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# Carbon Dioxide in Inland Waters

## *Drivers and Mechanisms Across Spatial and Temporal Scales*

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**Abstract**

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Inland waters are an essential component of the global carbon cycle as they are very active sites for carbon transformation processes. Much of this carbon is transformed into the greenhouse gas carbon dioxide ($CO_2$) and emitted into the atmosphere. The biogeochemical and hydrological mechanisms driving $CO_2$ concentrations in inland waters are manifold. Although some of them have been studied in detail, there are still knowledge gaps regarding the relative importance of the different $CO_2$-driving mechanisms, both on a spatial and a temporal scale. The main aim of this thesis was to fill some of the knowledge gaps by studying long- and short-term effects of enhanced dissolved organic carbon (DOC) concentrations on surface water partial pressure of $CO_2$ ($p$$CO_2$) as well as to investigate both internal (i.e., within the water body) and external (i.e., catchment) drivers of $p$$CO_2$ in inland waters. Based on analyses of long-term data from more than 300 boreal lakes and streams and on results from two mesocosm experiments as well as a detailed catchment study, one of the main results of the thesis was that DOC concentrations were, on a temporal scale, generally uncoupled to $p$$CO_2$. Indeed, additions of allochthonous DOC to lake water could result in increased $p$$CO_2$ in waters but not as originally expected by stimulation of bacterial activity but instead by light driven suppression of primary production, at least in mesotrophic waters. Changes in the carbonate system was also found to be a main driver for surface water $p$$CO_2$. Finally, also external processes such as groundwater inputs contributed substantially to variations of surface water $p$$CO_2$. In a detailed study on carbon in groundwater, $p$$CO_2$ in groundwater was found to decrease with soil depth and correlated negatively with pH, which increased with soil depth. Conclusively, this thesis show that $p$$CO_2$ does not follow the trends of increased DOC in boreal surface waters but instead correlates with changes in primary production and shifts in the carbonate system. Additionally, the dominating mechanisms driving $p$$CO_2$ clearly differ between lakes and streams. Consequently, simulations of future $CO_2$ dynamics and emissions from inland waters cannot rely on DOC concentrations as a $p$$CO_2$ predictor, but rather need to incorporate several $p$$CO_2$ driving mechanisms, and consider the difference between lakes and streams.

*Keywords:* carbon dioxide, dissolved organic carbon, inland water, lake, stream, groundwater, mesocosm, carbonate system, carbon

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"If you change the way you look at things, the things you look at change."

- Wayne Dyer

*To the people I love*

# List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

| | |
|---|---|
| I | Nydahl, A. C., Wallin, M. B., and Weyhenmeyer, G. A. (2017) No long-term trends in $p\mathrm{CO}_2$ despite increasing organic carbon concentrations in boreal lakes, streams and rivers. *Global Biogeochemical Cycles*, 31(6):985–995 |
| II | Nydahl, A. C., Wallin, M. B., Tranvik, L. J., Hiller, C., Attermeyer, K., Garrison, J. A., Chaguaceda, F., Scharnweber, K., and Weyhenmeyer, G. A. (2019) Colored organic matter increases $\mathrm{CO}_2$ in meso-eutrophic lake water through altered light climate and acidity. *Limnology and Oceanography*, 64(2):744-756 |
| III | Nydahl, A. C., Wallin, M. B., Laudon, H., and Weyhenmeyer, G. A. (2019) Groundwater carbon within a boreal catchment – spatiotemporal variability of a hidden aquatic carbon pool. *Submitted* |
| IV | Nydahl, A. C., Wallin, M. B., and Weyhenmeyer, G. A. (2019) Highly variable explanations of long-term $p\mathrm{CO}_2$ increases in boreal lakes and streams. *Submitted* |

