of natural improvement of the light climate in CDOM-rich lakes and the effect on future macrophyte colonisation.

**Oligotrophic soft water lakes**

Oligotrophic soft water lakes are widely distributed all over the Northern Hemisphere (Murphy 2002). Their waters are low in nutrients and dissolved inorganic carbon (DIC) because they receive most of their water through precipitation or groundwater from nutrient- and carbonate-poor catchments (Smolders et al. 2002). The low concentration of available CO$_2$ ($\approx 18$ $\mu$M), nutrients (phosphorus < 10 $\mu$g l$^{-1}$) and bicarbonate (<0.5 mM) in oligotrophic soft water lakes are insufficient to sustain high biomass of phytoplankton and the existence of macrophytes depends mainly on nutrient and carbon supply from the water column. This situation has resulted in lakes with clear waters and extensive colonisation of a diverse group of rosette plants, so-called isoeetids. These small plants have a short-leaf rosette with an extensive root system. They grow in the littoral zone where light is available, and they exploit nutrients and CO$_2$ from the sediment. The shallow lakes often have a low organic content in the sediment due to oligotrophic conditions and the continuous release of oxygen by the isoeetids facilitates mineralisation of organic material in the sediment (Møller and Sand-Jensen 2011). Furthermore, the oxygenation of the sediment results in strong absorption and precipitation of phosphorus by oxidized iron (Christensen and Andersen 1996). Thus, isoeetid vegetation is important for maintaining the oligotrophic state in many aquatic systems. The iconic isoeetid species *Lobelia dortmanna* has been used to name the lakes Lobelia lakes.

**Threats to Lobelia lakes**

Unfortunately, during the last century, the isoeetid vegetation has decline considerably in many lakes across the Northern Hemisphere (Murphy 2002). Many of these previous oligotrophic waters have received high amounts of nutrients, organic matter and DIC through increased anthropogenic activities from agricultural and urban areas as well as drainage and deforestation of catchment vegetation (Arts 2002; Jeppesen et al. 2005; Smolders et al. 2002). Systems enriched by nutrients and DIC have experienced algae blooms and fast-growing elodeid macrophyte species shifting the systems towards light limitations (Smolders et al. 2002). Even Lobelia lakes with restricted anthropogenic activity in the catchment have experienced
deterioration of the light climate. This is mainly due to increasing inputs of CDOM making the lakes increasingly more brownish; a tendency which was already recognised in the 1980’s (Forsberg 1992).

Dissolved organic matter in lakes
There are two main sources of dissolved organic carbon (DOC) and coloured dissolved organic matter (CDOM) in inland waters. It may derive internally from primary production within the lake (autochthonous) or from external input (allochthonous) from the surrounding terrestrial environment (Kragh and Sondergaard 2004). The allochthonous input is frequently the main contributor to DOC and CDOM in small lakes due to the large contact zone with the terrestrial surroundings compared to the lake surface (Sand-Jensen and Staehr 2007).

What is CDOM?
Although DOC mineralisation occurs in the soil environments of the catchment, substantial amounts of DOC are exported to the freshwater systems (Kritzberg 2017; Mulholland 2003). The mineralisation of DOC in soils alters the chemical composition resulting in a mixture of diverse molecular sizes, ages and biological availabilities in the soils (Neff and Asner 2001). The increased water colour associated with increasing DOC concentrations is due to the increased export of CDOM primarily from the terrestrial environment via surface waters or groundwaters (Xiao et al. 2015). A large fraction of this terrestrially derived CDOM consists of humic and fulvic substances (also referred to as humic substances (McDonald et al. 2004)). These component are relatively resistant to microbial degradation due to their complex molecular composition and high C:P and/or C:N ratios (McKnight and Aiken 1998).

CDOM effects on lakes
High CDOM concentrations in inland waters have profound effect on lake ecosystems, organism communities and human utilisation of lakes. CDOM affects lake ecosystems by reducing the downward penetration of solar radiation in the water column. This light reduction causes a decrease in lake productivity as well as secondary effects through changes of food webs and population structure of plants and fish (Jansson et al. 2007; Karlsson et al. 2009). The increased organic carbon has also resulted in an increase of organic carbon available for bacterial mineralisation intensifying freshwater CO₂ outgassing (Lapierre et al. 2013). Moreover, CDOM also affects the physical structure of the water column by absorbing heat in the surface waters and decreasing the extension of the mixed epilimnion with phytoplankton in the water surface (Solomon et al. 2015).

Nutrient limitation has been the common paradigm for understanding lake ecosystem behaviour. However, the brownification of lakes in the boreal region has resulted in lakes with high background light attenuation making these systems light limited rather than nutrient limited (Jones et al.
1996; Karlsson et al. 2009). The low-light conditions induced by CDOM absorbance reduce the depth colonisation of submerged macrophytes (Søndergaard et al. 2013). Especially the isoetid vegetation is vulnerable to deterioration of the light climate because of high light requirements. A reduction in the macrophyte abundance could further change the ecosystem due to is structural effect on habitats and their diversity (Hilt et al. 2017).

**Increasing DOC and CDOM and the reasons behind**

During the last decades, concentrations of DOC and CDOM have increased dramatically all over the Northern Hemisphere in the boreal regions (Monteith et al. 2007; Winterdahl et al. 2014). The increase of DOC has been followed by a parallel increase in water colour (CDOM) often referred to as browning or brownification (Kritzberg and Ekstrom 2012; Roulet and Moore 2006). The increase of DOC and CDOM concentrations in surface waters have been widely discussed and attributed to a combination of many different factors (Clark et al. 2010). Some drivers are directly linked to the increasing temperatures on a global scale (Pagano et al. 2014). The increasing temperatures have resulted in an associated increase in precipitation and runoff, leading to higher DOC and CDOM leaching from the catchment via both surface waters and ground water (Fasching et al. 2016; Weyhenmeyer et al. 2012). In addition, the increasing atmospheric CO$_2$ and global temperatures have increased plant growth and prolonged growing seasons referred to as “greening” and resulted in increased production of terrestrial organic matter in the catchment (Finstad et al. 2016).

Furthermore, changes in land-use, land management and afforestation have contributed to the increasing DOC and CDOM concentrations (Kritzberg 2017; Meyer-Jacob et al. 2015). Another proposed driver, which has gained attention in recent years, is the long-termed change in atmospheric deposition in which the strong reduction in anthropogenic sulfur (S) emission has allowed for recovery from acidification and low soil pH, thereby increasing the solubility and leaching of soil organic matter (Ekstrom et al. 2011; Monteith et al. 2007). This change in solubility by higher soil pH, could have resulted in a loss of organic matter in the terrestrial soils now being dissolved and leached to the aquatic system. The relative importance of different factors for the increasing brownification is hard to separate because they occur simultaneously and interact within the ecosystems (Clark et al. 2010; Evans et al. 2006; Roulet and Moore 2006).

Variation in catchment vegetation and soil management practices affect production and transport of DOC (Stanley et al. 2012; Wilson and Xenopoulos 2008). This notion is supported by the findings that the proportion of coniferous boreal forest in the catchment is the best predictor for DOC concentrations on a global scale (Sobek et al. 2007). Shifting land-use to coniferous forest induces an increase over time in the soil organic carbon pool (Guo and Gifford 2002) resulting in a higher terrestrial input over time to the freshwater system. The importance of riparian vegetation as a source of DOC (Bishop
et al. 2004) and the effect of vegetation type and cover (Kritzberg 2017; Larsen et al. 2011) suggest that controlling and understanding of water input associated with catchment vegetation should play a main role in predicting the change in browning of lakes.

UV-induced degradation of CDOM

It is commonly recognised that degradation of DOC and CDOM in inland waters is accelerated by UV-radiation. UV-induced DOC and CDOM mineralisation can play an important role in lakes as well as in running waters (Cory et al. 2014; Koehler et al. 2014). Especially the more recalcitrant coloured fraction originating from the terrestrial vegetation (CDOM) has a high solar absorbance (Kirk 1994).

The UV-mediated degradation and colour removal, also referred to as photo-mineralisation, transforms CDOM to more labile non-coloured DOC compounds which are more assessible to bacterial remineralisation (Kragh et al. 2008; Moran and Zepp 1997; Obernosterer and Benner 2004). Bacteria alone do mineralise some coloured humic substances at low rates in the aquatic systems (Tranvik 1988). Photobleaching in itself by UV-irradiation is also known to have an effect on colour removal (Obernosterer and Benner 2004). However, it is the combined effect of UV-irradiation and microbial mineralisation that results in the highest turnover of DOC and CDOM. This makes sunlight and UV-exposure an important factor in CDOM degradation and subsequent colour removal (Cory et al. 2014).

Ecological effects and CDOM removal

Given the fundamental ecological influence of browningification on environmental conditions, metabolism and food webs (Jansson et al. 2007; Karlsson et al. 2009; Sand-Jensen and Staehr 2007), it is important to understand the processes behind the internal colour removal and DOC turnover in lakes. In the past, freshwaters were considered as passive transport systems for DOC to the ocean. However, in recent years it has been recognised that a major fraction of the organic matter is processed and lost during transport through the freshwaters to the ocean (Battin et al. 2009; Cole et al. 2007; Tranvik et al. 2009). The freshwater conduit plays a much more important role in the global carbon cycle than previous assumed (Tranvik et al. 2009). This notion is supported by Catalán et al. (2016) who showed a much faster turnover of organic carbon in freshwaters compared to terrestrial soils and marine waters.

Turnover of organic carbon in inland waters is driven by two fundamental processes. The first process is the microbial degradation converting DOC to carbon dioxide (CO$_2$), which is then released to the atmosphere (Fasching et al. 2014; Lapierre et al. 2013). The second process consists of the production of organic aggregates sinking and accumulating in the sediment (Cole et al. 2007). Previous research has mainly focused on CO$_2$ production and outgassing to the atmosphere, but little is known regarding the removal of water colour. Surprisingly few studies have addressed the combined effect of UV-exposure and microbial degradation in the circulating water column waters.
Traditionally, the UV-degradation experiments have been carried out in different ways but with a common design that involved a high initial UV-treatment followed by bacterial degradation. Bacteria were added after UV-exposure due to the harmful effect of UV irradiation on organisms (Häder and Sinha 2005). Some experiments involved an initial UV-exposure until a certain proportion of CDOM had vanished due to bleaching (Moran et al. 2000), UV-exposure for a pre-determined time-period (Helms et al. 2008; Vachon et al. 2017) or a continued UV-exposure throughout the experiment to the entire sample volume has also been applied (Helms et al. 2014; Obernosterer and Benner 2004). The single dose approach has led to transformation of a certain pool of recalcitrant DOC to more labile compounds leading to the general understanding that decomposition of DOC and CDOM follows a first order decay (Weyhenmeyer et al. 2012). The more labile components are utilised first leaving more recalcitrant compounds in the water (Vachon et al. 2017). These previous experimental designs correspond poorly to the natural scenario in which water is constantly circulated from UV-exposure at the surface to no-exposure in the deeper layers of the water column and back again to the surface. This prompted me to develop a setup resembling a natural degradation scenario by having the upper 5% simulating the surface waters exposed to UV-radiation and the remaining 95% in darkness simulating bottom waters in a diurnal light scheme. The continuous mixing and transporting of compounds form “surface” water yielded a more continues transformation of refractory CDOM to labile compounds supporting a continuous bacterial mineralisation and associated with an increase in colour removal.

The motivation behind this study

The primary motivation behind this project was the observed reduction of isoetid vegetation in the small oligotrophic softwater lakes dispersed throughout the Thy National Park in north western Jutland, Denmark. These lakes have experienced a major deterioration of the light climate due to browning and experienced large-scale changes in the catchment vegetation going from heathland to mainly coniferous forest. The lack of ecological knowledge regarding natural colour removal and associated light climate recovery in these small aquatic systems have prevented the development of management schemes to secure the survival and colonisation of isoetids, and equally important, secure the ecological quality and diversity of flora and fauna of these small oligotrophic soft water lakes.
Thesis aims

The overall aim of this Ph.D.-thesis was to determine changes in the light climate as a response to changes in CDOM-induced light attenuations. The main focus was on UV-induced CDOM turnover and changes of CDOM inputs, but also the interaction with other light attenuation parameters were included to evaluate light climate development over time.

I attempted to achieve these goals by:

1) Investigating the effect of continuous CDOM turnover in circulating water between UV-exposure and darkness in a new experimental setup.
2) Investigating the effect of CDOM, derived from specific catchment vegetation, on the light climate in a natural lake.
3) Investigating the effect of different UV intensities on CDOM turnover to provide a better understanding of changes on seasonal and annual scales.
4) Testing and developing a new tool for tracking CDOM-rich groundwater input to a natural lake.
5) Investigating the natural interaction between different light attenuation parameters (CDOM, suspended particles and phytoplankton) to evaluate light climate development in a new lake.

My hope is that these results will result in new and better understanding of light regulation and carbon cycling from CDOM and DOC in freshwater lakes. I hope my contribution will enable better understanding management of the lakes in Thy National Park in order to secure the survival and colonisation of the pristine macrophyte vegetation.
Work included in the current thesis

Chapter 1 – Madsen-Østerbye, M., Kragh, T., Pedersen, O., & Sand-Jensen, K. 2018.

Coupled UV-exposure and microbial decomposition improves measures of organic degradation and light models in humic lakes

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Rare and pristine isoetids communities have been diminished due to increased browning by coloured dissolved organic matter (CDOM) and dissolved organic carbon (DOC) in softwater lakes around the Northern hemisphere. To examine the degradation of CDOM and DOC, we tested the effect of combined UV and microbial degradation in our new experimental setup, which mimics the natural water column circulation. We were especially motivated to see the extent of possible colour removal in the case of photo-mineralisation. Also, the study focuses on CDOM concentrations leaching from two specific vegetation types, heathland and coniferous forest, since these were the dominant vegetation surrounding the lakes in the focus habitat – Thy National Park. Our new experimental setup showed that DOC and CDOM degradation took place at a slow, but constant rate over 30 days. The traditional single dose design followed a 1st order decay with falling degradation rates over time and cessation of DOC degradation after 20 days. DOC concentration in the new experimental setup reached the same level after 30 days.

In regard to CDOM, we found that 84 % of the CDOM derived from heathland and 20 % derived from coniferous forest were removed in the new experimental setup compared to an average of only 3.5 % in the single dose design. This highlighted that especially CDOM degradation could be much more profound than previously assumed. Degradation rates of CDOM found in the new experimental design showed that a new steady state in water colour would be reached within 1-2 years. This finding indicated that the main delay to attain a new steady state in the light climate would be degradation of soil organic pools and rate of leaching from groundwater magazines. Furthermore, model estimates of the change in CDOM input, caused by a shift in the dominant catchment vegetation from coniferous to heathland vegetation, showed that the theoretical colonisation depth of macrophytes would increase from 0.6 m to 2.5 m, improving plant cover from 3 % to 35 %.
Chapter 2 – Madsen-Østerbye, M., Sand-Jensen, K., Kristensen, E., Pedersen, O., & Kragh, T. 2018.

High removal of dissolved organic matter under integrated photochemical and microbial degradation as a response to UV-intensities

Submitted to Biogeochemistry

This experimental study was conducted to further investigate the high CDOM removal highlighted in chapter 1. We investigated CDOM degradation as a response to increasing UV-intensities to evaluate the seasonal removal of CDOM. We hypothesised that the increase in UV-irradiation should result in a higher carbon turnover due to a transformation of recalcitrant organic matter to more labile compounds. In addition, we used the newly found relative degradation of CDOM as a response to UV-intensity to estimate annual changes of CDOM in the focus lake, Lake Tvorup. We found a linear relationship between UV-intensities and CDOM degradation up to a UV-intensity of 0.36 W m\(^{-2}\), while saturation of CDOM degradation occurred at higher intensities corresponding to \textit{in situ} summer UV-values. In contrast, DOC degradation did not show the same tendency to saturate as a response to increasing UV-intensities. As a consequence, we found systematic changes in the ratio between DOC and CDOM at high UV-intensities which did not occur at low intensities. Relating CDOM degradation to seasonal irradiation, we estimated that 92 \% of colour could be removed in a closed system with no additional CDOM input. However, \textit{in situ} water sample only showed a reduction in water colour of 22 \% from winter to summer because of continuous CDOM input. We were able to model the seasonal pattern found in \textit{in situ} water samples by accounting for CDOM input driven by precipitation and subsequent groundwater input. Overall, the results showed that colour removal is much more dynamic than hitherto believed and it has probably been underestimated due to the continuous input of new material to the system.
Groundwater fed lakes offer complex challenges to evaluate inputs of nutrients and organic carbon. We developed a new experimental approach for fast hydrological surveys using multiple groundwater-borne conservative and non-conservative tracers such as CDOM, DOC, nutrients, δ¹⁸O/δ¹⁶O ratios and Fluorescent Dissolved Organic Matter (FDOM) components derived from Parallel Factor Analysis (PARAFAC). All tracers were found in water samples taken directly from the focus lake (Lake Tvorup) and in a network of temporary groundwater wells surrounding the lake. By using the δ¹⁸O/δ¹⁶O ratios and FDOM component as tracers, we were available to estimate the recharge areas of water flowing from the lake. Discharge areas and fraction of water deriving from discrete recharge points were estimated using the community Assembly via Trait Selection Model (CATS). The CATS model utilises tracer measurements from the different groundwater samples related to lake components of tracers. A direct comparison between the sample concentrations was possible as degradation rates of each used tracer were considered and related to a range of water retention times. By using this new approach, we were able to pinpoint five groundwater discharge areas and estimate a maximum water retention time of 2 years.
Chapter 4 – Madsen-Østerbye, M., Kragh, T., Kristensen, E., & Sand-Jensen, K. 2018.

Light climate and submerged plants in a large re-established lake on agricultural land

Submitted to Aquatic Sciences

Four years of continuous data enabled us to examine the interaction between light attenuation parameters in the re-established Lake Fil on former agricultural land. We investigated the interaction between light attenuation parameters controlling the light climate in the lake and compared it to macrophyte colonisation. Our results revealed a highly dynamic system with a complex interaction between light attenuation components. Daily vertical attenuation coefficients ranged from 0.45 to 35.2 m$^{-1}$ with seasonal means between 2.7 to 10.2 m$^{-1}$ with lowest values in spring. The two main parameters contributing to the total attenuation were CDOM and suspended particles (30.5 % and 64.7 %, respectively), while phytoplankton attenuation only contributed with 4.8 %. Modest phytoplankton blooms were initiated by declining background attenuation, especially the decline of suspended particles, during calm weather. The high light attenuation only allowed continuous macrophyte colonisation to at water depth of 0.6 m, while scattered vegetation to 1.2 m was established during clear-water periods.
Conclusions and implications

Several important results have emerged from this Ph.D.-thesis. By applying an experimental setup mimicking circulation in the water column with periodic UV-exposure at the surface I was able to show that degradation of DOC and CDOM followed a linear decline over time and not a 1st order decay as previously shown. However, due to carbon limitation over time, the system would probably move towards a 1st order decay over time if no replenishment of organic matter takes place. This time course aspect of the carbon depletion with time, as revealed by the novel experimental setup, should be further investigated.

The work supports that exposure to sunlight with UV is important for DOC and CDOM degradation in the aquatic environment. However, it is a new insight that colour removal by CDOM degradation was so profound in my experiments. This was simply due to the continuous interaction between UV-exposure and generation of labile compounds followed by microbial degradation. Furthermore, the results of this Ph.D. suggest that colour removal could be much more profound than previously assumed and that observed colour degradation in situ is underestimated due to the continuous input of new material from the catchment. The saturation in CDOM degradation shown in Chapter 2, was carried out on a single type of water source. However, the point of saturation in water with different humic concentrations remains unknown, and similarly, it is not known if the molecular composition of the humic compounds affects the saturation point.

Investigating CDOM leaching from different catchment vegetation surrounding the focus lake, Lake Tvorup, supports the conclusion that changes in vegetation and land-use play a pivotal role in the brownification of lakes. CDOM deriving from coniferous forest had 4-fold higher concentration compared to CDOM deriving from heathland vegetation. Changing the dominant vegetation type in the catchment could remarkably improve the light climate when a new steady state of CDOM concentrations in the system was obtained. This Ph.D project focussed on the two most important vegetation types (coniferous forest and heathland) located in Thy National Park. However, I find it highly relevant to assess the influence of other types of vegetation and land use each resulting in different specific CDOM concentration and compositions. Addressing CDOM from these different land uses and vegetation types could strengthen future management of areas with a high CDOM input.

The monitoring data from Lake Fil supported the notion that light attenuation parameters interacted in a complex system. A decrease in CDOM or particle attenuation could allow phytoplankton to grow and thereby cause higher light attenuation. A decline in CDOM as a result of UV-induced mineralisation could also result in an increase in phytoplankton biomass and chlorophyll-induced light attenuation.

This thesis has implication for conservation, ecology and land-use management on a broad scale. I show that colour removal of lakes could be much more profound than previously assumed.
Furthermore, I show that different vegetation types in the catchment are important for the magnitude of CDOM input into lakes. Using the new developed methodology to trace groundwater, I was able to find the most important recharge and discharge areas of the focus lake. This could add to an improved management strategy of drainage to reduce nutrient and organic input to lakes. My focus on groundwater sources made it possible to estimate water retention time in lakes in which traditional hydrological approaches could not be applied. Following improvement of the light climate, re-colonisation of macrophytes would still depend on the suitability of the sediment. So even though a natural improvement of the light climate could take place due to combined photo- and bacterial mineralisation of organic matter, many years may still pass before mineralisation of high pools of organic matter in the sediments has rendered them suitable for macrophyte colonisation.

It’s is my hope that the results of this thesis may be an inspiration for general management of lakes colonised by the pristine isoetid vegetation by providing better understanding of catchment vegetation and hydrology and UV-effects on CDOM input and degradation and the resulting light conditions in these systems.
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Chapter 1:
Coupled UV-exposure and microbial decomposition improves measures of organic matter degradation and light models in humic lake

Lake Tvorup, Thy National Park, Denmark. Photo: Theis Kragh.
Coupled UV-exposure and microbial decomposition improves measures of organic matter degradation and light models in humic lake

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\begin{abstract}
Increasing terrestrial input of colored dissolved organic matter (CDOM) to temperate softwater lakes has reduced transparency, distribution of pristine rosette plants and overall biodiversity in recent decades. We examined microbial and UV-induced reduction of absorption by CDOM and dissolved organic carbon pools (DOC) in humic water from a groundwater-fed softwater lake as well as groundwater received from surrounding heathland and coniferous forest. An experimental setup that mimics naturally coupled continuous UV-exposure and microbial degradation was introduced and compared with experiments applying a single initial or no UV-exposure. We found that decreases of CDOM and DOC concentrations were negligible in groundwater and very small in lake water over 30 days in the absence of UV-exposure. Initial UV-exposure increased degradation rates, but further degradation ceased after 20 days preventing determination of the natural time course of degradation. Coupled continuous UV-exposure and microbial degradation showed high and constant degradation of CDOM (340 nm) over 30 days removing 87\% of the initial absorption in heathland groundwater, and 20\% in forest groundwater and lake water. Declines in DOC concentrations over 30 days were 34\%, 28\% and 13\% of the initial levels in heathland groundwater, forest groundwater and lake water, respectively. Model estimates showed that a shift in land use from a forest dominated to a heathland dominated catchment could increase lake transparency from 0.6 to 2.5 m. and expand plant-covered area from 3 to 35\%. The main time delay to a new steady state of better light climate would be degradation of soil organic pools and exchange of groundwater magazines, while the delay in the lake water after a complete shift to inflow of CDOM-poorer groundwater would last only 1–2 years. Consequently, changes in CDOM levels in groundwater input should have relatively rapid and marked influence on light conditions and plant distribution in shallow softwater lakes.
\end{abstract}

1. Introduction

Dissolved organic carbon (DOC) in lake water originates from primary production within the lake (autochthonous) and external input (allochthonous) from the surrounding terrestrial environment (Krågh and Sondergaard, 2004; Lampert, 1978; Sondergaard et al., 2000). The pool of autochthonous DOC in lakes is mainly of recent origin containing small amounts of structural material and more labile compounds resulting in faster turnover rates compared to allochthonous compounds (Sondergaard and Middelboe, 1995). Allochthonous input is frequently the main contributor to the DOC content in small lakes having a large contact zone with the terrestrial surroundings relative to lake surface area and a fast renewal of the lake water (Sand-Jensen and Staehr, 2007). Allochthonous DOC input derives from terrestrial primary producers and has undergone photochemical changes (Opsahl and Benner, 1998; Wetzel et al., 1995) as well as biological transformations (Agren et al., 2008; Marín-Spiotta et al., 2014; McCallister and Giorgio, 2008) before entering the lake via groundwater or surface water. The DOC input to groundwater fed lakes has primarily undergone biological transformation due to the lack of UV-exposure in soil water.

DOC undergoes all kinds of production, transformation and degradation processes in the terrestrial-aquatic continuum. It has a strong impact on the physicochemical and biological characteristics of lakes. The pool of DOC in lakes is rarely constant and particularly the labile DOC compounds undergo marked temporal variations, for example, as a result of bursts of release from phytoplankton blooms followed by fast degradation of labile compounds (Baines and Pace, 1991; Sondergaard et al., 2000). Much of the DOC pool in lakes (commonly 50–70\%) is composed of colored organic material (Gelbstoff or CDOM; (Aiken et al., 1985)), which is primarily refractory complex substances of low N and P content deriving from partial soil degradation of cellulose, hemicellulose and lignin from terrestrial plants before entering the lake.

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The inverse relationship between the concentration of each compound and its rate of degradation (Hopkinson et al., 2002; Ogura, 1972; Sondergaard et al., 1995) reflects that the degradation rate is an essential determinant of the resulting concentration. CDOM is very important for the light climate and thermal structure in lakes (Jones, 1998; Williamson et al., 1999). CDOM components are responsible for shielding organisms against harmful UV-exposure (Arrigo and Brown, 1996; Bricaud et al., 1981) and CDOM competes with pigment absorption in photoautotrophs for primary production (Krause-Jensen and Sand-Jensen, 1998). Brown-colored lake waters have a very restricted photic zone for pelagic and benthic photoautotrophs (Jones, 1992), and high nutrient levels are required for phytoplankton to form high biomasses capable of absorbing a substantial proportion of the incoming light (Krause-Jensen and Sand-Jensen, 1998). Moreover, refractory allochthonous CDOM promotes sedimentation of fine floucculent particles (von Wachenfeldt and Tranvik, 2008) that can further reduce growth and survival of rooted plants by smothering their surfaces (Barko et al., 1991).

During the recent decades, an increase in DOC has been observed in freshwaters across Europe and North America (Freeman et al., 2001; Monteith et al., 2007). Increasing water color and DOC concentration have been correlated with higher precipitation (Hongve et al., 2004), but climatic factors alone cannot be the main driver behind this change in CDOM and DOC (Evans et al., 2006). Studies have documented that high acid deposition and low soil pH can change the molecular size of organic compounds and make them less aromatic and hydrophobic, thereby decreasing the content and mobility of CDOM compounds (Ekstrom et al., 2011; Evans et al., 2012). These processes contributed to reduced CDOM leaching and the development of more transparent waters in acidified catchment soils (Hultberg and Grennfelt, 1986). Recent detailed evaluations have documented, however, the prominent influence of changes in land use, catchment vegetation and drainage on DOC concentrations in lake waters across Europe and North America (Freeman et al., 2001; Arts, 2002) located on sandy sediments with conifers or heathland vegetation along the West coast of Denmark and the Netherlands (Brouwer et al., 2002) leading to deterioration of some of the few oligotrophic lakes with a pristine vegetation of small rosette species in the two countries. For ecosystem ecology and nature conservation, it is, therefore, essential to evaluate sources to brownification and processes reducing it.

In the present study, a new experimental design was applied to obtain a better understanding of the interaction between photo-bleaching and microbial degradation of CDOM in a mixed water column. Traditionally these experiments have involved an initial UV-exposure until a certain proportion of CDOM-absorbance had vanished (Moran et al., 2000). UV-exposure for a specific time period (Helms et al., 2008; Vachon et al., 2017) or a continued UV exposure throughout the study (Helms et al., 2014; Obernosterer and Benner, 2004) to the entire sample volume have also been applied. The traditional approach with UV-exposure followed by gradual microbial degradation in darkness of the generated degradable carbon results in first order degradation kinetics. In our novel design, however, a small fraction of the water was exposed to UV-light and circulated directly to a reservoir chamber for bacterial mineralization in a continuous loop to mimic the natural situation with water constantly being transported from deeper layer to UV-exposure at the surface. This approach yields a continuous input of degradable carbon from the large pool of refractory CDOM and may result in different degradation kinetics over the 30-days experiments. Jones et al. (2016) also used a recirculation approach, but used high UV-exposure and ran experiments over a short period of only four days. Furthermore, our design used water circulation and UV-exposure estimated from in situ measurements in a dose that is not harmful to the bacteria.

The experiment was carried out on lake water and on groundwater below the two main vegetation types in the catchment, coniferous forest and heathland. While DOC mineralization in groundwater is restricted to microbial degradation due to lack of UV-exposure, lake water is exposed to UV-light enhancing the degradation of CDOM by photo-bleaching (Del Vecchio and Blough, 2002; Goldstone et al., 2004). When CDOM absorbs UV-light, the molecular and optical properties change (Bertilsson and Tranvik, 2000) resulting in transformation of refractory CDOM to more labile DOC as well as lesser refractory CDOM enhancing microbial degradation (Kragh et al., 2008; Mopper, 2002; Moran and Zep, 1997). The new experimental design offers a better understanding of how color absorption decreases in a lake, where constant input of previously none UV-exposed water releases labile compounds for bacterial degradation, compared to most previous experiment applying a single, initial UV-dose.

Our objective was to study the decrease in DOC concentrations and spectral light absorption of CDOM in the two main groundwater inputs and in the water of a humic softwater lake by using the new experimental design and comparing it with results obtained with a single or no UV-exposure. The obtained degradation rates were used in a theoretical model to estimate future scenarios for the light environment in the lake in which the main input of CDOM-rich coniferous groundwater is shifted to CDOM-poorer heathland groundwater and, thereby, potentially improves the vertical light penetration and cover of submerged vegetation.

2. Materials and methods

2.1. Study site and water sampling

Lake water and groundwater was collected in Lake Tvorup Hul and in the surrounding catchment in Nationalpark Thy in NW-Jutland, Denmark (56°91′N, 8°46′E). Lake Tvorup Hul is a mainly groundwater fed small (surface area 4 ha), shallow lake (mean depth 2.5 m, maximum depth 7.5 m) with a water retention time of about 1 year (Kristensen et al., 2017). Lake Tvorup Hul is an oligotrophic kettle lake, which before 1990 had transparent waters and a high diversity of submerged plants as well as loss of the above-mentioned nationally threatened species.

To reduce brownification, the Danish Nature Agency restructured ditches and closed surface drainage inlets to the lake already in 1992 and by-passed the former surface inflow of humic-rich water. The water sources to the lake today are small amount of surface water during snow melt and exceptional high rainfall and a greater amount of groundwater with high concentrations of humic substances from the two main vegetation types, coniferous forest and heathland in the catchment.

Water was collected at 1 m depth in the lake and in groundwater wells below the coniferous forest and heathland. The groundwater was collected in autumn 2015 and the lake water in February 2016 before the spring development of phytoplankton. Groundwater was collected from wells with water inflow at 2 m depth that were emptied twice before sampling to ensure that only “fresh” groundwater was retrieved. All samples were kept in darkness, at low temperatures (5°C) in large, acid-rinsed containers (10 L) for less than 6 months until start of each experimental run. Experimental water consisted of 90% GF/F (0.7 µm nominal pore size GF/F) filtered water to remove all heterotrophic
flagellates and microalgae and an inoculum of 10% GF/C (nominal pore size 1.2 µm) filtered water from the same source to secure the presence of bacteria (Kragh et al., 2008). The procedure ensured the same initial proportion of water with an inoculum of bacteria in the experimental water. Changes in DOC are linked to the quality rather than the size of the inoculum (Kragh and Sondergaard, 2004). Bacteria have no problem rebuilding their biomass after an inoculum of this magnitude from both soils, streams and lakes (Kragh et al., 2008; Risse-Buhl et al., 2013). The experimental bottles chosen for the experimental chambers in this experiment are large (0.75 L) and showed no “bottle effect” on the concentration of bacteria (Kragh et al., 2008; Risse-Buhl et al., 2013).

2.2. Experimental design

The novel experimental design was constructed to mimic natural conditions in the water column, where only a certain proportion of the water is exposed to UV-irradiance during the day, due to the low penetration depth of UV light in the humic-rich waters. In this design, the surface water is simulated by UV-exposure, while deep water without UV exposure is simulated by circulating the water to a dark reservoir chamber with only bacterial degradation. This ensures a constant input of labile organic material, generated by UV-exposure in the light chamber, into the dark chamber. The experimental setup consisted of a water reservoir (0.75 L) and a quartz-glass container (0.05 L) connected in a closed circuit by inert PTFE Teflon tubes. UV-exposure of water took place in the quartz container, while the reservoir was kept in darkness (see details below). A parallel experimental setup was kept in complete darkness or with only an initial 24-h UV-exposure to assess microbial degradation in the absence of constant UV-exposure. All experiments were run in four replicates. To correct for possible contamination and artifacts such as adsorption to container walls and tubing, control experiments were also run with Milli-Q water (Millipore Milli-Q academic water system, France) with no dissolved organic matter and in sterile water with known initial DOC concentration. The controls with Milli-Q water showed no systematic contamination throughout the 30-days experiments and there was no change in DOC concentration in the DOC controls. Water was circulated by a BT1000-L multi-channel peristaltic pump (Langer Instruments, USA) at a rate corresponding to a renewal time of 24 h. The renewal time on 24 h in the experimental setup was selected based on the average “Piston Velocity”. The Piston Velocity was estimated by wind speed measurements by a weather station located next to the lake. The piston velocity is a measure of the turnover of the water in the lake. As of that the average exposure time to UV light in the surface water is determined by the piston velocity. Higher piston velocity causes shorter exposure of a single molecule in the surface. However, the whole water column will be exposed to UV more frequent compared to a system with low piston velocity.

After each sampling, which slightly reduced the water volume, flow rate of the peristaltic pump was adjusted to maintain an unaltered renewal time in the circuit. Water in the quartz-glass container was exposed at a distance of 15 cm to UV-light from a Q-Panel UVA-340 lamp (Q-LAB, USA) in a 12–12 light/dark cycle. The emission flux and wavelength spectrum of the UV-light panel were measured with a StellarNet Inc. Black-Comet UV-VIS spectrometer model CXR (280–900 nm) and analyzed with SpectraWizz Spectroscopy software, light intensity 0.13 W m$^{-2}$ measured at 340 nm. The light intensity was measured over a year by the lake and the proportion of UVA in relation to total irradiance was established (details in UV-dose section). The experimental dose from the UVA-340 lamp (0.13 W m$^{-2}$) corresponds to the average daily UV-dose on the lake. Degradation experiments with the three water sources were performed over 30 days with 8 sampling points during the period. Water samples were analyzed for DOC and CDOM (see below). The traditional single dose design was a 24-h UV exposure followed by 39 days of microbial degradation in darkness.

2.3. Analyses

CDOM absorbance was measured on newly GF/F (0.7 µm nominal pore size) filtered water on a 1800 SHIMADZU UV-spectrophotometer fitted with a 1 cm cuvette at 440 and 340 nm. Absorbance at 440 nm has traditionally been used to describe water color in freshwater and light absorbance from CDOM (Cuthbert and Del Giorgio, 1992). Absorbance is also presented at 340 nm as this was the wavelength of the emission peak from the UV-lamp. Absorbance values were converted to absorption coefficient at a given wavelength, $\alpha(\lambda)$, (unit m$^{-1}$) by the relationship (Kirk, 1994):

$$\alpha(\lambda) = \frac{I_n}{A(\lambda)} = \frac{I(10)}{A(\lambda)}$$

in which $A(\lambda)$ is the absorbance at the given wavelength and $I$ is the light path (1 cm) through the cuvette in the spectrophotometer.

DOC samples were GF/F-filtered and conserved with 150 µL 2 M HCl per 15 mL sample to prevent degradation of organic matter. All DOC samples were analyzed on a Shimadzu Total Organic Carbon Analyzer according to methods of Kragh and Sondergaard (2004). All samples were visually checked and homogenized (whirl mixing and ultrasonic bath) to dissolve any colloids before measurements as described by Kragh et al. (2008). DOC concentrations were measured by the Non-Purgeable Organic Carbon method (NPOC) with a 3-point calibration curve ($R^2 = 0.998$) and with at least 3 injections for each water sample. For each sample run, a standard series and blanks were included. The accuracy of the analytic method is ± 1%.

2.4. UV-dose

The dose of UV-exposure in the experiments was adjusted conservatively to mimic natural conditions during an average day in the lake. Incoming UV-light on the lake at 340 nm was measured directly with a black-comet spectrometer (StellarNet Inc., Tampa, Florida, US) in August 2016. UV data was related to simultaneous in situ measurements of the total PAR irradiance (400–700 nm) from January to December. Assuming a constant proportion of UV to PAR irradiance enabled calculation of the annual mean incoming UV-light on the lake. The incoming UV-light from the Q-Panel UVA-340 lamp in the experimental setup was adjusted to 65% of the annual mean UV-340 nm radiation in situ. To confirm that all UV-light was absorbed in the experiment, like in the lake, light measurements were performed showing that less than 1% was left at a depth of 5 cm, which was the depth of the quarts bottle exposed to UV-light. The single dose design received a UV-dose of 0.65 W m$^{-2}$ (as in Kragh et al., 2008; Vachon et al., 2017) and corresponded to the accumulated UV-dose received over 30 days in the experimental design.

2.5. Statistical analysis

The statistical analysis was made with GraphPad Prism 6.1. The tools were linear and non-linear regression for comparisons of rates of change with time in the different experiments and at different wavelengths. Figures show means ± SEM unless specified otherwise. Traditionally, the decrease of CDOM and DOC are modeled as simple first-orders degradations processes (Hanson et al., 2014; Hanson et al., 2011; Molot and Dillon, 1997; Stets et al., 2010), where more labile compounds are degraded first, leaving more refractory DOC behind over time (Weyhenmeyer et al., 2012). First order degradation kinetics according to an exponential decline were used in the experiment with a single UV-dose, while 0 order kinetics according to linear regression provided the best fit in the new experimental design due to constant input of newly transformed compounds from continuous UV-exposure.
2.6. Model scenarios of future lake light climate

The light model applied is a simple steady state model in which changes in light absorbing compounds were used to estimate possible changes in the light climate for Lake Tvorup Hul driven by changes of the vegetation in the catchment. The calculations were based on current pool sizes and expected changes caused by UV-induced bacterial turnover of CDOM and the corresponding changes in light absorption. From these data, future CDOM absorption levels, new Secchi transparency and lake bottom area of possible reestablishment of underwater vegetation were calculated. The model takes all light absorbing compounds in the range of 400–700 nm (PAR) into account.