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# Contents

# Abbreviations

| | |
|---|---|
| $\delta^{13}C$-DIC | Stable carbon isotope of dissolved inorganic carbon |
| ANC | Acid neutralizing capacity |
| BCP | Bacterial carbon production |
| $CH_4$ | Methane |
| Chl *a* | Chlorophyll a |
| C:N | Carbon to nitrogen ratio |
| $CO_2$ | Carbon dioxide |
| $CO_3^{2-}$ | Carbonate |
| DIC | Dissolved inorganic carbon |
| DOC | Dissolved organic carbon |
| $H_2CO_3$ | Carbonic acid |
| $H_3PO_4$ | Phosphoric acid |
| $HCO_3^-$ | Bicarbonate |
| PAR | Photosynthetically active radiation |
| N | Nitrogen |
| $pCO_2$ | Partial pressure of carbon dioxide |
| P | Phosphorus |
| POC | Particulate organic carbon |
| TOC | Total organic carbon |
| WRT | Water retention time |

# Introduction

Carbon is the chemical basis of all known life and by definition is present in all organic compounds. The transformation of carbon compounds, from inorganic to organic during photosynthesis or from organic to inorganic during respiration and decomposition, essentially defines the actions of living organisms and therefore play a fundamental role for life on Earth (Brown *et al.* 2015). The carbon cycle is the biogeochemical cycle by which carbon is exchanged between land, water and the atmosphere, and comprises a sequence of events that are key to make life on Earth possible. Carbon in the atmosphere exists in two main forms, carbon dioxide ($CO_2$) and methane ($CH_4$), both of which are greenhouse gases (Wigley & Schimel 2005).

Since the industrial revolution, human activities have greatly modified the exchange of carbon between land, water and the atmosphere. Atmospheric $CO_2$ concentrations increased from about 280 ppm before the industrial era to about 410 ppm in 2019. Only about half of the $CO_2$ emissions from human activities are reflected in this increase, the other half has been sequestered on land, in inland waters and in the oceans (Battin *et al.* 2009).

## Carbon in inland waters

Although inland water ecosystems cover only about 1% of Earth's surface, inland waters play a major role in the global carbon cycle (Battin *et al.* 2009; Cole *et al.* 2007). Historically, inland waters have been considered passive pipes merely transporting carbon from land to the ocean (Cole *et al.* 2007). However, it is now well known that inland waters are also very active sites for transformation and storage of carbon (Aufdenkampe *et al.* 2011; Battin *et al.* 2009; Cole *et al.* 2007; Tranvik & von Wachenfeldt 2009). The most recent estimate of the terrestrial input of carbon to inland waters suggested that 5.1 Pg C is delivered to inland waters annually (Drake *et al.* 2018). Out of this 5.1 Pg C, 3.9 Pg is annually outgassed, mostly as $CO_2$ (Drake *et al.* 2018), which is even higher than the terrestrial carbon sink for anthropogenic emissions of 2.8 Pg C $yr^{-1}$ (Canadell *et al.* 2007). Inland waters also sequester as much, or more carbon as the oceans do (Clow *et al.* 2015).

Carbon compounds in inland waters can be either organic or inorganic. The organic carbon is composed of two major fractions, a dissolved and a particulate phase. These are defined based on isolation techniques using filtration through a membrane filter with a defined pore size. Dissolved organic carbon (DOC) is typically the fraction that passes through a filter with 0.2 to 0.45 µm pore size, whereas the fraction retained by the filter is termed particulate organic carbon (POC) (Tranvik & von Wachenfeldt 2009). More than 90% of the total organic carbon (TOC) in inland waters consist of DOC (Thurman 1985). The dissolved inorganic carbon (DIC) pool in inland waters consists of dissolved $CO_2$, carbonic acid ($H_2CO_3$), bicarbonate ($HCO_3^-$) and carbonate ($CO_3^{2-}$). These inorganic compounds readily interconvert from one to another as a function of their relative concentration, pH and temperature, a concept which is referred to as the carbonate system. In the 1990's it was realized that the majority of lakes worldwide are supersaturated with $CO_2$ relative to the water-atmosphere equilibrium (Cole *et al.* 1994), and since then subsequent research has shown that inland waters effectively emit large amounts of $CO_2$ to the atmosphere (Raymond *et al.* 2013).

$CO_2$ in inland waters can originate from internal or external sources (Cole 1999; Hotchkiss *et al.* 2015; Tranvik 1992). The key internal biogeochemical mechanisms that drive $CO_2$ concentrations in inland waters are microbial mineralization and photochemical oxidation of DOC, primary production and distribution of carbonate system variables (del Giorgio & Peters 1994; Hope *et al.* 1994; Jansson *et al.* 2007; Lazzarino *et al.* 2009; Tranvik 1992). Furthermore, DIC can be imported directly from the surrounding terrestrial environment where it is produced via respiration or weathering in the catchment soils. Hence, catchment hydrology is also of importance for $CO_2$ concentrations in inland waters. Although some of the biogeochemical and hydrological mechanisms driving $CO_2$ concentrations in inland waters have been intensely studied, there is still some controversy regarding which processes are most important. Understanding the mechanisms driving inland water $CO_2$ concentrations across spatial and temporal scales is important to allow for generalizations and predictions of inland water $CO_2$ responses to environmental change (Seekell & Gudasz 2016).

## DOC quantity and quality

DOC consists of a heterogeneous mixture of different carbon compounds that vary in quality. The quality of DOC can vary greatly due to differences in origin as well as transformation processes. In inland waters, DOC can originate either from surrounding terrestrial areas (i.e., allochthonous) or from in situ release of carbon from phytoplankton, algae and macrophytes (i.e., autochthonous).

Allochthonous DOC is derived from vascular plant material, root exudates and the primary and secondary metabolites of microorganisms, mostly accumulating in the organic horizon of the soil (Aitkenhead-Peterson *et al.* 2003; Mostofa *et al.* 2013). Allochthonous DOC is transported from the soil to aquatic ecosystems through advective transport in surface- and groundwaters (Aitkenhead-Peterson *et al.* 2003). Although DOC quality can vary considerably, it can generally be divided into humic-like or protein-like components (Kothawala *et al.* 2014). Allochthonous DOC is generally structurally complex and composed of colored, aromatic, high molecular weight compounds such as humic substances (Williams *et al.* 2010) and is thus often considered to be more humic-like. Due to the high molecular weight and aromaticity of humic-like DOC, it has been regarded as recalcitrant (Miller & McKnight 2010). However, it has also been shown that high molecular weight DOC is more bioreactive than low molecular weight compounds (Amon & Benner 1996), and that the most aromatic, highly colored fractions with high molecular weight have shorter half-lives (Köhler *et al.* 2013; Weyhenmeyer *et al.* 2012a).

Autochthonous DOC is mostly produced by algae and phytoplankton, however macrophytes and autotrophic bacteria also contribute to the synthesis of autochthonous DOC (Likens 1973). Autochthonous DOC is generally more protein-like, has low molecular weight and less color (Bertilsson & Jones 2003; Guillemette & del Giorgio 2011). Protein-like DOC, is often labile and biologically reactive (Guillemette & del Giorgio 2011; McKnight *et al.* 2001). Protein-like DOC can, however, be more persistent in inland water due to constant renewal despite the loss of other components (Kothawala *et al.* 2014).

Differences in the composition of DOC, and hence its quality, e.g. driven by its origin, affect its biogeochemical functions. DOC can be transformed through processes such as microbial mineralization, photochemical oxidation, flocculation and sorption to clay, and when DOC is transformed, its quality changes. All these transformations change the quantity and the quality of the DOC pool, which subsequently influences the fate of the carbon, whether it is degraded and emitted as $CO_2$ or $CH_4$, sequestered into sediments or exported to the ocean. One of the major pathways is the emission of $CO_2$ to the atmosphere, which is highly influenced by microbial and photochemical degradation of DOC (Graneli *et al.* 1996; Tranvik 1992).