Changes in lake CDOM absorption in the entire PAR range (400–700 nm) per unit time \( \frac{dC}{dt} \) was calculated according to the differential equation:

\[
\frac{dC}{dt} = C_{in} - C_{out} - C_{degredation}
\]

\( C_{in} \) was calculated from CDOM absorption in groundwater from the different catchment types before any UV-exposure had taken place. These absorption values were then multiplied by water input according to lake water volume and water retention time (WRT). Lake volume was calculated from lake bathymetry (see below) and a WRT-level set at 1 year (Kristensen et al., 2017). \( C_{out} \) was calculated from CDOM absorption in lake water before UV-exposure multiplied by lake water volume and WRT. \( C_{degredation} \) was calculated from the degradation rates of CDOM absorption in the experimental approach with combined UV-exposure and microbial degradation and corrected to the field situation by adjusting the rates according to the \( \text{in situ} \) UV-exposure. The experimental UV-dose was 65% of the \( \text{in situ} \) UV-dose. Model estimates assume constant water renewal with time and do not take seasonal changes into account because the model is used to establish the long-term steady state of the system and not the seasonal changes. Consequently, the photo-induced degradation has been averaged over the year disregarding seasonal changes. The calculations were performed by iteration in time steps of one day. Initial lake concentration was set as the measured lake values in February 2016. The model was run for 10,000 days, though steady state was obtained within 2 years in all scenarios reflected by a constant absorption values at zero values of \( \frac{dC}{dt} \) (simulation in supplementary data). Simulations were made for the contemporary situation, for an altered catchment covered solely by

Fig. 1. DOC concentration with incubation time in groundwater from heathland (a and b), groundwater from coniferous forest (c and d) and water from Lake Tvorup Hul (e and f). Left column (a, c and e): degradation in complete darkness (solid symbols) or assisted by continuous UV-exposure (open symbols). Right column (b, d and f): degradation in complete darkness following initial UV-exposure for 24 h. Mean ± SEM (n = 4) are presented. Horizontal and vertical scales differ between panels.
heathland, with lower CDOM concentrations in groundwater than forest groundwater, and a contemporary groundwater input of a mixed composition from both forest and heathland.

To estimate different scenarios for future plant cover, plant depth limits were set at the Secchi transparency, which was estimated as the depth receiving 10% of subsurface irradiance (Wetzel, 2001). The attenuation coefficient was defined as the average absorption, after averaging the measured CDOM absorption for every 1 nm across the PAR spectrum (400–700 nm). Bathymetry of the lake was determined with a Lowrance HDS-12 Gen2 Touch chart plotter by recording water depth 6132 times for every 1 m along transects across the lake. Maximum interpolation between data point was 10 m. Data was analyzed with Reefmaster 1.8 pro software to calculate the area of lake bed in depth intervals of 0.5 m.

Model calculations used two parameters. Firstly, CDOM absorption in the inflowing groundwater was determined by mixing of heathland- and coniferous groundwater in proportions from 0% to 100% heathland water with an increase of 25% in each step. Secondly, water retention time (WRT, which could increase photo bleaching by prolonged exposure) was varied between 0.5, 1.0, 1.5 and 2.0 years. The mean summer Secchi transparency measured at different times over 2 years was 72 ± 6.8 cm (n = 9), which is close to the model estimate for 50% forest water which is 70 cm for a WRT of 1 year. The measured transparency in the lake when samples were taken in February was 35 cm. This finding supports the application of WRT of 1 year used in the model, which yields a Secchi transparency of 31 cm.

3. Results

3.1. Changes in DOC over time

DOC concentrations in all three water types with UV-exposure decreased substantially from the beginning of the experiment. Over 30 days, DOC declined in groundwater from the coniferous forest (28.2%) and heathland (34.9%) and in the lake water (13.6%) (Fig. 1a, c and e). In contrast, the decline of DOC in darkness was negligible and insignificant in groundwater from the coniferous forest (0.05%) and heathland (2.2%), but significant in lake water (5.0%), though markedly lower than with supplementary UV-exposure.

Decline in the DOC concentration was linear over time, in contrast to the single dose experiment which resembled a first order decay (Fig. 1b, d and f and Table 2). In the single dose experiment the concentration had reached a constant level after app. 20 days compared to the ongoing linear degradation in the continuous exposure experiment. The strongest effect of the initial UV-exposure was a DOC loss of 1.42 mg C L⁻¹ corresponding to 70% of the total loss realized after 30 days of bacterial degradation in darkness. In forest groundwater the initial DOC loss due to direct UV-exposure was only 13% of the total DOC loss, but corresponded to a loss of 1.38 mg C L⁻¹.

The initial DOC concentration was 10-fold higher and degradations with UV-exposure were 7-fold higher in groundwater from the coniferous forest than from the heathland (Table 1); lake water had intermediate DOC concentrations. The time required to degrade 25% of

Table 1
Linear regression analysis of changes in DOC concentrations in two groundwater types and lake water from day 0 to 30. Slope of the regression, r² and P value are shown.

<table>
<thead>
<tr>
<th>Water source</th>
<th>Treatment</th>
<th>Slope (mg C L⁻¹ d⁻¹)</th>
<th>r²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heathland</td>
<td>UV-exposure</td>
<td>−0.054</td>
<td>0.859</td>
<td>0.0009</td>
</tr>
<tr>
<td>Heathland</td>
<td>Darkness</td>
<td>−0.001</td>
<td>0.005</td>
<td>0.8639</td>
</tr>
<tr>
<td>Coniferous forest</td>
<td>UV-exposure</td>
<td>−0.391</td>
<td>0.962</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Coniferous forest</td>
<td>Darkness</td>
<td>0.002</td>
<td>0.001</td>
<td>0.9218</td>
</tr>
<tr>
<td>Lake (0–30 days)</td>
<td>UV-exposure</td>
<td>−0.074</td>
<td>0.838</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lake (0–30 days)</td>
<td>Darkness</td>
<td>−0.023</td>
<td>0.004</td>
<td>0.0058</td>
</tr>
</tbody>
</table>

3.2. UV-induced changes of CDOM absorption

CDOM-absorption declined significantly over time in all three water types when microbial degradation was assisted by UV-exposure. In contrast, CDOM-absorption declined at a much lower rate in lake water and non-significantly in groundwater without UV-exposure (Fig. 2, Table 3) or with only initial UV-exposure. CDOM degradation assisted by UV-exposure started immediately in lake water, while degradation in groundwater was delayed by about 10 days.

CDOM-absorption was much higher at 340 nm than at 440 nm. As a result, degradation rates were also faster at 340 nm than at 440 nm (Table 3). The linear decline in CDOM absorption (Table 3), calculated from day 10 to day 30, was approximately 2-fold higher in the two groundwater types than in the lake water. Groundwater from the coniferous forest showed a shift in the absorption spectrum over time (Fig. 2c and d), because absorption decreased at 340 nm and increased at 440 nm. In the single dose experiment no decrease in the absorption coefficients was observed over time in any of the three water types (Fig. S2).

3.3. DOC and CDOM relations

UV-induced microbial turnover showed a relationship between DOC and CDOM (Fig. 3), in which a decrease in color was accompanied by a lower DOC pool. CDOM-absorption and DOC concentrations in groundwater kept in the dark remained almost constant over time (Fig. 3). In the UV-exposed groundwater, DOC concentrations initially declined much faster than CDOM-absorption at 340 nm (Fig. 3b and d, open symbols), but after several days both variables declined in parallel. CDOM and DOC concentrations in lake water, which had recently been exposed to UV in the lake, decreased slightly and in parallel in darkness.

Initially, CDOM-absorption of visible light at 440 nm in forest groundwater increased whereas the DOC concentration decreased suggesting conversion between organic compounds of different spectral absorption (Fig. 3c). In the UV-exposed lake water, CDOM-absorption at all three wavelengths and DOC concentration declined in parallel during the experiment.

3.4. Changes in light climate

The measured decline in CDOM absorption by microbial decomposition assisted by continuous UV-light was used to calculate scenarios for the light climate following changes in land use in the catchment at variable water retention time. The scenarios were established by replacing groundwater from the forest with groundwater from the heathland and applying the present water retention time in the lake (1 year) and longer retention times (1.5 and 2 years). Changing the origin of water and the water retention time had profound effect on the light attenuation coefficient in the lake water (Fig. 4). By replacing all groundwater currently derived from the coniferous forest with

Table 2
First order decay of DOC with time in groundwaters and lake water in darkness following a single initial UV-exposure for 24 h; see Fig. 1b, d and f. K-values (with 95% C.L), half-life and r² values are shown.

<table>
<thead>
<tr>
<th>Water source</th>
<th>Treatment</th>
<th>K (d⁻¹ 95% C.L)</th>
<th>T½ (d)</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heathland</td>
<td>Initial UV-dose</td>
<td>0.20 (0-0.40)</td>
<td>3.5</td>
<td>0.526</td>
</tr>
<tr>
<td>Coniferous forest</td>
<td>Initial UV-dose</td>
<td>0.08 (0.02-0.13)</td>
<td>9.2</td>
<td>0.789</td>
</tr>
<tr>
<td>Lake</td>
<td>Initial UV-dose</td>
<td>0.09 (0-0.23)</td>
<td>7.2</td>
<td>0.411</td>
</tr>
</tbody>
</table>

the initial DOC pool assisted by continuous UV-exposure and microbial metabolism was 22 days for heathland groundwater, 27 days for forest groundwater and 56 days for lake water.
groundwater from the heathland, the attenuation coefficient was reduced by 85–88% for water retention times of 1–2 years. An increase of water retention from 1 to 2 years reduced the attenuation coefficient by about 40% almost independent of the origin of water (Fig. 4a). These profound reductions in the light attenuation coefficients with higher input of heathland groundwater and longer water retention time markedly improved the Secchi transparency (Fig. 4b).

The model does not include any direct change in light absorption by planktonic algae, as the aim was to model changes in light climate caused by alterations in CDOM. Nevertheless, the lake has low (below 10 µg l⁻¹) P concentrations available for primary production, which could only sustain minimal phytoplankton growth. Increased transparency in the lake would increase the macrophyte cover and cause further decrease in available P for phytoplankton production. Changes in attenuation coefficients and Secchi transparency are, therefore, a result of changes in CDOM input and in-lake degradation by combined microbial degradation and UV-exposure (Fig. 4b). Improvements in light penetration in the lake with all water derived from heathland and a retention time of 1 year could allow the vegetation cover to expand 10-fold from presently only 3% to 35%. The expansion of vegetation cover, assuming a water retention time of 2 years was also substantial from initially 5% to 69% cover of the lake bottom according to model calculations (Fig. 4c).

4. Discussion

The present study demonstrated that a combination of UV-exposure and bacterial degradation greatly accelerated degradation of CDOM regardless of the origin of water compared to classic UV-degradation experiments. We found that groundwater formed in the forest catchment had substantially higher concentrations of CDOM compared to groundwater formed on heathland. Below we discuss these findings and the implications for the pristine submerged rosette vegetation in the

<table>
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<th>Water source</th>
<th>Wavelength</th>
<th>Slope (m⁻¹ d⁻¹)</th>
<th>r²</th>
<th>P-value</th>
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<td>0.907</td>
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</table>

Table 3

Linear regression analysis of changes in CDOM absorption in experiments assisted by UV-exposure in groundwaters and lake water from day 10 to 30; see Fig. 1a, c and e. Slope of the regression, r² and P values are shown.

Fig. 2. Absorption coefficient (m⁻¹) at 440 nm and 340 nm with time in groundwater from heathland (a and b), groundwater from coniferous forest (c and d) and water from Lake Tvorup Hul (e and f). Degradation experiments were performed in complete darkness (solid symbols) or assisted by UV-exposure (open symbols). Mean ± SEM (n = 4) are presented. Vertical scales differ between panels.
4.1. Determinants of DOC and CDOM degradation

In both groundwater types and in lake water, the DOC pool was recalcitrant in darkness as the reduction of DOC concentrations during 30 days was less than 5%, which is typical of recalcitrant DOC (Moran and Hodson, 1990). Nonetheless, the degradation rate of DOC was higher in lake water (5%) than in groundwater (0.05–2.2%) suggesting the presence of a more labile pool DOC in lake water compared to groundwater (Sondergaard and Middelboe, 1995). Before sampling the lake, water has been exposed to natural UV-light, making organic matter more labile for bacterial degradation (Kragh et al., 2008; Mopper, 2002; Moran and Zepp, 1997). This may have contributed to the faster degradation rate in lake water compared to groundwater, which had not been exposed to UV-light prior to the experiment. This former UV-exposure could also form some labile carbon working as a primer for a higher microbial degradation rate in the lake water (Kuzyakov, 2010). Furthermore, the continuous input of fresh labile carbon owing to the UV exposure could enhance or sustain a steady state priming effect throughout the entire experiment and, thereby, support a higher microbial activity compared to a single UV-dose releasing a single pulse of fresh carbon. A comparison of DOC reduction in the two groundwater sources showed the lowest values in water from the coniferous forest perhaps owing to a higher proportion of aromatic structural compounds than from heathland vegetation (Sondergaard and Middelboe, 1995).

DOC and CDOM degradation was enhanced by supplementary UV-exposure because the degradation rates of DOC and CDOM were several-fold higher with UV-exposure, even though 5% of the DOC loss in lake water could be due to UV-exposure prior to collection of water. Thus, the interaction between photo-chemically induced changes of organic compounds and microbial degradation is essential for reducing both concentrations and light absorption of DOC. The greater decline of the DOC pool in groundwater (heathland 34%, coniferous forest 28%) than lake water (13%) over 30 days agrees with studies showing that previously unexposed DOC has the highest photochemical degradation rate when exposed to solar radiation (Lindell et al., 1995; Salonen and Vahatalo, 1994; Vahatalo and Wetzel, 2002).

The decline in absorption at 340 nm during 30 days was 86% of the initial value in groundwater from heathland and 20% in groundwater from coniferous forest. However, the degradation rate in absolute terms was higher in groundwater from the forest owing to much higher DOC concentrations. The higher percentage decrease of CDOM absorption at 340 nm in heathland groundwater could be due to greater UV-exposure.
of the individual organic molecules in water of lower total absorption, while organic molecules in CDOM rich groundwater are partially shielded by higher internal self-shading. It is also possible that the molecular composition of the leaching CDOM from the two largely different vegetation types have different degradation rates. Whether photo-bleaching varies among different CDOM sources could not be addressed. We are unable to estimate the effect on degradation rates because of variable self-shading from colored organic molecules present at variable concentrations (Krause-Jensen and Sand-Jensen, 1998). As a consequence, the lower absorption and thus self-shading in heathland water results in higher UV-exposure per molecule of CDOM.

4.2. Coupling of DOC and CDOM degradation

Changes in the relationship between CDOM and DOC over time reflect whether the colored or uncolored fraction of DOC is the primary carbon source for bacterial degradation. Changes in the relationship between DOC and CDOM for both groundwater types suggested removal of an uncolored and more labile fraction of DOC in the beginning of the experiment, where a declining DOC concentration was not accompanied by a decrease in CDOM absorption. The results showed that UV-light alone does not have a direct bleaching effect on CDOM from heathland and coniferous vegetation. The initial UV-exposure in the single dose experiment did not show any direct effect on the CDOM absorption during bacterial degradation.

After 10 days delay, however, a common linear decline of CDOM and DOC suggested a shift to the more refractory CDOM as the primary carbon source for bacterial degradation. This finding demonstrated that bacteria in the added inoculum over this period had formed a sufficiently active population capable of degrading the more refractory CDOM after UV-exposure. However, single wavelength analysis does not reflect directly DOM quality, but only to which extent degradation occurs.

Studies have shown that bacterial growth in some situations is not nutrient but organic carbon limited (Kragh et al., 2008). This is reflected by higher degradation rates of DOC, which is made labile by UV-exposure, compared to rates in samples kept in darkness. Lake water had, prior to collection, been exposed to natural UV causing the degradation of DOC and CDOM to start after a very short time lag in both dark and UV-experiments. The pattern of degradation in the dark experiment with lake water followed first order exponential decay in accordance with our and previous results from single dosage UV-experiments (Del Vecchio and Blough, 2002). Therefore, the linear decline of DOC concentrations in our degradation experiments assisted by UV-exposure is particularly noteworthy. This pattern is a consequence of the experimental design which mimics the natural situation in a mixed water column where new organic molecules are constantly circulated to the surface waters and becoming exposed to UV. Thus, a constant pool of DOC is continuously made susceptible to bacterial degradation after UV-exposure. The reason why a constant DOC pool is becoming exposed to UV is that the UV-attenuation coefficient in the water increases linearly with CDOM concentrations provided the specific attenuation coefficient per molecule remains constant. Also, the constant circulation allows repeated exposure of organic molecules to UV-light, while the coupled microbial driven and UV-assisted degradation of organic molecules takes place. For these reasons, this novel long-term experimental design offers a new and better understanding of the degradation of the colored and uncolored fraction of DOC compared to more traditional designs (Helms et al., 2014; Helms et al., 2008; Moran and Zepp, 1997; Vachon et al., 2017). Over extended time the large pool of CDOM will slowly be exhausted supposedly causing the degradation to follow first order degradation kinetics (Weyhenmeyer et al., 2012). However, the time span for this experiment was not sufficiently long to test this development to take place due to the constant input of none UV-exposed material.

4.3. Lake brownification

Overall, our results confirmed that UV-exposure combined with microbial degradation had a profound effect in all types of water on mobilizing recalcitrant CDOM and DOC to more labile compounds (Kragh et al., 2008; Mopper, 2002; Moran and Zepp, 1997). Furthermore, the study showed that CDOM content was much higher in groundwater from coniferous forest than heathland. These findings are important considering that Lake Tvorup Hul has experienced a 10-fold increase of light absorption at 440 nm from mean levels of only 2.1 m$^{-1}$ in 1970 (Rebsdorf, 1981) to 22.2 ± 0.46 m$^{-1}$ in 2015 (Madsen-Østerbye, unpublished). The observed photodegradation rates of

![Fig. 4. Steady state scenarios of Secchi transparency and plant cover in Lake Tvorup Hul as a result of manipulating water retention time (WTR) between 1.0, 1.5 and 2.0 years and gradually increasing the proportion of groundwater input (0% to 100%) from the low CDOM source in heathland relative to the high CDOM source in coniferous forest (100% to 0%).](image)
leached CDOM imply that the light climate in Lake Tvorup Hul, and other similar lakes, can be improved and the depth limit for growth of rosette species expanded if input of brown-colored substances is reduced.

Lake recovery could be accelerated by reducing input of CDOM-rich groundwater and by increasing lake water retention time allowing more time for combined UV-mobilization and bacterial degradation of the recalcitrant CDOM to take place. Those effects would be most pronounced in shallow water bodies such as dune slacks, which are abundant in Nationalpark Thy, compared to deeper lakes such as Lake Tvorup Hul where only the upper 5 cm of the water column experience substantial UV-exposure.

The vegetation type in the catchment is crucial for lake brownification because CDOM absorption was 5-fold higher in forest than heathland groundwater reaching the lake which further supports the strong effect of land use and catchment vegetation on CDOM content in freshwaters (Kritzberg, 2017). Historically, these differences in CDOM absorption between groundwater from heathland and coniferous forest could have contributed to increased brownification of waters in Nationalpark Thy through the replacement of heathland by coniferous forest from 1870 and onwards. Considering that the profound changes of brownification took place from 1970 to 2015, however, changes in catchment vegetation are probably not the only reason for the observed changes, though gradual formation of larger organic soil pools over centuries may increase the impact. It seems likely that increasing precipitation and drainage of the forest soils as well as reduced atmospheric deposition of acids during the last decades (Driscoll et al., 2001; Kristensen et al., 2017; Kritzberg, 2017; Stoddard et al., 1999) have played an additional role in the more recent brownification.