## Microbial mineralization of DOC and its influence on $CO_2$ concentrations

Heterotrophic bacteria are the most abundant organisms in all ecosystems, and a great fraction of the annual primary production is metabolized by them. Consequently, they play a critical role in the carbon cycle and the largest fluxes of carbon in inland waters is that from the pool of organic matter into microorganisms (Cole 1999). Bacteria respire organic carbon to gain energy which is used to sustain cellular processes. Thereafter, the respired fraction is released as $CO_2$, which can subsequently be emitted to the atmosphere. Terrestrially sourced DOC can fuel secondary production within inland waters by heterotrophic bacteria. In many inland water ecosystems, the mineralization of organic carbon by microorganisms outweighs carbon fixation by primary producers, hence these systems are heterotrophic (where respiration is greater than primary production) and are thus sources of $CO_2$ to the atmosphere.

Several studies have shown that, on a spatial scale, there is a positive relationship between DOC and the partial pressure of $CO_2$ ($pCO_2$) due to microbial respiration of terrestrial DOC (e.g. Lapierre & del Giorgio 2012; Sobek *et al.* 2003). This relationship between DOC and $pCO_2$ has been suggested to be the main reason for the $CO_2$ supersaturation observed in the majority of inland waters across the globe (del Giorgio *et al.* 1997; Hope *et al.* 1996; Jonsson *et al.* 2001). On a temporal scale, however, much less is known about the relationship between DOC and $pCO_2$. Surface water DOC concentrations have been increasing in the boreal region since the 1990's (Filella & Rodriguez-Murillo 2014; Monteith *et al.* 2007), suggesting that $pCO_2$ might have concomitantly increased. Therefore, assessing the long-term DOC and $pCO_2$ relationships in boreal inland waters is an important undertaking.

## Photochemical oxidation of DOC and its influence on $CO_2$ concentrations

Solar radiation provides the primary driving force for biogeochemical cycles and is fundamental to the cycling of organic matter in inland water ecosystems. Most of the solar radiation that reaches the Earth's surface is converted into thermal energy, however a significant part is diverted into photochemical processes (Zepp *et al.* 1995). Photochemical reactions caused by solar radiation can change the physical, chemical and optical properties of water and play an important role in transforming DOC, subsequently affecting $CO_2$ production (Kopacek *et al.* 2003; Vähätalo *et al.* 2003). Photochemical transformations of DOC can have both direct and indirect effects on surface water $pCO_2$. Photochemical transformation of allochthonous DOC has been demonstrated to produce bioavailable substrates and increased bacterial activities, thereby also producing $CO_2$ through respiration, however, concurrently autochthonous DOC was converted to substances of lower microbial substrate quality during radiation, thus decreasing DOC bioavailability which could lead to decreased $CO_2$ production (Tranvik & Bertilsson 2001). This suggests that phototransformation of DOC can both enhance and reduce DOC bioavailability and $CO_2$ concentrations. Furthermore, DOC can be directly converted to DIC through photochemical oxidation (Graneli *et al.* 1996).

# Primary production and its influence on $CO_2$ concentrations

Primary production is the synthesis of organic compounds from inorganic compounds, such as $CO_2$ and water, through photosynthesis. Eventually, much of the primary production is converted back to $CO_2$ via respiration, however some carbon accumulates in biomass and organic matter. Consequently, primary production could be a strong driver of $p$$CO_2$ in inland waters. Primary production in inland waters is regulated by factors such as light, temperature and the supply of nutrients. The light climate in lakes and streams can be highly impacted by the water color. The water color could vary greatly depending on the DOC concentration since terrestrially derived DOC contains large amounts of colored humic-like DOC giving the water a brownish color (Roulet & Moore 2006). This browning effect has been particularly evident in surface waters across the boreal region where water color has been increasing since the 1990's (Haaland *et al.* 2010). Increased water color could lead to less light being available for photosynthesis, and subsequently less $CO_2$ bio-uptake (Jones 1992). However, it is still largely unknown if and how this increase in water color will alter $p$$CO_2$ through a change in primary production in inland waters.

# The role of the carbonate system for $CO_2$ concentrations

Aquatic primary production is affected by the presence of dissolved carbonate species, and is thus tightly linked to the carbonate system. The carbonate system is the set of DIC species produced by the equilibria:

$$1)\ H_2CO_3 \rightleftharpoons HCO_3^- \leftrightarrows CO_3^{2-}$$

When $CO_2$ dissolves in water it exists in chemical equilibrium with $H_2CO_3$:

$$2)\ CO_2 + H_2O \leftrightarrows H_2CO_3$$

Hence, in inland waters the concentration of $H_2CO_3$ can be considered equivalent to the $CO_2$. The concentrations of the various carbonate species depend on the pH of the solution. Concomitantly, the pH in inland water is susceptible to biological influence from $CO_2$ consumption (photosynthesis) and $CO_2$ production (respiration, decomposition), which affect the ratio between $CO_2$:$HCO_3^-$:$CO_3^2$ (Talling 2010). Hence, the pH of a solution is essentially controlled by the carbonate system (Roberts & Allen 1972). Increased $CO_2$ in inland waters due to reduced photosynthesis could lead to more acidic waters as pH decreases. However, inland waters have a natural protection against acidification, a buffering capacity, referred to as alkalinity. Alkalinity is a measure of the amount of bases in a solution which can neutralize hydrogen ions from strong acids and is usually determined by titration against sulfuric acid ($H_2SO_4$) to the endpoint of the acid-base reaction (Mattson 2009). Alkalinity can also be referred to as a conservative property regarding the addition or the removal of $CO_2$ through biological activity, which respectively decrease or raise pH. A closely related term is acid neutralizing capacity (ANC), which may include any type of buffering. The major buffers in most inland waters are $HCO_3^-$ and $CO_3^{2-}$. Alkalinity in inland waters is derived from several sources: weathering of rocks and soil, exchange reactions in soils, biological uptake and reduction of strong anions, evaporation and precipitation of minerals and atmospheric deposition of dust (Mattson 2009).

## Influence of catchment processes on $CO_2$ concentrations in inland waters

It is not only the processes occurring within lakes and streams that are of importance for carbon dynamics in inland waters. Much of the DOC and DIC in inland waters originates from the surrounding catchment, hence catchment processes could be key regulators of surface water carbon concentrations. Groundwater inputs can be strong regulators of $CO_2$ concentrations in both lakes and streams (Hotchkiss *et al.* 2015; Leith *et al.* 2015; Marce *et al.* 2015; Weyhenmeyer *et al.* 2015; Winterdahl *et al.* 2016). Groundwater has been shown to be oversaturated in $CO_2$, produced through microbial respiration of organic matter in soil and root respiration (Crawford *et al.* 2014; Leith *et al.* 2015; Macpherson 2009). Mineral weathering can also be a strong driver of groundwater $CO_2$ (Boerner & Gates 2015). The two key types of mineral weathering occurring in soils are carbonate weathering and silicate weathering. Both carbonate and silicate weathering consumes soil $CO_2$, which is produced through soil respiration, and produces $HCO_3^-$. Mineral weathering can also lead to increased ANC and enhanced pH through the release of base cations and consumption of $CO_2$. Hence, the carbonate system within a lake or a stream could be as important as the carbonate system in the surrounding catchment for regulating surface water $CO_2$ concentrations. For instance, a carbon budget for Sweden showed that $CO_2$ consumption through mineral weathering corresponded to 25% of the $CO_2$ efflux from lakes and streams (Humborg *et al.* 2010).