4.4. Deforestation and modeled future lake climate

According to the differences of absorption characteristics between groundwater from coniferous forest and heathland, the effect of replacing coniferous forest with heathland vegetation in the catchment could be a 6.5-fold decline of light absorption in the water entering Lake Tvorup Hul. This difference corresponds to changes to water with a Secchi transparency of 1.18 m in heathland groundwater compared to 0.35 m in forest groundwater. The time needed for a 50% decrease of the light absorbing CDOM is five times longer for the forest ground-water (64 days) than the heathland groundwater (13 days). However, these linear degradations rates estimated during the 30-days long experiments would probably slow down during extended degradation with rates changing in proportion to falling concentrations, though increasing UV-exposure of individual organic molecules at lower concentrations could extend the period of linear decline beyond the experimental period of 30 for all three water sources.

Model estimates for Lake Tvorup Hul result in an average Secchi transparency of 0.31 m with the present input of brown-colored water and a water retention time of one year. Changing the entire catchment to heathland vegetation, Secchi transparency could reach a new steady state level of 2.0 m with the current water retention of 1 year. In a scenario where water retention time was 2 years and the catchment consisted of heathland, the Secchi transparency could reach almost 3.5 m. As much as 69% of the lake area has water depths below 3.5 m. Such strong effect on the light climate is the result of much longer time available for photo-induced degradation to take place. Also, with the current vegetation in the catchment, the Secchi transparency could rise to 1.2 m solely if retention time in Lake Tvorup Hul increased to 2 years.

An increase of the Secchi transparency to 2 m in the scenario with the present water retention time of 1 year and complete heathland cover would lead to expansion of the possible plant covered area from 3% to 35% of the lake bottom. The time delay for obtaining this new steady-state of light penetration in the lake depends on the time frame for depletion of the terrestrial soil organic carbon pool and leaching of CDOM from the soils. Studies show that the immediate effect of clear-cutting of the catchment vegetation could be a 2 to 5-fold increase of leaching of DOC and CDOM from former forest soils (Kreutzweiser et al., 2008) associated with an increased input of nutrients that could affect phytoplankton and bacterial growth and, thus, DOC and CDOM dynamics (Niinemäe, 2004). This initial input could then be followed by a reduction after three-five years, while the soil pools are gradually being depleted (Hinton et al., 1997; Startsev et al., 1998). A new steady-state of reduced leaching could be attained after about 10 years (Murty et al., 2002).

Consequently, after clear-cutting and establishment of heathland vegetation, Lake Tvorup Hul could ideally reach a new steady state of high light penetration after 11–13 years assuming that no major phytoplankton production develops. With the short water retention time in the lake in mind, the determining factor for improvement of light penetration would be depletion of the pool of soil organic matter and associated nutrients in the catchment. The depletion would be influenced by precipitation causing leaching of soil water (Evans et al., 2006; Roulet and Moore, 2006; Worrall and Burt, 2007). This aspect is relevant for future measurements and modelling considering the ongoing increase in precipitation which affects both leaching of soil organic pools and retention time and organic degradation in the lakes.

Managing a lake to optimize conditions for one single organism group, in this case the pristine rosette species, would affect other species differently (some negatively et al., positively) and overall lake ecology would change. However, expanding the cover of rosette species would reduce internal nutrient loading through plant uptake and root oxidation of sediments, enhancing denitrification and phosphorus (Frandsen et al., 2012; Petersen and Jensen, 1997) providing habitat and shelter for invertebrates and fish (Jeppesen et al., 2012) and thereby, have a general beneficial influence on lake ecology.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecoleng.2018.04.018.

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Chapter 2: High removal of dissolved organic matter under combined photochemical and microbial degradation as a response to UV light intensities

Älvand Dune Heath, Thy National Park, Denmark. Photo: Ole Pedersen.
High removal of dissolved organic matter under combined photochemical and microbial degradation as a response to UV light intensities

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Abstract

Throughout the freshwater continuum, Dissolved Organic Carbon (DOC) and the coloured fraction, Coloured Dissolved Organic Material (CDOM) are continuously being added, removed and transformed resulting in changes in colour and lability over time. Our objectives were to examine experimentally the effect of increasing UV intensities on the combined photochemical and microbial degradation of CDOM and DOC over 37 days in a simulated mixed water column. We found a linear relationship between the degradation of CDOM and UV intensities up to UV intensities of 0.36 W m$^{-2}$, while degradation saturated at higher intensities both at specific wavelengths and for broader intervals. After 37 days at high UV intensity, CDOM absorption had been reduced by 41%, while DOC degradation continued to increase at higher UV intensities reaching a total removal of 48%. The two high UV intensities systematically changed the ratio between DOC and CDOM throughout the 37 days. In situ estimation of UV intensity suggested that up to 92% of the inherent CDOM could possibly be removed annually by combined photochemical conversion and bacterial degradation in the humic lake in which the water was collected. The results confirmed that CDOM degradation can be remarkably higher in lakes than previously thought and that high CDOM levels are maintained by continuous input of new terrestrial CDOM.
Introduction
Concentrations of Dissolved Organic Carbon (DOC) in surface waters have increased over the past 30 years throughout the Northern Hemisphere (Monteith et al. 2007). This increase in DOC has been accompanied by a parallel increase in Coloured Dissolved Organic Material (CDOM) exported primarily from the terrestrial catchment (Xiao et al. 2015). The process is often referred to as browning or brownification of the water (Kritzberg and Ekstrom 2012; Roulet and Moore 2006).

Increasing CDOM concentration has been attributed to a combination of mainly three different factors (Clark et al. 2010). These are: (i) the warmer climate (Pagano et al. 2014) and the associated increasing precipitation and runoff from land (Fasching et al. 2016; Weyhenmeyer et al. 2012), (ii) the recovery from decades of acidification in the 1970-1980s now resulting in higher pH and increased mobility of DOC in the catchment soils (Ekstrom et al. 2011; Monteith et al. 2007), and (iii) changes in catchment vegetation driven both by climate-induced “greening” (Finstad et al. 2016) and afforestation (Kritzberg 2017). The importance of these three factors for the increasing brownification is hard to separate because they occur simultaneously and interact within the ecosystems (Clark et al. 2010; Roulet and Moore 2006).

The input of CDOM and DOC to the lakes can have a profound effect on the ecosystem. Brownification impairs the light climate in the water and thus reduces the primary production potential and distribution of submerged aquatic plants in the affected lakes (Karlsson et al. 2009; Sondergaard et al. 2013). Increasing DOC input also increases the inorganic nutrient pool through photochemical and microbial degradation (Berggren et al. 2015). Given the fundamental ecological influence of brownification on environmental conditions, metabolism and food webs (Jansson et al. 2007; Sand-Jensen and Staehr 2007) it is imperative to understand the processes behind the internal colour removal in lakes and wetlands.

In the near past, freshwaters were considered as passive transport systems for DOC to the ocean. In the last decade it has been recognised, however, that a major fraction of the organic carbon exported from land is processed and lost on its way through the freshwater conduit (Battin et al. 2009; Cole et al. 2007; Tranvik et al. 2009), emphasising that inland waters play a much more important role in the global carbon cycle than hitherto acknowledged (Tranvik et al. 2009). As an example, Catalán et al. (2016) showed a much faster turnover of organic carbon in freshwaters than in terrestrial soils and marine waters. Two fundamental processes remove or transform DOC in inland waters. Part of the DOC is degraded microbially and converted to carbon dioxide (CO₂), which is released to the atmosphere (Fasching et al. 2014; Lapierre et al. 2013), while another part forms aggregates that sink to the lake sediment where it becomes buried (Cole et al. 2007). The latter
Degradation processes in the water are accelerated by UV radiation, which can play an important role in DOC mineralisation – particularly in shallow streams and lakes in which the UV impacted surface waters represent a high proportion of the total water volume (Cory et al. 2014; Koehler et al. 2014). The UV mediated degradation, also referred to as photobleaching, transforms CDOM to more labile non-coloured DOC compounds, which are more assessible for bacterial remineralisation (Del Vecchio and Blough 2002; Moran and Zepp 1997). However, formation of reactive oxygen species (ROS) can impede bacterial DOC degradation to some extent (Scully et al. 2003). The actual lake concentration of DOC is thus influenced by a combination of in-lake processes related to the variable magnitude and composition of DOC input, lake water depth as well as water retention time (Kohler et al. 2013).

Previous studies on DOC degradation have mainly focused on the formation of CO₂ and emission to the atmosphere, but less so on the removal of coloured components. Bacteria mainly utilise the uncoloured fraction of DOC, which does not have a significant influence on colour removal in itself (Madsen-Østerbye et al. 2018). This makes sunlight and UV exposure of CDOM a main factor in the degradation and colour removal (Cory et al. 2014).

Few studies have addressed the direct effect of UV intensity on the loss of CDOM in a circulating lake water column. Madsen-Østerbye et al. (2018) showed a linear CDOM degradation over time under simulated natural conditions with coupled UV exposure and microbial degradation. However, the influence of increasing UV intensity on the colour removal and CDOM lability is not known. Changes in the ratio between non-coloured DOC and CDOM express which fraction is mainly utilised by bacteria over time. An increase in the ratio over time reflects a higher loss of non-coloured DOC compared to coloured DOC and vice versa.

We have introduced an experimental approach in order to quantify the coupled degradation of DOC and CDOM by UV light and microbial degradation over weekly periods. We use this experimental system to quantify degradation of DOC and CDOM as well as unravel the possible shifts in the ratio between DOC and CDOM, as organic molecules are degraded and transformed over time. We evaluate the relevance of our results further by estimating the degradation and resulting CDOM concentrations in comparison with measured CDOM concentrations over a year in the humic lake from which the experimental water derives.

Materials and methods

Sample collection

Experimental water was collected from 2 m depth in the humic Lake Tvorup located in Thy National Park in north west Jutland, Denmark (56°91’N, 8°46’E) in February 2017. The lake has no surface inlets and is surrounded by conifer plantations and
heathland and resembles humic lakes in the Scandinavian boreal zone (Algesten et al. 2004; Verpoorter et al. 2012). The lake water was aged in darkness for six months at 4°C to reduce labile DOC compounds prior to the experiment. For each experimental replicate, 90% of the initial water volume was passed through a Whatman GF/F filter (nominal pore size 0.7 µm) to retain all heterotrophic flagellates and microalgae. 10% of the water volume was passed through a GF/C filter (nominal pore size 1.2 µm) in order to act as an inoculum with bacteria. Previously, it has been shown that bacteria from an inoculum of this filtered size range can rebuild their biomass within 0-7 days in similar experimental setups (Kragh et al. 2008; Risse-Buhl et al. 2013).

**Experimental design**

In order to quantify the effect of combined UV- and microbial degradation of CDOM and DOC, we conducted an experiment in which lake water was subjected to different UV intensities while maintaining the ongoing microbial degradation. The experimental setup was designed to mimic the slow vertical mixing of a natural water column with only organic matter in surface waters being exposed to UV radiation (Morris et al. 1995) and subsequently being transported to deeper layers while undergoing microbial degradation. The circulating system was designed according to Madsen-Østerbye et al. (2018) and consisted of three replicates and a control at each UV intensity.

In brief, the system included a small quarts-vial (0.05 L) connected to a larger water reservoir (1 L) by an inert PTFE Teflon tubing in a closed circulation driven by a BT100-L multi-channel peristaltic pump (Langer Instruments, USA) at a rate corresponding to 100% water exchange within 24 hours. Each sampling slightly reduced the water volume and the flow rate was adjusted to maintain an unaltered exposure time to UV radiation. The water in the quarts-vials was exposed to UV radiation in a 12/12 day-night cycle by a Q-Panel UVA-340 lamp (Q-LAB, USA), which provided a spectral composition similar to natural sunlight in the wavelength region 295-365 nm (Q-lab 2012). This spectral range from 395-365 nm is the most important for environmental photoreactions of DOC (Stubbins et al. 2010). The water circulated from the UV chamber to the larger water reservoir, which was maintained in complete darkness for microbial degradation of labile compounds produced by UV radiation. Thus, 5% of the water was exposed at any point corresponding to a circulating water column, in which the upper 5% of the water column received UV light. The selected reservoirs for microbial degradation had no bottle effect with respect to abundance and maintenance of the bacterial biomass (Kragh et al. 2008; Risse-Buhl et al. 2013) and, thereby, did not affect the DOC degradation. All equipment was acid rinsed before use and the experiment was conducted at 20°C.

The choice of UV intensities in the experiment was based on a comparison between the light spectrum of the Q-Panel
UVA-340 lamp and a field measurement using a Black Comet UV-VIS spectrometer model CXR (StellarNet Inc., USA). UV intensities were extrapolated to a whole year using field measurements from a stationary HOBO PAR sensor (400-700 nm): S-LIA-M003, Onset Computers. The highest measured summer UV radiation was 0.62 W m⁻², which was close to the maximum dose in previous studies using 0.65 and 0.68 W m⁻² (Kragh et al. 2008; Vachon et al. 2017). Our experimental lamp design and setup restricted maximum UV exposure to a UV dose of 0.58 W m⁻², which was used as the maximum summer intensity. The highest and lowest spring UV radiation was used as well, which corresponded to 0.16 and 0.36 W m⁻² as these periods seem especially important for the CDOM degradation (Gonsior et al. 2013). The UV intensity of 0.1 W m⁻² corresponded to winter radiation. A set of replicates was kept in complete darkness to measure microbial degradation without UV exposure.

Analyses and sampling
The experiment lasted for 37 days and water samples were retrieved every week and analysed for DOC and CDOM after having been filtered through pre-combusted GF/F filters. Combusted GF/F filters, although with a nominal pore size of 0.7 µm, have shown indications of reducing the effective pore size to 0.3 µm thereby retaining even smaller particles (Nayar and Chou 2003). CDOM absorbance was measured on a UV-1800 spectrophotometer (Shimadzu instruments, USA) fitted with a 1 cm quartz-cuvette across the spectrum 300-750 nm with scanning intervals of 1 nm. Absorbance values were converted to the Napierian absorption coefficients (αCDOM (λ)) by multiplying the absorbance at wavelength λ (nm) by 2.303 (i.e. ln(10)) and dividing with the path length of the cuvette in meters (Stedmon and Markager 2001). CDOM absorption is presented at 340 and 440 nm, which corresponds to the highest UV intensity at 340 nm, while 440 nm is often used as a reference wavelength of water colour (Cuthbert and Del Giorgio 1992). CDOM absorption was also calculated for the photosynthetically active spectrum (PAR, 400-700 nm) and the entire analysed spectrum, 300-700 nm, which is suitable to evaluate the effect of changes in size of organic molecules.

DOC samples were conserved with 150 µL 2 M HCL per 15 mL sample and measured on a Total Organic Carbon Analyser (Shimadzu instruments, USA) and analysed according to Kragh and Sondergaard (2004). DOC was measured using the non-purgeable organic carbon method with a 3-point calibration curve (r² = 0.998) and with at least three technical replicates/injections of each DOC sample to ensure statistical confidence. Standard series and blanks were included for each experimental run and showed an accuracy of the analytical method of ±1%. All DOC samples were checked for colloids created by changes in pH and treated in an ultrasonic bath and whirl mixed to separate them (Kragh et al. 2008).

Also, DOC and CDOM data were used to assess the CDOM aromaticity by normalising the CDOM absorption coefficients
at 340 nm to the coherent DOC concentration. The absorption coefficient at 340 nm was used because the peak of CDOM is located here and the UV source had the highest UV radiation intensity at this wavelength.

Field measurements and modelling
In order to evaluate whether degradation followed natural conditions, we monitored Lake Tvorup closely for a year. A meteorological station was established 15 m from the lake shore placed 2 m above ground. The station was equipped with sensors for daily radiance (HOBO PAR sensor (400-700 nm): S-L1A-M003, Onset Computers) with a data logger (HOBO micro station, H21-002, Onset Computers) and a tipping bucket for precipitation measurements (HOBO rain gauge datalogger: RG3, Onset Computers) with a recorder (H07-002-04, Onset Computers). In addition, water samples were collected weekly from the lake and analysed for CDOM (as described above) to examine the in situ development of CDOM absorbance. The daily solar radiation between 9 a.m. and 3 p.m. was used to estimate the average radiation and was related to the five different experimental CDOM removal rates. This information was used to estimate the theoretical seasonal colour degradation in a closed system. In an open system like Lake Tvorup, CDOM is transported into the lake with the groundwater. In order to compare the seasonal degradation with seasonal measurements of CDOM, we used an estimated inflow concentration of 1.37 m$^{-1}$ at 340 nm with a mean water retention time (WRT) of one year. These parameter values accord with measurements in Lake Tvorup (Kristensen et al. 2018). WRT was varied seasonally in 14-day intervals according to the magnitude of precipitation. The modelled concentration of CDOM at 340 nm was described by the following differential equation:

$$\frac{dc}{dt} = C_{prv} - C_{degra} + C_{in} \quad \text{Eq. 1}$$

where the change in concentration over time (dc/dt) is determined by the previously estimated CDOM concentration ($C_{prv}$), the fraction of degradation based on the average UV between 9 a.m. and 3 p.m. multiplied by the total CDOM pool ($C_{degra}$) and the inflow of CDOM as the running average of precipitation over 14 days normalised to a WRT of one year multiplied by the estimated inflow concentration ($C_{in}$).

All statistical analyses and graphs were made using the GraphPad Prism 7 software package (GraphPad Software, San Diego, CA, USA) or R (R Core Team 2017).
**Results**

**CDOM response**

CDOM degradation over 37 days at 340, 440 nm and at two wavelength intervals (300-700 nm and 400-700 nm) followed a saturating function with time (Fig. 1). Mean CDOM degradation increased from 22% (± 2%, SEM) at a UV intensity of 0.1 W m\(^{-2}\) to 39% (± 5%, SEM) at a UV intensity of 0.58 W m\(^{-2}\). CDOM degradation increased linearly with UV intensity from 0 to 0.36 Wm\(^{-2}\) and between 0.36 and 0.58 W m\(^{-2}\) CDOM degradation saturated. In the interval 400-700 nm, CDOM degradation remained constant between UV intensities of 0.1 and 0.16 W m\(^{-2}\), but increased above 0.16 W m\(^{-2}\) reaching maximum degradation (46%) at 0.36 W m\(^{-2}\).

**DOC response**

DOC degradation increased at higher UV intensities (Fig. 2). The increase in DOC degradation with UV intensity was modest from 0 to 0.16 W m\(^{-2}\), but at higher intensity, DOC degradation increased towards 0.36 W m\(^{-2}\) and increased moderately above this UV intensity. DOC degradation approached, but did not reach, a plateau at the highest UV intensities and as a percentage removal surpassed the degradation of CDOM observed at the same intensities. In contrast, at low UV intensities, the decrease of CDOM exceeded that of DOC (Fig. 1 and Fig. 2).
Fig. 2. DOC removal over 37 days as a percentage of the initial concentration for each applied UV intensity. Means (±SEM, n = 3).

**DOM relative to DOC**

The absorption coefficient at 340 nm relative to the DOC concentration in the same sample was calculated (Fig. 3). At 0, 0.1 and 0.16 W m\(^{-2}\) UV intensities, there were no systematic changes over time in the CDOM absorption coefficient at 340 nm relative to the corresponding DOC concentration (p = 0.16). This suggested the same proportional loss of CDOM and DOC during the experiment.

However, at higher UV intensities (0.36 and 0.58 W m\(^{-2}\)), the ratio between CDOM and DOC increased significantly over time due to a faster decrease of DOC than CDOM (t-test, p < 0.001). No systematic difference was observed in the time-change of the ratio between 0.36 and 0.58 W m\(^{-2}\) (p = 0.43).