The importance of groundwater input for surface water chemistry can vary depending on a number of factors such as stream order, time of year, groundwater level and catchment hydrology (Hotchkiss *et al.* 2015; Peralta-Tapia *et al.* 2015). Furthermore, the relative importance of shallow and deep groundwater input for surface water chemistry can also vary depending on similar factors (Tiwari *et al.* 2014). For instance, due to the strong hydrochemical connectivity between the catchment soil and headwater streams, the concentration of $CO_2$ in headwater stream water is largely dependent on the $CO_2$ concentration in the surrounding terrestrial areas (Vidon *et al.* 2010). Further downstream, the importance of in situ carbon transformation processes increases (Hotchkiss *et al.* 2015). Concurrently, the ratio of shallow to deep groundwater input changes towards greater influence from deeper groundwater further downstream (Peralta-Tapia *et al.* 2015). The importance of deep groundwater input to stream surface water chemistry also varies seasonally due to changes in flow rate. During spring, the contribution of deeper groundwater to surface water chemistry is diluted by increasing inputs of shallow groundwater and surface runoff, whereas during winter, at base flow, the relative contribution from deeper groundwaters increases (Humborg *et al.* 2010). Further, it has been suggested that deep groundwater inputs can be more influential at regulating stream chemistry than in-stream processes in downstream rivers (Tiwari *et al.* 2014). Despite this importance of deep groundwater, most studies on groundwater contributions to surface water chemistry have been using information from shallow depths down to ca. 1 m (i.e., Deirmendjian & Abril 2018; Grabs *et al.* 2012; Ledesma *et al.* 2016; Rasilo *et al.* 2017). Studies investigating the water chemistry of deeper groundwater are required to accurately assess how water chemistry from deeper groundwater could contribute to surface water chemistry. Consequently, there is a need for comprehensive studies and quantifications of carbon species in deeper groundwater, across both spatial and temporal scales.

Water residence time (WRT), both in the landscape and within the water body could also be a key regulator of surface water $CO_2$ concentrations (Algesten *et al.* 2004; Catalán *et al.* 2016). Organic carbon loss was found to increase rapidly with increasing WRT up to 2-3 years (Algesten *et al.* 2004) and $CO_2$ production has been shown to be more efficient in waters with long WRT due to more time available for microbial mineralization (Hanson *et al.* 2011). Runoff is predicted to decrease due to global warming, subsequently increasing WRT (Catalán *et al.* 2016) and with increasing WRT, $CO_2$ production may increase. Changes in precipitation can also impact WRT, and with climate change predicted to induce significant changes in many parts of the world in regards to precipitation, with increases in some areas and decreases in others (Martel *et al.* 2018), this could potentially have a large impact on $CO_2$ in inland waters. With decreased precipitation, WRT, and subsequently $CO_2$ production, may increase. Decreased precipitation would also lead to reduced discharge and consequently lead to a higher relative contribution from deeper groundwater than surface runoff and shallow groundwater to surface water chemistry (Carroll *et al.* 2018).

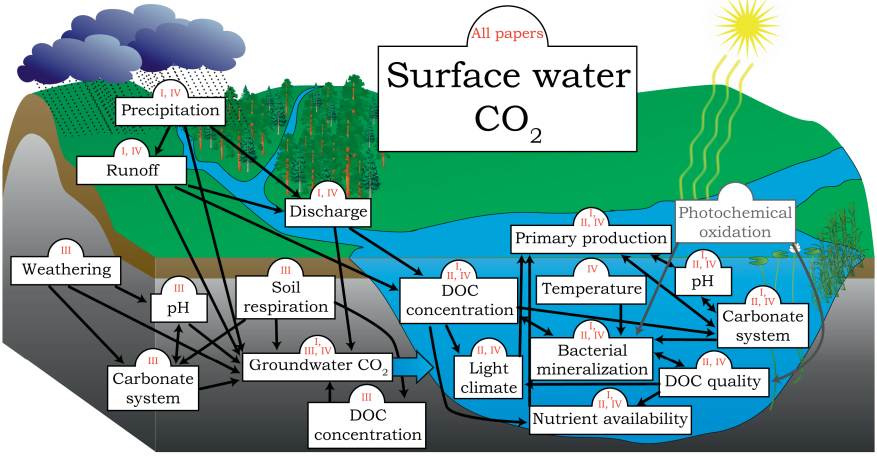

*Figure 1* Conceptual figures illustrating the biogeochemical and hydrological $CO_2$ driving mechanisms that could be of importance for controlling $CO_2$ concentrations in surface water of boreal lakes and streams. Photochemical oxidation is, however, not covered in the studies of this thesis.