Fig. 3. Ratio between the CDOM absorption coefficient at 340 nm (m\(^{-1}\)) and the DOC concentration (mg L\(^{-1}\)) over time at different UV intensities (W m\(^{-2}\)). Means (±SEM, n = 3). Sampling at UV intensity 0 on day 14 is missing.

**Field measurements and model estimations**

Daily measurements in 2015 at Lake Tvorup showed that UV intensities in the winter period were too low to support photochemically induced organic degradation. The winter period included 125 days, or 34% of the year, primarily between 13 November and 1 February. Twenty-five additional days with low UV radiation and no photochemical degradation were scattered throughout summer and spring. In comparison, 109 days, or 30% of the year, had intensities that supported the
highest photochemically induced degradation rates. Moreover, 98 days, or 27% of the year, exhibited low photochemical induced degradation and 33 days, 9% of the year, supported the lowest photochemically induced degradation. Using the 2015 UV radiation data, 91.7% of the initial CDOM would be degraded within one season.

### Discussion

The general reduction in CDOM absorption and DOC concentrations as a response to UV exposure observed in our study complies well with previous studies (Cory et al. 2014; Del Vecchio and Blough 2002; Koehler et al. 2014). However, our results showed a clear saturation of CDOM degradation as a response to increasing UV intensities. This finding contradicts previous studies showing that prolonged UV exposure, in a single initial dose, resulted in a higher transformation to more labile compounds available for bacterial utilisation (Kragh et al. 2008; Moran et al. 2000). In decomposition experiments of DOC and CDOM after a single UV dose, the transformation from recalcitrant to labile compounds is dependent on the initial pool of

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**Fig. 4.** The change in CDOM absorbance at 340 nm over a year at Lake Tvorup as a response to inflow of new material and UV-induced removal. The solid line represents the model data and the filled circles represent *in situ* water samples.

Overall, observed and modelled seasonal changes of CDOM concentrations followed the same course over the year (Fig. 4). Weekly water samples showed a CDOM reduction of 27% from winter to summer, while model calculations predicted a larger colour loss during summer (33%, Fig. 4).
humic substances in the water. Consequently, the initial UV decomposition is not linear with dose (Kragh et al. 2008), as water with high humic content has a higher pool of recalcitrant DOC available for transformation to labile compounds compared to water with low humic content.

The UV accumulated dose in our experiment simulated spring conditions and corresponded to the intensity used in a previous study by Kragh et al. (2008). Compared to Kragh et al. (2008), who found a total DOC loss of 27%, we found a remarkably higher loss exceeding 47% at 0.36 W m$^{-2}$. This suggests that DOC turnover is higher in an experimental setup that mimics a circulating water column compared to the single initial dose design. However, Madsen-Østerbye et al. (2018) showed similar DOC losses in three different water sources when comparing the single dose experimental design to the continuous mixing design while the same radiation intensities were used (0.65 W m$^{-2}$). In both experimental setups, DOC losses were 28% in coniferous forest groundwater, 35% in heathland groundwater and 14% in lake water. In contrast, different CDOM degradation was found between the two experimental designs. In the single dose experiment, the initial UV exposure resulted in a decline in CDOM absorption at 340 nm of only 2.7% in heathland groundwater, 5.8% in lake water and 1.5% in forest groundwater. The continuous mixing system resulted in a decline in CDOM absorption of 87% in heathland water and 20% in forest and lake water. This result supported the notion that UV induced CDOM turnover could be much higher in lake waters than previously assumed.

No previous studies have, to our knowledge, shown a saturation of CDOM degradation with increasing UV intensities under natural conditions as mimicked in our experiment. In an experimental setup resembling our approach, Jones et al. (2016) showed an increase in CDOM removal (ABS$_{254}$) over four days in response to accumulated UV exposure. However, the percentage loss depended largely on the initial amounts of light absorbing humic compounds and, thereby, on the water source. Compared to our study, Jones et al. (2016) did not show saturation of colour removal in DOC-rich peatland water, which almost had the same initial DOC concentration as our experimental water. This result is likely due to the fact that Jones et al. (2016) use much shorter experimental time in which 25% of the water volume (including bacteria) was exposed to continuous UV radiation for four days instead of UV exposure of 5% of the water in a 12/12 light dark cycle over 37 days, as is the case in the present study.

In contrast to the positive effect of UV radiation on bio-availability of CDOM and DOC, which results in higher bacteria utilisation of the organic carbon, some studies have reported a negative effect of solar radiation on bacterial growth with marine derived DOC (Benner and Biddanda 1998; Obernosterer et al. 1999). This difference in response to UV exposure is reported to be a result of DOC quality and the amount of
humic compounds in the water (Tranvik and Bertilsson 2001). Water with a high humic content has a higher pool of recalcitrant DOC compared to waters with low humic content. The recalcitrant pool of DOC reacts to UV exposure and results in transformation to more labile compounds available for bacterial degradation. In contrast, waters more dilute in humic substances, but high in labile DOC, respond to UV exposure with transformation of a higher proportion of labile compounds to more refractory compounds (Scully et al. 2003). The transformation from labile to more refractory compounds by UV exposure is the effect observed in the marine waters (Benner and Biddanda 1998; Obernosterer et al. 1999). We suggest that the high CDOM removal in the present study at high UV intensities could have created a similar effect over time resulting in the observed saturation of CDOM degradation, but with an ongoing degradation of non-coloured DOC.

Bacterial inhibition by high UV intensities could also be a reason for the observed saturation of total CDOM degradation. The transport of bacterial biomass to the UV exposed quartz chamber would impair the bacteria due to high UV exposure above 0.36 W m⁻², since UV is harmful for organisinal DNA (Häder and Sinha 2005). This could limit the bacterial biomass to a certain degree thus making it unable to utilise the extra pool of carbon made available by the UV exposure. If so, a higher loss of the total DOC pool would be observed at the high UV intensities if the samples were transferred to darkness for further bacterial degradation after the experiment. Besides the harmful UV radiation, increased production of free oxygen radicals at high UV intensities could limit bacterial activity (Blough and Zepp 1995). While radicals have a negative effect on bacterial growth, they can react with recalcitrant non-coloured DOC transforming it into more labile components. This results in a higher consumption of non-coloured DOC, which could be the main reason for the observed change in the ratio between the coloured fraction and the total DOC pool at higher UV intensities (Fig 3). Co-precipitation could also contribute to the higher DOC removal rates at increasing initial UV dosage. Co-metabolism is reported from terrestrial environments (Fontaine et al. 2007) and occurs when an increase in the availability of substrate for microbial degradation induces enzyme production or increased enzyme activity, which leads to higher decomposition of DOC (Kuzyakov 2010).

Interpretation of the degradation rates obtained in the present study was done by relating these to in situ measurements of CDOM in the lake from which the samples originated. Firstly, we evaluated the degradation in the lake for a situation where no new CDOM was added to the lake and found that 91.7% of the coloured substances would be removed during the year as a response to UV-induced degradation. The high removal illustrates how extensive the removal is if no new CDOM is added to the lake. In a more realistic scenario, which also considered lake water retention time, precipitation, UV intensity mediated degradation and the
concentration of inflowing CDOM, we were able to closely predict the annual temporal course of CDOM absorption in the lake. Small differences between observed and predicted values are likely related to wind induced mixing and the effect of temperature on microbial degradation that are not considered in our model.

We found that low UV intensities did not support photochemical degradation during the winter months in Lake Tvorup. Together with higher precipitation and intensified groundwater input and surface runoff from the catchment, the winter is often characterised by a net input of new organic material leading to higher in-lake CDOM concentration compared to summer (Fasching et al. 2016; Weyhenmeyer et al. 2012). Previous studies have shown a higher photodegradation in early spring (Gonsior et al. 2013) as also supported by our in situ CDOM samples in Lake Tvorup. No photodegradation takes place during winter, which results in accumulation of otherwise photo-labile CDOM. Consequently, the same UV intensities can result in higher degradation rates in spring compared to summer. Nonetheless, when UV intensity is increased with the same parent material, degradation rates increase in both spring and summer. This response is most important in the summer time when UV intensities are highest. The colour loss found in the collected water samples and by the model corresponded with the removal range of 25-50% estimated by Muller et al. (2014). However, the higher CDOM loss in our combined laboratory model study compared to in situ colour loss, could indicate that lakes are much more dynamic regarding CDOM degradation than previously assumed. In situ colour degradation is masked by the constant input of new material often associated with rain events (Raymond and Saiers 2010). In addition, a lake such as Lake Tvorup is highly susceptible to major water pulses due to the short water retention time, which means that the entire pool of CDOM can be renewed over a short period (Berggren et al. 2018). Such pulses of water adding new material to the lake could explain some of the observed variation of CDOM absorbance in the collected water samples.

Catchment vegetation and water retention time that influenced input and degradation of CDOM, respectively, are essential when forecasting future CDOM levels and optical conditions (Madsen-Østerbye et al. 2018). Establishing a correct response of CDOM and DOC degradation to increased UV radiation is another important step towards improved modelling of carbon dynamics in aquatic systems. Further work on the combined effect of water column mixing and temperature can increase our understanding of the carbon cycle in humic lakes.

In summary, we showed a clear saturation of CDOM degradation as a response to increasing UV intensities in an experiment, which mimicked vertical circulation of the water column. We suggest that colour removal is markedly higher compared to previous findings because of ongoing UV exposure. Our study supports the notion that large proportions of organic carbon are processed
and lost during transport through freshwater systems.

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**References**


Chapter 3:
Catchment tracers reveal discharge, recharge and sources of groundwater-borne pollutants in a novel lake modelling approach

Lake Tvorup, Thy National Park Thy, Denmark. Photo: Theis Kragh.
Catchment tracers reveal discharge, recharge and sources of groundwater-borne pollutants in a novel lake modelling approach

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Abstract. Groundwater-borne contaminants such as nutrients, dissolved organic carbon (DOC), coloured dissolved organic matter (CDOM) and pesticides can have an impact on the biological quality of lakes. The sources of pollutants can, however, be difficult to identify due to high heterogeneity in groundwater flow patterns. This study presents a novel approach for fast hydrological surveys of small groundwater-fed lakes using multiple groundwater-borne tracers. Water samples were collected from the lake and temporary groundwater wells, installed every 50 m within a distance of 5–45 m to the shore, were analysed for tracer concentrations of CDOM, DOC, total dissolved nitrogen (TDN, groundwater only), total nitrogen (TN, lake only), total dissolved phosphorus (TDP, groundwater only), total phosphorus (TP, lake only), δ¹⁸O/δ¹⁶O isotope ratios and fluorescent dissolved organic matter (FDOM) components derived from parallel factor analysis (PARAFAC). The isolation of groundwater recharge areas was based on δ¹⁸O measurements and areas with a high groundwater recharge rate were identified using a microbially influenced FDOM component. Groundwater discharge sites and the fractions of water delivered from the individual sites were isolated with the Community Assembly via Trait Selection model (CATS). The CATS model utilized tracer measurements of TDP, TDN, DOC and CDOM from the groundwater samples and related these to the tracer measurements of TN, TP, DOC and CDOM in the lake. A direct comparison between the lake and the inflowing groundwater was possible as degradation rates of the tracers in the lake were taken into account and related to a range of water retention times (WRTs) of the lake (0.25–3.5 years in 0.25-year increments). These estimations showed that WRTs above 2 years required a higher tracer concentration of inflowing water than found in any of the groundwater wells around the lake. From the estimations of inflowing tracer concentration, the CATS model isolated groundwater discharge sites located mainly in the eastern part of the lake with a single site in the southern part. Observations from the eastern part of the lake revealed an impermeable clay layer that promotes discharge during heavy precipitation events, which would otherwise be difficult to identify using traditional hydrological methods. In comparison to the lake concentrations, high tracer concentrations in the southern part showed that only a smaller fraction of water could originate from this area, thereby confirming the model results. A Euclidean cluster analysis of δ¹⁸O isotopes identified recharge sites corresponding to areas adjacent to drainage channels, and a cluster analysis of the microbially influenced FDOM component C4 further identified five sites that showed a tendency towards high groundwater recharge rate. In conclusion, it was found that this methodology can be applied to smaller lakes within a short time frame, providing useful information regarding the WRT of the lake and more importantly the groundwater recharge and discharge sites around the lake. Thus, it is a tool for specific management of the catchment.

1 Introduction

Most lakes are connected to the groundwater, which to some degree defines their chemical and biological characteristics (Lewandowski et al., 2015). Particularly in smaller lakes and ponds, the groundwater contributes nutrients, dissolved...
organic carbon (DOC), coloured dissolved organic matter (CDOM) or other contaminants, which can have a negative impact on the biological quality of the lakes (for nitrogen and phosphorous, see review by Lewandowski et al., 2015). These inputs often result in unfavourable light conditions for submerged macrophytes due to either increased phytoplankton biomass (Smith, 2003) or increased light absorption from high CDOM concentrations. The negative impacts of the contaminants make the identification of pollutant sources an important management issue for lakes, which, however, is complicated for groundwater due to temporal and spatial differences in discharge and associated pollutant concentrations (e.g. Meinkamm et al., 2013). In addition, the lake hydrology itself may be important, particularly in small water bodies. For example, low or fluctuating water level can have a large influence on the biodiversity of the lake (Chow-Fraser et al., 1998). This illustrates the need for approaches to quickly identify discharge (i.e. groundwater exfiltrating to the lake) and recharge (i.e. lake water infiltrating to the groundwater) areas on the lake scale and thereby provide the necessary tools for an effective management strategy for ponds and small lakes.

Groundwater discharge and recharge are traditionally identified and quantified by measurements of the hydraulic head through the installation of piezometers around the lake and of the hydraulic conductivity in the sediments (Rosenberry et al., 2015). This method is often combined with the use of seepage meters, which quantify the water entering or leaving through the lake bottom (Lee and Cherry, 1979). However, this method is challenged by the heterogeneous nature of groundwater seepage related to the specific hydraulic conductivity of the lake bed sediments (Cherkauer and Nader, 1989; Kishel and Gerla, 2002; Rosenberry, 2005). Furthermore, these methods are also time-consuming as they have to be carried out several times throughout the season. The heterogeneity and annual variability in groundwater seepage call for a robust, easier and faster method to determine groundwater inputs and influences.

Various conservative tracers have been used to achieve estimates of groundwater flow and water retention times (WRTs) in lakes. These tracers are divided into three main categories: (1) environmental tracers (naturally derived tracers from the atmosphere or catchment that are transported to the system), (2) historical tracers (anthropogenic tracers such as $^3$H or $^{36}$Cl from nuclear testing) or (3) applied tracers (tracers added to the system such as Br, Cl or fluorescent dyes) (Stets et al., 2010). Precipitation-derived environmental tracers, such as the isotope $\delta^{18}$O (reported in the Vienna-standard mean ocean water (SMOW), where $\delta_{\text{sample}} \%e = 1000(R_{\text{sample}}/R_{\text{smow}}) - 1$ and $R$ is the $\delta^{18}$O/$\delta^{16}$O ratio (Turner et al., 1987)), have been used to trace the interaction between groundwater and surface water. As evaporation occurs in the surface water it becomes enriched with $\delta^{18}$O, producing a unique lake $\delta^{18}$O/$\delta^{16}$O ratio, which can be traced in the areas with groundwater recharge (Krabbenhøft et al., 1990). The isotopic composition can also be related to evaporation lines (from the local evaporation line describing the $\delta^{18}$O and $\delta^{2}$H relationship) to estimate WRT (Gibson et al., 2002). Overall, these tracers provide information on flow patterns in the terrain or WRT, but they do not provide information regarding discharge in specific areas or the concentrations of the previously mentioned pollutants in the discharging water. We propose a different approach utilizing both conservative and non-conservative tracers such as dissolved carbon and nutrients, which are partly transferred to the percolating groundwater on its way to the lake (Kidmose et al., 2011) and have a direct influence on the lake’s biological structure.

Some non-conservative tracers such as fluorescent dissolved organic matter (FDOM), which can be determined using parallel factor analysis (PARAFAC), have been used to trace DOM in aquatic environments (He et al., 2014; Massicotte and Frenette, 2011; Stedmon et al., 2003; Stedmon and Markager, 2005b; Walker et al., 2009). PARAFAC analysis is a modelling tool that can separate multiple FDOM samples (emission and excitation matrices) into specific fluorescent components (Stedmon et al., 2003). The fluorescent components can be biochemically produced proteins derived from bacteria or molecules from the degradation of terrestrial organic material. These components have previously been found visually using a single excitation emission matrix and the observed fluorescent peaks (Coble, 1996). The differentiation between the fluorescent components are both a strength and a weakness as we can isolate many different components, but all of them can differ in both degradation and production rate in the lake and groundwater. Furthermore, these FDOM components have not yet been investigated as tracers in groundwater-fed lakes, as they, just as the rest of the non-conservative biological tracers, are volatile.

This is observed as a change in tracer concentrations (often a decrease) after the groundwater is discharged to the lake. The speed at which the change in concentration occurs is typically related to seasonal variations (e.g. temperature, mixing of the water column and UV radiation) and the WRT of the lake, e.g. the amount of time the tracer has been in the lake. The removal and degradation rates have been examined in many instances, e.g. for phosphorus (Larsen and Mercier, 1976; Vollenweider, 1975), nitrate (Harrison et al., 2009; Jensen et al., 1995), CDOM and DOC (Madsen-Ostbye et al., 2018). In a modelling approach, these rates are important as they provide information about the change in tracer concentration from the time when the tracer entered the lake. From this, it is possible to back-calculate the mixed inflow concentration of specific tracers when they were discharged to the lake. These estimations are crucial when working with non-conservative tracers, as they enable a direct comparison between the tracer concentration found in the catchment and the estimated lake concentration before degradation took place, which originates from the mixed inflow of groundwater.
As the concentrations of both conservative and non-conservative tracers in a groundwater-fed lake correspond to the mixed concentrations of discharging groundwater, taking degradation and atmospheric deposition into account, it is possible to utilize the Community Assembly via Trait Selection (CATS) approach. This model has been used to predict the relative abundances of a set of species from measures of community-aggregated trait values (average leaf area, root length, etc.) for all plant species at a site (Shipley, 2009; Shipley et al., 2006, 2011). The CATS model has three main parameters: (1) it models the probabilities that (2) maximize the entropy, which (3) is based on a set of constraints (Laliberté et al., 2014; Shipley et al., 2011). In reality, the model (1) predicts the relative abundances of species at a location from their (3) average trait values by (2) minimizing the number of species that explain the mean trait values. Maximum entropy (2) is the maximizing of “new knowledge gained” related to plant communities. This means moving from “all species have the same relative abundances” to “a few species have a high relative abundance”. When applying the model to the lake–groundwater interaction, we use the measured tracer concentrations at groundwater well sites around the lake as the individual plant species and the estimated mixed lake concentration before degradation took place as the community-aggregated trait values.

Determining groundwater movement using both conservative and non-conservative tracers found around the lake shore overcomes some fundamental shortcomings related to traditional sampling. Firstly, we often measure tracers, which do not have a direct impact on the lake ecosystem and therefore do not provide meaningful information regarding the inflow of nutrients or CDOM. Furthermore, the sampling is only performed in a few places throughout the catchment, which does not necessarily provide all the information on the groundwater flow patterns or to which degree water enters the lake and where. To overcome this, we measured conservative and relevant non-conservative tracers in and around a small lake with the aim of developing a novel approach to identify groundwater discharge and recharge areas on a high spatial scale. Thus areas that deliver pollutants to the lake, in which groundwater recharge happens and where recharge occurs with an increased flow rate, were pinpointed. The latter can spark further investigations into the lake WRT. Information regarding the WRT of the lake is especially useful when investigating how the concentrations of pollutants in the lake will develop after future restoration attempts. In the present study, the eight following tracers were measured: FDOM, CDOM, DOC, total dissolved phosphorus (TDP), total dissolved nitrogen (TDN), total phosphorus (TP), total nitrogen (TN) and $\delta^{18}O / \delta^{16}O$ isotope ratios. For these tracers, we tested (1) if groundwater discharge sites and pollutant sources can be estimated with the CATS model based on tracer concentrations, (2) whether conservative and non-conservative tracers can be used to detect groundwater recharge areas as well as provide insights into which areas possess a high groundwater recharge rate and (3) if catchment-derived tracer concentrations can be used to estimate a range of WRTs, which can be used with the CATS model.

2 Materials and methods

A small groundwater-fed lake in the sandy northwestern part of Denmark was chosen for this study (Tvorup Hul, area: 4 ha, mean depth: 2.4 m, 56°91′N, 8°46′E, UTM Zone 32). Coniferous forest and heathland dominate the catchment, although some agricultural activities are found in the eastern part of the catchment (Fig. 1a). Various isoetids including the rare nationally threatened species *Isoetes echinospora* and *Subularia aquatica* inhabited the lake until some decades ago when brownification increased significantly (based on Rebsdorf, 1981, and the present study), probably due to increasing soil pH (Ekström et al., 2011). This led to a restoration attempt in 1992; a channel was established to bypass the stream going through the lake, thus making the lake fed by groundwater. CDOM, DOC and the hydrologic conditions in the lake have since been investigated in several projects (Madsen-Østerbye et al., 2018; Solvang, 2016). This has resulted in extensive background data as well as estimations of WRTs between 0.4 and 3.3 years based on water table heights, hydraulic conductivity and seepage meter samplings (Solvang, 2016, and preliminary work Peter Engesgaard, personal communications, 2017).

2.1 Sampling and laboratory analysis

A total of 30 groundwater samples were taken every 50 m around the lake within a distance of 5–45 m to the shore in temporary groundwater wells at 1.25 m depth in February 2016. The data preparation, analysis and results are visualized in Fig. 2. The water in the wells was replaced three times before transferring the sample water to an acid-rinsed container. The samples were filtered through pre-combusted 0.7 μm nominal pore size Whatman GF/F filters the same day and kept cool and dark in hermetically sealed acid-rinsed BOD flasks until examination. Unfiltered samples were also collected from the lake.

DOC concentrations were measured using a total organic carbon analyser (Shimadzu, Japan) in accordance with Kragh and Søndergaard (2004). The CDOM absorbance was measured on a spectrophotometer (UV-1800, Shimadzu, Japan) between 240 and 750 nm in 1 nm intervals in a 1 cm quartz glass cuvette and expressed as the absorbance at 340 nm ($A_{\text{CDOM}}$ (340) cm$^{-1}$). The samples were analysed for $\delta^{18}O$ and $\delta^{16}O$ isotopes at the Department of Geosciences and Natural Resource Management (University of Copenhagen) using mass spectrometry in accordance with Appelo and Postma (2005). $\delta^{18}O$ is presented in the standard $\delta$ notation V-SMOW as $\delta^{18}O‰$ (Vienna Standard Mean Ocean Water).
2.2 PARAFAC modelling

The fluorescent properties of DOM samples were investigated using PARAFAC. The FDOM samples were initially diluted 2–12 times to account for self-quenching, also referred to as inner filter effect, which occurs with high CDOM absorbance in the sample (Kothawala et al., 2013). Sample and blank fluorescence were measured using a spectrophotometer (Cary Eclipse, Agilent Technologies, USA) by excitations between 240 and 450 nm, in 5 nm steps, while scanning the emissions from 300 to 600 nm in 2 nm increments. Prior to PARAFAC analysis, fluorescence data were processed in R (3.3.1) (R Core team, 2017) using the eemR (0.1.3) package. Blank values were subtracted following the documentation provided in the eemR package to remove Raman and Rayleigh scattering (Bahram et al., 2006; Lakowicz, 2006; Zepp et al., 2004). The data were then Raman normalized by dividing the fluorescent intensities by the integral of the Raman peak of the blank sample (Lawaetz and Stedmon, 2009) and lastly corrected for the inner filter effect (Kothawala et al., 2013) before being exported to MATLAB (2015b). In MATLAB, the fluorescence data were combined with a larger dataset (> 1000 fluorescent samples from Masciotte and Frenette (2011) originating from a range of diverse aquatic systems) in order to increase the diversity of FDOM components. This allows for the detection of components insufficiently represented in the collected samples (Fellman et al., 2009; Stedmon and Bro, 2008; Stedmon and Markager, 2005a). The drEEM package was used to perform the PARAFAC modelling following the same procedure as described in Murphy et al. (2013). A split-half analysis, in which the dataset is split into two parts and compared multiple times, was used to test the results found in the PARAFAC model. A contour map showing the measured FDOM concentrations in groundwater was plotted in ArcMap (ArcMap 10.4.1, ESRI, USA) using the inverse-distance-weighted (IDW) function with barriers fitted around the lake and drainage channels.

2.3 Groundwater recharge and areas with a high groundwater recharge rate

A hierarchical Euclidean cluster of $\delta^{18}O\%e$ was employed to determine groundwater recharge areas using the Stat base package in R. $\delta^{18}O\%e$ was chosen as it is both conservative and biologically inert. Groundwater well sites that formed a cluster together with the lake samples were considered as being groundwater recharge sites, e.g. water originating from the lake, and were excluded for the later estimations of groundwater discharge sites. The groundwater recharge sites were further investigated using a range of non-conservative tracers influenced by biological degradation. We found that some of the tracer concentrations changed when moving from the lake to the groundwater. For example, CDOM showed a decrease in concentration when entering the groundwater, which is properly due to pH changes in the soil. An inspection of the results revealed that a protein-based fluorescent component met our criteria of being (1) non-conservative, (2) not afflicted by the lake–groundwater interface and (3) not too easily degraded or produced in...
From the lake concentrations calculate the amount of degraded tracer for a range of WRTs to estimate the mixed concentration of tracers in the discharging groundwater. Relate these estimates with measured groundwater concentrations to find a maximum WRT.

Possible groundwater discharge sites are incorporated in the CATS model together with estimates of the mixed inflowing tracer concentration related to a range of possible WRTs.

The model isolates the least number of sites that can explain the measured tracer concentration in the lake and the fraction of discharging groundwater water each site is expected to deliver to the lake.

Isolate one or more tracers that are not afflicted in the lake–groundwater interface while still being affected by natural degradation over time.

Incorporate the tracer/tracers into a hierarchical Euclidean dendrogram to isolate sites that resemble the lake and indicate low retention time in the soil, e.g. high recharge rate.

The model isolates the least number of sites that can explain the measured tracer concentration in the lake and the fraction of discharging groundwater water each site is expected to deliver to the lake.

Find appropriate degradation models for the desired tracers related to the lake type and catchment area.

From the lake concentrations calculate the amount of degraded tracer for a range of WRTs to estimate the mixed concentration of tracers in the discharging groundwater. Relate these estimates with measured groundwater concentrations to find a maximum WRT.

Possible groundwater discharge sites are incorporated in the CATS model together with estimates of the mixed inflowing tracer concentration related to a range of possible WRTs.

Isolate one or more tracers that are not afflicted in the lake–groundwater interface while still being affected by natural degradation over time.

Incorporate the tracer/tracers into a hierarchical Euclidean dendrogram to isolate sites that resemble the lake and indicate low retention time in the soil, e.g. high recharge rate.

Choose a conservative tracer and incorporate it into a hierarchical Euclidean dendrogram to isolate sites that receive lake water.

Determine the concentrations of the chosen tracers in the samples.

Split the dataset into groundwater recharge sites and possible groundwater discharge sites.

Isolate one or more tracers that are not afflicted in the lake–groundwater interface while still being affected by natural degradation over time.

Incorporate the tracer/tracers into a hierarchical Euclidean dendrogram to isolate sites that resemble the lake and indicate low retention time in the soil, e.g. high recharge rate.

Find appropriate degradation models for the desired tracers related to the lake type and catchment area.

From the lake concentrations calculate the amount of degraded tracer for a range of WRTs to estimate the mixed concentration of tracers in the discharging groundwater. Relate these estimates with measured groundwater concentrations to find a maximum WRT.

Possible groundwater discharge sites are incorporated in the CATS model together with estimates of the mixed inflowing tracer concentration related to a range of possible WRTs.

Isolate one or more tracers that are not afflicted in the lake–groundwater interface while still being affected by natural degradation over time.

Incorporate the tracer/tracers into a hierarchical Euclidean dendrogram to isolate sites that resemble the lake and indicate low retention time in the soil, e.g. high recharge rate.

Figure 2. Diagram showing the workflow from data preparation to analysis to the results.

high amounts, which could create false positive groundwater recharge sites. The PARAFAC component was related to the lake concentration with a hierarchical Euclidean cluster dendrogram, and the sites that clustered together with the lake samples indicated a high groundwater recharge rate.

2.4 Non-conservative tracer degradation and lake WRT

Lake WRT was found using traditional hydrological methods combined with non-conservative tracer concentrations, which were related to their degradation rates to form a proxy

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Biogeosciences, 15, 1203–1216, 2018
for the maximum WRT. Previous hydrological models suggested that the lake had a WRT between 0.4 and 3.3 years. To further narrow this range, we estimated WRT by relating the concentrations found in the lake to their respective degradation rates related to increasing WRT, e.g. by adding the estimated removed tracer since the groundwater entered the lake to the measured concentration in the lake. This enabled us to narrow the span of the WRT based on the estimated mixed inflowing tracer concentration related to the actual catchment concentrations. For example, if the estimated inflowing concentration of a tracer is 100 µmol L\(^{-1}\) at a WRT of 2 years, and the highest catchment tracer concentration is 50 µmol L\(^{-1}\), then the catchment does not support a WRT of 2 years. In this instance, we estimated lake tracer concentrations of TN, TP, CDOM and DOC for WRTs from 0.25 to 3.5 years in 0.25-year increments following Eq. (1):

\[
\text{MIC} = \frac{\text{tr}_{\text{lake}}}{\text{ret (frac)}},
\]

where MIC is the mixed inflow concentration, \(\text{tr}_{\text{lake}}\) is the tracer concentration found in the lake and ret (frac) is the retention fraction of the tracer at a known WRT. Retention models used in this study were based on the lake type as well as the geographical location of our lake. As there is not one model that can provide removal rates across all lakes, we encourage the readers to find models related to their specific lake type. Thus, phosphorus equilibrium concentration in this study was found using Eq. (2) modified from Larsen and Mercier (1976), which describes phosphor retention in lakes with low productivity:

\[
\text{retP (frac)} = 1 - \frac{1}{1 + \sqrt{	ext{WRT}}},
\]

where retP (frac) is the retention fraction of phosphorus and WRT is the water retention time in the lake. Similarly, nitrate inflow concentrations were estimated using a modified Danish nitrate removal model derived from Jensen et al. (1995) describing retention for shallow lakes with a short WRT (0–6 years) Eq. (3):

\[
\text{retN (frac)} = \frac{59 \cdot \text{WRT}^{0.29}}{100},
\]

where retN (frac) is the retention fraction of nitrate and WRT is the water retention time in the lake. The corresponding retention fractions removed at different WRTs were related to the lake concentrations to estimate what the mixed inflow concentration must have been to produce the present lake concentration. The combined summer UV radiation and bacterial degradation rates of DOC and CDOM in groundwater from the dominating catchment vegetation type of the lake (Madsen-Østerbye et al., 2018) were extrapolated to the rest of the year. This was performed by relating the degradation rates to the mean monthly UV index (DMI, 2015) while assuming a linear relationship between the UV index and degradation rates. Thus we were able to estimate the specific removal of DOC and CDOM on a monthly basis related to the concentration measured in the lake at the time of sampling following Eq. (4):

\[
\text{tr}_{\text{lake}} = \text{tr}_{\text{lakepm}} - \text{tr}_{\text{lakepm}} \cdot \text{degra (frac)} - \text{tr}_{\text{lakepm}} \cdot \text{mf} + \text{tr}_{\text{inflow}} \cdot \text{mff},
\]

where \(\text{tr}_{\text{lake}}\) is the lake concentration in the specific month, \(\text{tr}_{\text{lakepm}}\) is the lake tracer concentration in the previous month, mff is the monthly flushing fraction (\(\text{mff} = 1/\text{WRT}/12\)), degra (frac) is the degradation fraction in the present month related to UV radiation and \(\text{tr}_{\text{inflow}}\) is the inflowing tracer concentration. Equation (4) was solved for \(\text{tr}_{\text{inflow}}\) and calculated using the same WRTs as the nitrate and phosphorus models.

### 2.5 The CATS model

Since the concentrations of both conservative and non-conservative tracers in a groundwater-fed lake correspond to the mixed concentrations of discharging groundwater, while taking degradation and atmospheric deposition into account, it is possible to utilize the CATS model. In the present study, the concentrations of non-conservative tracers (DOC, CDOM, TDP and TDN) at groundwater well sites around the lake acted as the individual plant species at a site and the equilibrium tracer concentrations derived from Eq. (1) (DOC, CDOM, TP and TN) acted as the community-aggregated trait values. When choosing tracers, it is important that there is differentiation between the concentrations measured at the sites. This means that a higher number of tracers and higher uncorrelated concentration differences between the sites result in a more secure determination of groundwater discharge sites. All tracers were investigated as a combined package, e.g. a single site is described by all the tracers mentioned above, and was run using the maxent function in the FD (functional diversity) package in R to compute the CATS model (Laliberté et al., 2014). Further information on the calculations can be found in the supplementary material for the FD package (Laliberté et al., 2014). From this, the model predicts the minimum number of groundwater well sites along the lake shore, which explains the measured concentrations in the recipient lake by maximizing the sites’ relative contribution. The model also computes lambda values from the least-squares regression measuring which tracers are most influential on the relative fractions of water originating from the groundwater well sites. Consequently, lambda values quantify how much the relative contribution from the sites change when one tracer is changed a unit while the rest of the tracers are kept constant.
3 Results

3.1 Groundwater recharge

Recharge areas were identified with a Euclidean hierarchical cluster dendrogram of $\delta^{18}O\%e$. The cluster revealed two main groups marked with orange and light blue in Fig. 3. The first group (orange) shows the groundwater well sites, ranging from sites 18 to 29, which clustered together with lake samples. The samples in this orange cluster share a clear resemblance with lake $\delta^{18}O\%e$ measurements and were therefore considered as groundwater recharge sites. The recharge sites were located in the north and western part of the lake and are marked with orange in Fig. 1a.

3.2 Fluorescent DOM

PARAFAC and split-half analysis modelling identified five distinct fluorescent DOM components (C1–C5, explained variance 96.79%). The spectral properties of the five fluorophores (components) identified by the PARAFAC analysis (Fig. 4) revealed that the DOM pool had both terrestrial and microbial influence. Component C1 was similar to previously found components from terrestrial humic-like material (Stedmon et al., 2003). The component absorbs in the UV-C region, which is absorbed by the ozone layer and atmosphere (Difffey, 2002), and is for this reason expected to be mainly photo-resistant (Ishii and Boyer, 2012). Component C2 has been reported to be both marine and terrestrial humic-like (Coble, 1996; Murphy et al., 2006) and seems to be degraded by visible light and produced by microbial degradation in equal amounts (Stedmon and Markager, 2005b). Component C3 was also believed to be of terrestrial humic-like origin and was similar to the fluorescent peak C described by Coble (1996). The component absorbs in the UV-A region and is susceptible to both microbial and photochemical degradation (Stedmon et al., 2007; Stedmon and Markager, 2005a). Component C3 may be an intermediate product or produced biologically since changes in the concentration have been observed in the open oceans and in sea ice that has no apparent connection to the terrestrial environment (Ishii and Boyer, 2012). Component C4 is similar to component 5 found in Stedmon et al. (2003) and is believed to be a combination of fluorescent labile materials named peak N and T, which are produced biologically associated with DOM degradation (Coble, 1996; Stedmon and Markager, 2005b). Component C5 is linked to free tryptophan, which is a product of microbial activity (Determann et al., 1998). This component has been found to decrease during dark incubations and UV exposure (Stedmon et al., 2007), but component C5 is also associated with the degradation of DOM (Stedmon and Markager, 2005b) and autochthonous production (Murphy et al., 2008).

The highest fluorescence concentrations were found in the groundwater while the lake water fluorescence concentrations were generally lower (Table S1). Component C1 had the highest fluorescence with a value of 7.8 Raman’s units (R.U.) in the lake and a maximum fluorescence of 47.1 R.U. at groundwater well site 7. Component C5 had the lowest fluorescence in the lake (0.27 R.U.) and a maximum fluorescence of 2.9 R.U. at groundwater well site number 8. Component C5 also varied much between groundwater samples with the lowest value of 0.1 R.U. or 28 times lower than the maximum concentration. Components C1, C2 and C3 had low lake-like concentrations in recharge areas (orange sites in Fig. 1a). Concentrations of C4 were generally higher in groundwater around the lake than in the lake (1.1–1.5 vs. 1.1 R.U. visualized in Fig. 1b). Component C4 was chosen as a proxy for groundwater recharge as the concentration of the C4 component increase with biological activity and there were no apparent concentration changes in the lake–groundwater interface. The cluster diagram of component C4 showed that especially site 24 grouped with lake samples, but sites 20, 21, 23 and 26 also showed high comparability with the lake (Fig. 5), which can also be observed from the IDW map of component C4 around the lake (Fig. 1b).

3.3 Groundwater discharge areas and lake WRT

Tracer concentrations of the lake narrowed down the possible WRT. Equilibrium tracer concentrations of DOC, CDOM, TDP and TDN (found using Eqs. 1–4) for WRTs between 0.25 and 3.5 years in 0.25-year increments revealed that concentrations of TDN in the catchment are not high enough
Groundwater discharge areas were found employing the CATS model on nutrient concentrations and DOM fractions estimated using Eq. (1). related to WRTs between 0.25 and 2 years. The estimated phosphorus concentrations ranged from 46 to 80 µg PL$^{-1}$ (Eqs. 1 and 2) while nitrate concentrations ranged from 1113 to 2417 µg NL$^{-1}$ (Eqs. 1 and 3). CDOM and DOC degradation rates were related to the UV index and varied from 0.64 % in December to 28 % per month in June for DOC and between 0.46 and 20 % for A$_{CDOM}$ (340) in the same months and were significantly different from each other ($P < 0.001$). Thus, estimated mixed inflow concentrations of CDOM ranged from A$_{CDOM}$ (340) = 0.43 to 1.04 cm$^{-1}$ while DOC ranged from 1205 to 3160 µmol L$^{-1}$ for a WRT between 0.25 and 2 years (Eqs. 1 and 4). The CATS model isolated the minimum number of sites that explained the estimated lake concentrations. The model identified sites 1, 9, 11, 13 and 14 as the groundwater discharge sites delivering more than 0.1 % of the water throughout the different WRTs (Fig. 6). Changes in site distribution and fractions of discharging water were observed between the different WRTs, but in general, groundwater from 3–4 sites explains the estimated concentrations in the lake (Fig. 6). Site number 14 delivers more water with a higher WRT (to a maximum of 54 % of the total discharge). Site 1 peaks at a WRT of 1.25, providing 27 % of the water to the lake. Site number 9 delivers less water with increasing WRT, but 49 % at the lowest WRT of 0.25 years. Site number 11 delivers 26 to 34 % of the water to the lake until a WRT above 1.5 years is reached, at which site 13 explains the concentration in the lake better and provides 29 and 19 % of the water to the lake. Overall, 73 to 96 % of the water is estimated to arrive from the eastern part of the lake, while site number 1 (in the southern part) is estimated to deliver 4 to 27 % of the water. Lambda values, explaining which tracers are the most important when predicting the fractions of water originating from groundwater well sites, showed that CDOM was the most important tracer when determining which sites delivered water to the lake with a mean lambda value for all WRTs of 24.2 vs. 0.1–5.9 for the other tracers.

4 Discussion

The combination of biological and hydrological methods in a novel approach provided a better estimate of the WRT, an identification of groundwater recharge and discharge areas, and the fractions of water coming from each site. Based on the model results and earlier hydrological studies, we will discuss the main questions from the introduction (1) if groundwater discharge sites and pollutant sources can be estimated with the CATS model based on tracer concentrations, (2) whether conservative and non-conservative tracers can be used to detect groundwater recharge areas as well as provide insights into which areas have a high groundwater recharge rate and (3) if catchment-derived tracer concentrations can be used to estimate a range of the WRTs, which can be used with the CATS model. Furthermore, we will discuss which of the tracers work and which could possibly work with refined methods, as well as how these findings could benefit lake restoration programmes.
Figure 5. Euclidean hierarchical clustering of fluorescent component C4 from recharging groundwater sites. The fluorescence found at sites 20, 21, 23, 24 and 26 clusters together with lake fluorescence (marked red). This indicates that these sites have a high degree of groundwater recharge. Groundwater well site 24 seems to be especially important in this regard.

Figure 6. Results derived from the CATS model shown in a bar plot in which the groundwater well sites (their numbering) are seen on the top x axis and the fractions of groundwater discharge estimated to derive from the sites are shown on the y axis (only the sites that deliver more than 0.1 % water to the lake are shown). The bottom x axis denotes the different water retention times used in this model. Three to four sites generally explain the estimated concentrations in the lake.

4.1 Determination of groundwater recharge areas

Groundwater recharge sites were identified along the northern and western part of Tvorup Hul with a hierarchical cluster analysis of the conservative δ18O tracer. The exact same areas are also the ones with adjacent drainage channels (Fig. 1a), which facilitate the areas as recharge sites. While δ18O‰ worked well as a general groundwater recharge estimator, it does not indicate which sites deliver more water. An indication of this can be found when examining the non-conservative tracers such as the fluorescent components.

Sites resembling the fluorescence found in the lake will indicate a high groundwater recharge rate, while a difference in concentration between lake and groundwater sites will indicate a lower groundwater recharge rate where there is sufficient time for a significant modification of the components representing the DOM pool of the lake. The fluorescent component C4 has previously been found to increase with biological activity (Coble, 1996), which is why we used it as a proxy to estimate the sites with a high groundwater recharge rate. The hierarchical Euclidean cluster dendrogram of component C4 showed that sites in the northern part of the lake formed a group (sites 24, 20, 21, 23 and 26) (Fig. 5 and visually in Fig. 1b). This information can be of importance related to placement of seepage meters, which will result in better estimations of the groundwater discharge and recharge and as such the modelled WRT of the lake. In other words, it might be advantageous to carry out groundwater sampling first to estimate sites with high discharge rates, then estimate WRT, utilizing these sites and finally model groundwater discharge areas by using the improved and narrowed WRT range.

CDOM generally showed much lower absorbance at groundwater recharge sites than in the lake, making it less suitable for estimating recharge areas. The decrease in absorption is possibly due to low soil pH, causing flocculation of CDOM in the soil matrix (Ekström et al., 2011). The same was observed with fluorescence of component C1, which had lower intensities in recharge areas, indicating that component C1 is linked to CDOM. While component C1 was not particularly useful for estimating groundwater recharge, it could be useful to estimate discharge sites. To utilize the component for discharge estimates there is a need for an assessment of the degradation rate. While it has been shown that component
C1 is largely photo-resistant, as it does not absorb the UV-A radiation areas and is largely resistant to microbial degradation processes (Ishii and Boyer, 2012), no reliable rates for the degradation have been found. In this study, we found that only sites number 9 and number 11 hold concentrations lower than the lake (Table S1), indicating that most groundwater discharge would originate from these sites if little to no degradation takes place.

4.2 Determination of groundwater discharge areas

Neither $\delta^{18}O$ nor previous seepage meter samplings have achieved a similar understanding of groundwater recharge areas in Tvorup Hul as compared to the present approach. While the $\delta^{18}O$% provides a way of separating groundwater and surface water, using it to determine groundwater discharge sites is simply not possible due to the homological distribution seen in groundwater (Krabbenhoft et al., 1990). Previous seepage meter samplings provided scattered and momentary estimations of discharge sites, indicating that groundwater entered the lake from the southern bank (Solvang, 2016). This does not correspond to tracer concentrations found in the southern area, which show very high CDOM absorbance at 340 nm ($A_{CDOM}(340) = 1.3–3.1 \text{ cm}^{-1}$) and DOC concentrations (3114–10 467 μmol L$^{-1}$) in relation to the lake ($A_{CDOM}(340) = 0.4 \text{ cm}^{-1}$/DOC 1058 μmol L$^{-1}$). This hints that the lake is influenced by groundwater discharge from other areas as well. The lowest DOC concentrations in the southern area were several times higher than those from the equilibrium estimation, suggesting a WRT above 6 years, which is well beyond previous estimates of WRT. Samples from the eastern shore had lower concentrations all around, proposing that water from this area influences the lake water. Thus, if the water actually originated from the southern area, the lake would need to have a prolonged WRT, resulting in increased removal of tracers from the lake. This requirement conflicts with the remaining tracers, for which especially TDN sets an upper limit of 2 years to the WRT.

The CATS model used in this study shows that while a fraction of groundwater enters the lake from the southern bank, most of the water originates from the eastern shore (Fig. 1a). Seepage meter measurements from the eastern shore showed both discharging and recharging of groundwater (Solvang, 2016). The same was observed for $\delta^{18}O$% samples from the eastern part of the lake, which were lower than in the groundwater from the southern shore, indicating an influence of newly precipitated water or influence from the lake. Sampling in the northeastern and eastern part of the lake revealed an area with little groundwater and a clay deposit layer that possibly reduces infiltration to deeper groundwater layers. As a result, precipitations could enter the lake as surface and subsurface run-off water originating from the hills to the east and the plateau in the northeastern corner (Fig. 1a), resulting in short bursts of discharging water. The multi-tracer approach enables the determination of discharge areas much more precisely and on a temporal scale related to the WRT of the lake (in this instance the previous 3 to 24 months). Consequently, the model is able to track uncommon events such as heavy precipitation events in which a large amount of water is discharged to the lake during a short period. These events are often difficult to track as seepage meters need to be deployed in this exact period as well as in the right places.

4.3 Tracer influences

Most tracers used in this study are less conservative compared to $\delta^{18}O$ and can therefore change both in the lake water and in the catchment soils. This entails an understanding of processes and rates that influence the concentrations. The temporal variability in nitrate concentrations in groundwater are related to the flow rate rather than seasonal changes (Kennedy et al., 2009). The same was observed for phosphorus, where particularly dry periods followed by heavy rain increased the phosphorus concentration measured in groundwater-fed springs (Kilroy and Coxon, 2005). Thus, in the case of northern Europe, sampling during late winter might be the best solution because soils are saturated at this time of year (Sand-Jensen and Lindegaard, 2004). Previously polluted areas, e.g. from wastewater infiltration, with increased concentrations of DOC and nutrients are likely to be in a state of imbalance, resulting in a reduction in concentrations over time (Repert et al., 2006). For this reason, in these areas, it is important to conduct temporal sampling following decreases in concentrations and to relate the samples to lake concentrations during sampling. Lake inter-annual DOC and CDOM changes were generally low in our study with an annual $A_{CDOM}(340) = 0.41 \text{ cm}^{-1} \pm SD 0.05$, corresponding to what is observed in larger water bodies where WRT integrates inflowing DOC and CDOM (Winterdahl et al., 2014). Inter-annual DOC and CDOM variations in groundwater from the lake catchment (Fig. S1) showed the same tendency as described for nutrients, and this suggests that sampling should be performed at multiple times or in a period without drought or high rainfall. On a broader scale, the variation in DOC is known to be related to hydrology (Erlandsson et al., 2008), mean air temperature (Winterdahl et al., 2014) and the recovery from acid deposition (Evans et al., 2006; Monteith et al., 2007). Sampling from wet areas with standing surface water resulted in high concentrations of most tracers (Table S1). Consequently, these areas should be avoided, seeing that they provide no information regarding the discharge of groundwater. The removal of CDOM and DOC also changes on an annual basis in lakes and is related to bacterial degradation, photodegradation, sources and mixing of the water column. A sensitivity analysis of the results was conducted by running the CATS model with a ±10 % change in tracer concentrations. The results showed that sites generally remained unchanged with only smaller deviations.
in percent-wise distribution in discharge up to a WRT of 1.25 years (Fig. S2). Above this point, there are some differences in sites, which change between sites number 11 and number 13. In conclusion, even when changing multiple parameters in the model, the same five groundwater wells are identified, explaining the measured lake concentrations. Future investigations into variation in tracers in groundwater and degradation rates in lakes will likely strengthen this model.

The processes that influence changes in FDOM are still being investigated (Ishii and Boyer, 2012). Tracing FDOM has been conducted in both rivers and open waters (Baker, 2001, 2002; Stedmon and Markager, 2005a), but only a few studies have been conducted in groundwater. These studies have focused on changes in FDOM from deep groundwater wells (Lapworth et al., 2008) or tracing FDOM using samples that are collected very far apart (Chen et al., 2010). Specific fluorescence intensity of components showed large differences among sites in this study, up to a factor of 28, between groundwater well sites, with the lowest at site 11 and highest at site 8, around the relatively small lake. These findings illustrate the problem when applying FDOM as a tracer over large distances in groundwater. In addition to bio- and photodegradation of fluorescent components, absorption changes have also been observed in relation to Fe(III) concentrations (Klapper et al., 2002). This might change the concentrations of FDOM components as they travel from anoxic groundwater with reduced iron into the oxic lake water. Overall, PARAFAC components have the potential to work as groundwater tracers, but there is a need for a better understanding of the processes that cause changes in fluorescence characteristics of DOM and hence concentrations of FDOM components both in the lake and in the lake–groundwater interface.

4.4 Potential lake management influence

The determination of discharge sites can result in direct management related to specific problematic areas. The model used in this study showed that water entering the lake primarily originated from the catchment to the east of the lake. If water from this part were diverted around the lake, there would be a reduction in CDOM absorbance of 61–89% based on calculations relating percent-wise discharge, its concentrations and WRTs from 0.25 to 2 years in 0.25-year increments. Conversely, diverting water around the lake at site number 1 would only result in a lowered inflowing CDOM absorbance of 11–39%. Moreover, in both cases, there would be an increase in photobleaching of present CDOM in the lake caused by the increased WRT. Furthermore, huge reductions would occur for TP and TN, with a decrease of 82–96% if diverting water from the eastern shore, in contrast to the southern shore with a modelled decrease of 4–18% in TP and TN. In the future, hydrology is likely to be the main driver of variability in DOM (Erlandsson et al., 2008) with an estimated increase in CDOM by a factor of 4 in lakes with short WRT (Weyhenmeyer et al., 2016). This makes it critical to establish a modelling tool that is capable of pinpointing sites delivering pollutants to lakes and provide us with the ability to take action and reduce the impact on the ecological state of lakes.

5 Conclusions

The present method and modelling tool can improve estimates of recharge and discharge areas as well as WRT in smaller lakes on a temporal scale. The model provides accurate estimates of discharge fractions, related to field measurements, and can be used for precise management of problematic pollution areas. The hierarchical clustering can be used to estimate groundwater recharge sites, which can be incorporated as a guideline for a better estimation of WRT in lakes. Furthermore, the use of multiple tracers strengthens the model and keeps a certain degree of freedom in regard to the choice of tracers related to laboratory capabilities.

Data availability. The underlying data can be accessed in the Supplement (Table S1).

The Supplement related to this article is available online at https://doi.org/10.5194/bg-15-1203-2018-supplement.

Competing interests. The authors declare that they have no conflict of interest.

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E. Kristensen et al.: Catchment tracers reveal groundwater flow


Chapter 4:
Light climate and submerged plants in a large re-established lake on agricultural land

Lake Fil, Denmark. Photo: Theis Kragh.
Light climate and submerged plants in a large re-established lake on agricultural land

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Abstract

Lake establishment on former agricultural fields is usually associated with high nutrient loading, high phytoplankton biomass and limited colonisation of submerged macrophytes. In contrast, Lake Fil in Denmark attained a remarkably high species richness immediately after establishment. Our objectives were to examine the light climate and its regulation in Lake Fil and to evaluate whether transparent water could account for the high macrophyte richness. Four years of measurements of light attenuation showed high daily (0.45 to 35.2 m\(^{-1}\)) and seasonal medians (2.7 to 10.2 m\(^{-1}\)) with the lowest values in spring. Dissolved coloured organic matter (CDOM) and suspended sediment particles were responsible for the largest mean proportions of total light attenuation (30.5% and 64.7%, respectively), while the contribution of phytoplankton was small (4.8%). CDOM had a particularly high attenuation in the first year following establishment due to release from flooded soils and grain-stubbles left on the fields. Sediment particles formed the main attenuation component because of frequent wind-induced resuspension in the shallow wind-exposed lake. During calm periods, particle attenuation declined, and phytoplankton established higher biomasses. Well-develop colonisation of macrophytes only reached 0.6 m depth at 2-21% subsurface light. However, scattered macrophyte populations were established down to 1.2 m during clear water spring periods and they survived turbid summer periods by extending their shoots into high light at the water surface. Despite unclear waters and low depth penetration, macrophyte colonisation was successful on open lake shores caused by physical exposure, water level fluctuation and cattle grazing.
Introduction

During the past 200 years, more than half of Europe’s wetlands have been drained and reclaimed for agriculture (Moreno-Mateos et al. 2012; Zedler and Kercher 2005). Especially small shallow lakes and ponds have been lost in the intensively cultivated European lowland regions (Sand-Jensen 2001; Williams et al. 2001), but also many large lakes have been drained and converted to crop land (Hansen 2014). In Denmark alone, more than 200 large lakes (> 10 ha) have vanished by conversion to agricultural fields between 1780 and 1980 (Hansen 2014).

The long-term loss of wetlands, cultural eutrophication of lakes and decline of biodiversity have recently stimulated the interest in re-establishing wetlands and lakes and improving their ecological quality. These management initiatives have had different objectives such as reducing and retaining nutrients in the aquatic continuum (Strand and Weisner 2013; Zedler and Kercher 2005), improving aquatic biodiversity (Mitsch et al. 1998) as well as generating recreational areas to the public and stimulate their appreciation of nature quality (Hoffmann and Baattrup-Pedersen 2007). During the past two decades, more than 50 larger lakes (>10 ha) have been re-established in Denmark for these reasons (Hansen 2014). In this paper, we examine the light conditions that are essential determinants of macrophyte colonisation and richness during four years after re-establishment of Lake Fil, the largest (970 ha) new lake in Denmark.

In re-established lakes on former agricultural land, the large phosphorus pools in the former cultivated soils may have a large impact on the ecological quality (Pant and Reddy 2003; Steinman and Ogdahl 2011). Because the organic and nutrient soil pools usually are much richer compared to the original lake sediment before drainage, the new lakes may face high internal nutrient loading and release of chromophoric dissolved organic matter (CDOM) to the lake water. Re-established lakes, mostly shallow, can also experience a high wind-induced physical stress on the sediments suspending particles into the water column and, thereby, release phosphorus, stimulate phytoplankton growth and increase vertical light attenuation further (Søndergaard et al. 2003).

Regulation of water clarity is complex (Kirk 1994). Vertical light penetration is reduced by CDOM, suspended sediment particles and phytoplankton pigments (Kirk 1994). Leaching of CDOM from flooded terrestrial soils and vegetation can cause strong light attenuation and thus decrease the light availability for both phytoplankton and benthic phototrophs (Karlsson et al. 2009). However, the light-absorbing CDOM initially released from flooded terrestrial soils may decrease over time due to gradual reduction of leaching from organic soil pools and ongoing photochemical degradation and bacterial mineralisation of CDOM in the lake water (Madsen-Østerbye et al. 2018). Re-location of fine soil particles from exposed shallow waters to deeper waters with less physical shear may also reduce the likelihood of particle resuspension over
time, depending on lake size and bathymetry (Kragh et al. 2017). Phytoplankton development in nutrient-rich lakes is dependent on the magnitude of the other attenuation components and phytoplankton may flourish and contribute substantially to overall light attenuation when CDOM and particle attenuation is low, while it may be low when CDOM and particle attenuation is high.

The submerged vegetation in lakes plays a key role in ecosystem functioning when it reaches a high areal cover (Hilt et al. 2017; Sand-Jensen and Borum 1991). Macrophytes can form a refuge to zooplankton grazers, reduce release of sediment nutrients, enhance particle sedimentation and compete directly with phytoplankton in shallow waters for nutrients, thereby increasing water clarity (Scheffer et al. 1993). Moreover, the development of submerged vegetation is strongly dependent on the light climate and, thus, on the combined light attenuation of CDOM, particles and phytoplankton in the water (Pérez et al. 2010). These different components interact in such a way that increased light attenuation results in declining algae biomass and chlorophyll concentrations (Pérez et al. 2013). The lower depth limit of macrophyte growth is often close to the Secchi-transparency at 8-15% of subsurface light (Middelboe and Markager 1997). The key to regulation of submerged macrophyte depth limits and areal cover is lake bathymetry and water transparency.

We examined the temporal dynamics of light attenuation and related it to submerged vegetation development over four years in the large, shallow Lake Fil in Denmark following its re-establishment on agricultural land in the winter of 2012-2013. Already two years after its re-establishment, the lake has experienced rapid development of a species-rich submerged vegetation although high nutrient concentrations in the water should support phytoplankton blooms and restrict submerged plant growth (Bastrup-Spohr et al. 2016). Our general objective was to quantify the dynamics of CDOM, suspended particles and phytoplankton, which controls the light regime in Lake Fil and influences the establishment of submerged vegetation. The specific objectives were: 1) to determine the temporal development of light attenuation over four years following lake re-establishment, 2) to determine the main parameters that control the light attenuation coefficient in the water, and 3) to examine the relationship between macrophyte development and light climate.

**Materials and Methods**

**Study site**

The study was conducted in Lake Fil, which is located 3 km behind the sand dunes in South West Jutland facing the North Sea (55°42′N, 8°14′E). Previously, Lake Fil was the second largest lake in Denmark (2185 ha, before 1850), but it was drained and reduced in size on several occasions from 1852 and onwards, and finally totally reclaimed for agriculture in 1952. Until 2012, the reclaimed lake area was intensively cultivated and received large amounts of inorganic fertilisers
and manure (Hansen 2014). The result was soil with a high phosphorus content (Kragh et al. 2017).

**Re-establishment of Lake Fil**

In 2010, the area was purchased by Aage V. Jensen Nature Foundation to re-establish the lake and the surrounding meadows and grasslands. The new Lake Fil was established in 2012 at approximately one third of its original size (889 ha). The new lake is divided into a southern (Søndersø) and northern (Mellemsø) basin of approximately equal size. The catchment area is 104 km², with the inlet located in the southern basin and the outlet located in the northern basin. The two basins are separated by a 7 m wide dam and interconnected by a 5 m wide canal through the dam. The lake is shallow (mean depth: 1.03 m; maximum depth: 3.5 m). Mean nutrient concentrations from weekly water samples were 102 µg L⁻¹ of total phosphorus (P) and 1741 µg L⁻¹ total nitrogen (N) and chlorophyll α concentrations were 13.3 µg l⁻¹ (n = 92) (Bastrup-Spohr et al. 2016).

The lake is highly exposed to wind because of its large surface area, shallow water and location close to the windy North Sea resulting in a well-mixed water column and frequent resuspension of sediment particles (Kragh et al. 2017). Water retention time averaged 70 days (range: 20-180 days). Prior to flooding in autumn 2012, grain-stubbles were left on the fields to stabilise the sediment and reduce resuspension of particles and phosphorus release to the water immediately after flooding.

**Field measurements**

Field measurements started in summer 2013, approximately seven months after flooding was commenced and continued through 2017, and consequently we have data from four years. A meteorological station was established on the lake shores 2 m above the water surface. The station was equipped with sensors for light (HOBO PAR sensor (400-700 nm): S-LIA-M003, Onset Computers), wind speed and wind direction (HOBO anemometer and S-WSET-A, Onset Computers). Data was stored on a data logger (HOBO micro station, H21-002, Onset Computers). Incident PAR and lux were also logged separately (Odyssey PAR logger, Dataflow Systems; HOBO UA-002-64, Onset Computers). Due to technical problems in 2014, data was retrieved from a neighbouring meteorological station located at Skjern Enge 28 km from Lake Fil. Comparisons of data between the two stations were made when both stations were in operation and were used to calibrate data from Skjern Enge to Lake Fil in 2014.

At four open water sites (two in each basin), light measuring stations were set up. Each station was equipped with either two-five lux loggers (HOBO UA-002-64, Onset Computers), two-three PAR sensor (Odyssey PAR logger, Dataflow Systems) or a combination of these two measuring systems placed 50-100 cm apart from each other from the lake bottom and up through the water column. Daily measurements of water levels with 2 mm precision were recorded by pressure differences between a submerged water level data logger and a similar one in air (HOBO U 20-001-04, Onset...
The measured water level was calibrated to current depth of deployment for each light measuring station. Water level measurements were used to determine the specific depth of lux and PAR loggers at each site and specific time. Calculations of vertical attenuation coefficients from HOBO and PAR sensors yielded closely similar results (F-test, \( p < 0.001 \)). Continuous light measurements were collected, except in cold winter months with ice formation. Data was used to calculate the daily mean vertical light attenuation coefficient through the water column, \( k \) (unit m\(^{-1}\)) by equation (1) (Kalff 2002).

\[
k = \ln\left(\frac{I_z}{I_0}\right) / z
\]

\( I_z \) is the light at a given depth (z, unit m) and the incoming light \( (I_0) \) was adjusted to sub-surface light assuming a 10% surface reflection (Kirk 1994).

Continuous measurements of phytoplankton chlorophyll \( a \) fluorescence were measured in the southern basin by a CYCLOPS-7 fluorescence probe (Turner Design, CA, USA) placed 30 cm below the water surface. The fluorescence signal was calibrated against water samples analysed for chlorophyll \( a \) by extraction in ethanol and measurement on a Shimadzu UV-1800 spectrophotometer according to Jespersen and Christoffersen (1987). Chlorophyll-specific light attenuation coefficients were calculated assuming a mean value of 10 m\(^{-1}\) (g chlorophyll \( a \) m\(^{-3}\)) according to Krause-Jensen and Sand-Jensen (1998).

Vegetation surveys at Lake Fil showed a remarkable species richness and colonisation depths of 0.6 m in the summers of 2014 and 2015 (Bastrup-Spohr et al. 2016). Simultaneously with our field campaign, macrophyte colonisation zones in both basins were investigated by sonar side scan according to Bastrup-Spohr et al. (2016). The presence or absence of macrophytes was controlled by sampling with a rake at the specific depths.

**Water sampling**

Water samples were collected on a weekly basis from the inlet, outlet and the channel connecting the southern and northern basin. The channel water represented the water in the southern basin and the outlet water the northern basin. Water was filtered through pre-combusted GF/F filters (0.7 µm) and CDOM absorbance in the filtered water was scanned across the PAR spectrum (400-700 nm) on a spectrophotometer (UV-1800 Shimadzu). CDOM light attenuation coefficients (m\(^{-1}\)) were calculated according to Kirk (1994). Gaps between data points were filled by linear interpolation to estimate the daily CDOM light attenuation coefficients in the lake. For POC analysis, GF/F filters of water samples were dried at 50°C and stored at room temperature until analysis. Carbon content on filters were analysed by high-temperature combustion using a system of a Struers Carbolite Furnace set at 650°C, an ADC-225-Mk3 NDIR IRGA analyser and a Picotech ADC-20 data logger. The filters were not exposed to HCL fumes prior to the analyses due to insignificant carbonate content.
Statistical analysis
The standard statistical analyses, Two-way ANOVA, One-way ANOVA and graphs were made in R (R Core Team 2017). Concerning the ability of wind speed to increase particle light attenuation (Fig. 6), data from all four stations showing an increase in light attenuation compared to the previous day were included. The 95 percentile was calculated based on a running average of 25 data point and the line between 95 percentiles was smoothed by using the Hill growth curve using the growthcurver package (Sprouffske 2018).

Results
Seasonal and annual light attenuation
Vertical light attenuation coefficients were high and variable at the four sites in the two basins over four or five years (Fig. 1). Medians ranged among seasons from 2.7 to 10.2 m\(^{-1}\) (n= 59) and daily values varied from 0.45 to 35.2 m\(^{-1}\) (n= 6933). For all years, median light attenuation coefficients were significantly lower during spring compared to summer (Two-way ANOVA, F = 1108, p < 0.001) and autumn (Two-way ANOVA, F = 529.6, p < 0.001). The same seasonal pattern with lower light attenuation during spring compared with summer and autumn was observed at all four sites in the two basins. Over the years, seasonal medians did not change systematically for any season. Spatial and temporal changes of total light attenuation are due to changes in the three main attenuation components: coloured dissolved organic matter (CDOM), phytoplankton and suspended non-algal particles.
Fig 1. Vertical light attenuation coefficients over seasons and five years in Lake Fil at four sites. Two sites in the southern basin (a and b) and two sites in the northern basins (c and d). Box-whisker plots are calculated for each season based on daily mean values based on measurements every 10 minutes. Boxes with medians include 25-75 percentiles and bars cover the range.

**Attenuation by CDOM**

The first year after the re-flooding of Lake Fil, CDOM light attenuation was significantly higher in both the southern and the northern basins compared to the following years (One-way ANOVA, $F = 56.66$, $p<0.001$) (Fig. 2). The high initial CDOM attenuation likely derives from CDOM release from the flooded soils and stubbles of crops left on the fields. No systematic seasonal changes of CDOM attenuation were found in the two basins except that values appeared to be slightly higher in winter compared to the other seasons (except in 2017). In the inlet, no systematic seasonal or year-to-year CDOM variations were observed, but the variation within seasons was extensive because of changes in stream discharge (Fig.2).
Attenuation by phytoplankton

Chlorophyll concentrations in phytoplankton were low in Lake Fil because of high background light attenuation in the water, despite the high nutrient concentrations. Consequently, light attenuation from phytoplankton was low throughout the summer and always less than half compared with attenuation from CDOM and suspended particles. The median chlorophyll attenuation was highest in spring 2014 and lowest in summer 2016 (Fig.3). Temporal development of phytoplankton was closely dependent on light attenuation in the water column. During periods of calm weather and little
sediment particle resuspension, the light attenuation decreased by 1.25-1.5 m\(^{-1}\), thus increasing the mean available light in the water column. As a response, the phytoplankton biomass and, thereby, the chlorophyll attenuation increased by an average of 0.69 ± 0.29 m\(^{-1}\) (n= 14). Subsequently, the chlorophyll biomass and chlorophyll light attenuation declined when windy weather again took over, which caused sediment resuspension and reduced light availability in the water column (Fig. 4).

![Fig. 3 Chlorophyll attenuation coefficient over seasons and five years in Lake Fil. Box-whisker plots are calculated for each season based on continuous measurements of chlorophyll fluorescence in the southern basin. Boxes with median include 25-75 percentiles and bars cover the range.](image-url)
Particles and partitioning of attenuation

Light coefficients of different attenuation components (CDOM, chlorophyll and particles) are shown together for the two basins in 2014 (Fig. 5). In both the southern and northern basins, non-algal particles represented, on average, more than half of total light attenuation. CDOM was the second most important contributor to light attenuation. On occasions of high external input, CDOM concentration increased and caused the highest proportion of light attenuation. In contrast, chlorophyll attenuation represented the smallest proportion of total light attenuation (mean 4.9%, range 0.1% -54%) both seasonally and annually.
Wind and sediment particle resuspension

Increasing wind velocity caused resuspension of sediment particles up to an upper asymptotic level. Wind from the north-east and south-east lead to increase of total light attenuation at lower mean wind speeds than wind from the prevailing directions from north-west, west and south-west (Fig. 6). Easterly wind increased particle attenuation at wind speeds exceeding only 1.25 m s$^{-1}$ to reach a maximum at 3.75 m s$^{-1}$, while attenuation increased above wind speeds of 2.5 m s$^{-1}$ and reached a maximum at 4-5 m s$^{-1}$ when
Wind came from westerly directions. The main direction and highest wind speeds across the lake is from the west. In the lake, the light attenuation coefficient increased as a response to increasing wind speeds with a maximum of about 1.25 m\textsuperscript{-1} compared to the day prior to the wind event. Regardless of wind direction, the same maximum increase of the light attenuation coefficient was found at high wind speed.

Fig. 6. The increase of the light attenuation coefficients from one day to the next as a function of the highest average wind speed over two coherent hours from different wind directions (open dots). (a) North-east (b) South-east (c) South-west and (d) North-west. The solid line represents the smooth Hill growth curve through 95 percentiles derived from running averages of 25 points.
Macrophyte depth distribution

The majority of submerged macrophytes penetrated to only 0.6 m depth in the lake during low water levels in July. However, during March to May water levels are 0.06-0.47 m higher and the macrophyte colonization depths are then located at 0.66-1.08 m (Fig. 7). Consequently, when calculating the irradiance at the lower depth boundary it is essential to correct for the variations in lake water level. If uncorrected, we would grossly overestimate the available irradiance at the depth boundary in March-May. For all four years, we calculated median daily irradiances over monthly periods between March and September at the depth boundary which receives between 0.95 and 6.31 mol m$^{-2}$ d$^{-1}$ (PAR, 400-700 nm) corresponding to 2.1-20.6% of subsurface irradiance.

After macrophytes have sprouted and extended into the water column, they will experience higher irradiances closer to the water surface. Thus, if plants manage to sprout at greater water depths than 0.6 m during short periods of high irradiances, like in May 2015, they can form scattered populations at greater depths as observed for the tall robust *Potamogeton praelongus* and *Potamogeton natans* growing at 1.2 m in 2016 and 2017.
**Discussion**

We used four years of *in situ* light measurements to evaluate the daily and seasonal development of the light attenuation in Lake Fil, which was recreated on former agricultural fields. We evaluated the role and mutual dynamics of the three main attenuation components in the lake – coloured dissolved organic matter, suspended particles and phytoplankton chlorophyll. Finally, we evaluated the variability of light intensity at the depth boundary of macrophyte colonization. Below, we discuss the findings and the implications.

**Light conditions and regulation in Lake Fil**

Four years measurements of light attenuation coefficients at four different stations did not show...
any systematic change of light conditions in Lake Fil in the comparisons of median values for different seasons over the years with one exception. In 2013, the first year after flooding the fields, CDOM absorbance in both basins was remarkably higher compared to the following years. This higher CDOM absorbance was likely a result of the soil organic carbon and the grain-stubbles left on the fields leaching humic compounds to the water after flooding. Already in the second year after flooding, the CDOM absorbance was close to that measured in the inlet water implying that the internal organic sources in the stubbles and the former soils had markedly declined and accumulated CDOM had been flushed from the lake. Previous analysis supports this finding, which shows rapid decline of organic matter in surface sediments (0-9 cm) during the first three years due to frequent sediment resuspension in the shallow wind-exposed lake (Kragh et al. 2017). Because of the short water retention time between 20 and 120 days, CDOM of terrestrial origin, which was received via the inlet, becomes of much greater importance compared to CDOM of internal aquatic origin. Furthermore, terrestrial CDOM has a much higher light absorbance and consequently represents a higher background attenuation (Astoreca et al. 2009).

The grain-stubbles could have been removed before flooding but were left to aid in sediment stabilisation during the early phases of the new lake. Also, the high external CDOM input would still result in high background attenuation irrespective of whether stubbles had been removed before flooding or not. It is even likely that removal of stubbles would have caused higher light attenuation by suspended sediment particles.

Resuspension of sediment material often induces phytoplankton growth caused by the release of nutrients (Schallenberg and Burns 2004). However, considering the large pool of dissolved inorganic phosphate and nitrogen in the lake due to the high external loading, this is not an important mechanism for phytoplankton development in Lake Fil (Kragh et al. 2017). Phytoplankton biomass did not usually contribute much to total light attenuation. Phytoplankton growth is regulated by a fine balance between light and nutrient availability (Jones et al. 1996). Although nutrients are available in ample amounts to support phytoplankton growth, it can be highly restricted by light limitation when high concentrations of coloured organic material form a main background attenuation (Jones et al. 1996; Karlsson et al. 2009). This is the case in Lake Fil in which background light attenuation by CDOM and suspended particles prevented the formation of phytoplankton blooms. During periods of reduced CDOM and particle attenuation, higher phytoplankton biomass contributes to control further phytoplankton development by self-shading (Krause-Jensen and Sand-Jensen 1998).

Besides resulting in a release of nutrients to the water, wind-driven resuspension of sediment particles changes the light attenuation (Schallenberg and Burns 2004). This is also the case in Lake Fil because of its large surface area,
shallow depths and location close to the North Sea (Kragh et al. 2017). Frequent sediment resuspension in Lake Fil was closely related to high wind speeds. Regardless of the wind direction, the same maximal increase of particle-induced light attenuation occurred. This finding suggested that the pool of particles subjected to resuspension was of a certain magnitude. However, to reach the same particle light attenuation, wind speeds must be higher from the prevailing western rather than eastern directions. Thus, we assume that more particles have accumulated in the shelter along the western coast and these particles are more easily resuspended when the wind on rare occasions shifts from west to east. Overall, when rapid changes in wind directions occur, much lower wind speeds are likely to cause resuspension of recently settled and un-consolidated sediment particles.

The dynamic light climate in Lake Fil
Regulation of light attenuation in lakes is the result of a complex interaction between CDOM, suspended particles and phytoplankton pigments (Kirk 1994; Van Duin et al. 2001). In Lake Fil, this complex interaction is evident. Periods of low wind exposure resulted in sedimentation of particles. This will enhance the UV-induced photochemical and microbial degradation of CDOM (Madsen-Østerbye et al. 2018). Furthermore, these periods with calm weather are often associated with low precipitation and, thereby, reduced input of CDOM via the inlet. Subsequently, phytoplankton biomass can increase and substitute for the decline in background light attenuation until higher total light attenuation reduces further biomass growth (Krause-Jensen and Sand-Jensen 1998). This scenario was supported by the fact that the increase of attenuation from a growing phytoplankton biomass did not exceed the decline in particulate light attenuation. When background attenuation from particles and CDOM increased again in windy and wet weather, phytoplankton biomass decreased because of stronger light limitation. Thus, Lake Fil is a light-limited system strongly controlled by background light attenuation.

Macrophyte distribution in Lake Fil
Previous studies have estimated colonisation depths of caulescent angiosperms to 4-10% of subsurface irradiance (Middelboe and Markager 1997). In Lake Fil, mean irradiance was 2-21% in spring and summer months at the main colonisation depth at 0.6 m. In 2016 and 2017, scattered macrophyte colonisation to 1.2 m depth had occurred, which suggested that periods with lower light attenuation had been present in certain areas of the lake. Rooney and Kalff (2000) showed that biomass distribution was affected by inter-annual changes between growing seasons. Good light conditions early in the year resulted in increasing macrophyte colonisation depths. If macrophyte species can manage to sprout and grow to a certain size in a period of higher light early in the season, they can reach a better light climate closer to the lake surface and survive despite high background attenuation. By building organic carbon reserves in rhizomes or bulbils in sediments, they may even be able to exploit these
reserves to sprout and form elongated shoots next spring despite insufficient light at the depth limit.

Increase of macrophyte cover may play an important role for lake ecosystem functioning (Hilt et al. 2017; Madsen et al. 2001; Sand-Jensen and Borum 1991). Macrophyte cover can reduce resuspension of particles and create refuge for zooplankton controlling the phytoplankton biomass and, thereby, reduce light attenuation and increase macrophyte cover further (Lauridsen and Lodge 1996; Madsen et al. 2001). However, in a shallow and highly wind exposed lake such as Lake Fil, the mechanical stress on the surface sediment combined with the relatively high and steady background attenuation from CDOM via the inlet water and in-lake particle resuspension can restrict further macrophyte colonisation in the lake. Moreover, if CDOM and particle attenuation decline, phytoplankton light attenuation will increase in the nutrient-rich water. Consequently, we suggest that the macrophytes will have difficulty extending the main colonisation depth much deeper than the present 0.6 m in the future. Nonetheless, the re-established Lake Fil supports one of the highest species richness of aquatic plants in the country due to extensive and variable shallow zones kept open by cattle grazing (Baastrup-Spohr et al. 2016).

Acknowledgement
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Reference
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Madsen JD, Chambers PA, James WF, Koch EW, Westlake DF (2001) The interaction between water movement, sediment dynamics and submerged macrophytes. Hydrobiologia 444:71-84


Supporting Information to Chapter 1 and 3
Fig. S1 Steady state modeled changes of CDOM absorption over time in the lake assuming all input from the present stage is altered to coniferous groundwater (A) or to heathland groundwater (B). Absorption of CDOM is shown as a function of variable water retention time in the lake (WRT, 1, 1.5 and 2 years). Longer WRT results in a higher photo-induced microbial degradation and thereby a higher removal of light absorbing CDOM.
Fig. S2. Absorptions coefficients (m$^{-1}$) of CDOM versus time in the single dose UV-experiment at two different wavelengths 340 nm (a) and 440 (nm). The three different water sources are labelled forest, lake and heathland. The symbol represents the mean of 3 replicates at each point in time. Vertical scales differ between panels. There was no observed decline in CDOM absorption over time in any of the tree water sources after exposure to one single large UV-exposure.
Figure S1: Weekly CDOM absorption ($A_{\text{CDOM}(340)}$ cm$^{-1}$) and DOC (mg C l$^{-1}$) measurements from a drainage channel in the catchment area and precipitation (mm) data from a weather station placed at the lake shore. Data are shown as the running average of four weeks.
Figure S2: Bar plot showing the results from the sensitivity analysis derived from the CATS model. The top x-axis shows the different concentrations used for the lake e.g. no change (Lake), 10 % reduction (Lake -10 %) and 10 % increment (Lake +10 %). The left y-axis denotes the estimated fractions of inflowing groundwater originating from the groundwater well sites (bottom x-axis, only sites delivering more than 0.01 are shown) and the second y-axis denotes the corresponding WRT. In general, a smaller variance between sites and discharge fractions is seen up to a WRT of 1.25 years. Above this point there is some changes between site 11 and 13. Overall, the same 5 groundwater well sites are isolated which explain the measured lake concentrations.
Table S1: Tracer concentrations of δ¹⁸O (‰), dissolved organic carbon (DOC µmol l⁻¹), coloured dissolved organic matter (ACDOM(340) cm⁻¹), total dissolved phosphorus (TDP µg l⁻¹), total phosphorus (TP µg l⁻¹), total dissolved nitrate (TDN µg l⁻¹), total nitrate (TN µg l⁻¹) and maximum fluorescence of component C1-C5 in Raman’s units (R.U.) found around the lake (1-30), in the lake (a, b and c) and at a water locked (WL) site. Dissolved nutrients are measured in groundwater while total fractions are found in lake water. For site reference see figure 1a.

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