# Aims of the Thesis

The overarching aim of this thesis was to get a better understanding of the biogeochemical and hydrological mechanisms driving $CO_2$ concentrations in inland waters (Figure 1). The studies on which the thesis is based upon include both internal (within lake and streams) and external (catchment) carbon transformation processes, cover a range of spatial scales, stretching from the entire Sweden, to a single catchment, to a mesocosm experiment in a lake, as well as different temporal scales ranging from four weeks to 21 years. Different spatial and temporal scales were combined to provide a comprehensive understanding of the mechanisms driving surface water $p\mathrm{CO}_2$, which is urgently needed for the prediction of future $CO_2$ emissions from inland waters.

More specifically, the aims of this thesis were to:

- Investigate long-term effects of increased DOC on $p\mathrm{CO}_2$ in boreal inland waters (Paper I).
  *The investigation was performed using long-term monitoring data from lakes, streams and river mouth systems distributed all across Sweden.*

- Examine short-term effects of increased DOC on $p\mathrm{CO}_2$ in lake water (Paper II).
  *The short-term effects of DOC on lake water $pCO_2$ were examined through two mesocosm experiments in a lake in eastern Sweden.*

- Assess internal drivers of surface water $p\mathrm{CO}_2$ (Paper II, IV)
  *Internal $pCO_2$ drivers were assessed through two mesocosm experiments in a lake in eastern Sweden and by investigating long-term trends in lakes and streams using monitoring data.*

- Investigate external drivers of surface water $p\mathrm{CO}_2$ (Paper III, IV)
  *Catchment drivers of surface water $pCO_2$ were investigated by exploring the spatial and temporal variability of organic and inorganic carbon concentrations in groundwater at intermediate depth in a catchment in northern Sweden as well as assessing long-term trends in catchment hydrology using monitoring data*

# Methods

## Study sites

The study sites for the individual papers of this thesis ranged spatially from the national scale of Sweden (Paper I and IV), to the catchment scale (Paper III), to the mesocosm scale (Paper II) (Figure 2). In Paper I and IV, water chemical data acquired from the Swedish national freshwater monitoring program were used (Fölster *et al.* 2014). The data are made freely available by the Swedish University of Agricultural Sciences (SLU). For Paper I, an initial data set comprising 178 lakes, 86 streams and 42 river mouth systems was used. To get a complete data set with a minimum of four samples for each year for the period 1997 to 2013 a subset of the 306 waters, which resulted in a total of 71 lakes, 30 streams and 4 river mouths, was used. The same 105 water systems were initially used in Paper IV, and from these, the waters which had increased significantly in $p\mathrm{CO}_2$ during the study period were used for further analysis, resulting in eight lakes and five streams. In Paper III, groundwater in the Krycklan catchment in northern Sweden, a boreal catchment dominated by forest and peatlands, was sampled across three seasons. For Paper II, two mesocosm experiments were conducted in the meso-eutrophic Lake Erken in eastern Sweden.

## Mesocosm Experiment set-up

Two mesocosm experiments were performed, each lasting four weeks (Paper II). The first experiment was conducted in June-July (Experiment A) and the second in August-September (Experiment B) 2016. Both experiments had five replicates of four treatments (Figure 3a). In Experiment A, the four treatments were; (1) addition of DOC concentrated by reverse osmosis from a humic stream draining a forested wetland (i.e., reverse osmosis); (2) DOC from HuminFeed® (Humintech, GmbH, Grevenbroich, Germany), an alkaline extract of Leonardite (i.e., HuminFeed); (3) a mix of reverse osmosis concentrate and HuminFeed (i.e., mixed); and (4) no addition of DOC (i.e., control). Starting DOC concentrations for Experiment A were: 18.4, 18.1, 23.5 and 13.0 mg $L^{-1}$ for the reverse osmosis, HuminFeed, mixed and control treatments, respectively. In Experiment B the four treatments consisted of; (1) addition of reverse osmosis concentrate of DOC from the same humic stream water as in

Experiment A (i.e., reverse osmosis); (2) shading by covering of the outside of the mesocosms using black polyethylene film and on top using black chiffon fabric (i.e., shading); (3) addition of reverse osmosis concentrate of DOC and shading (i.e., DOC-shading); and (4) no DOC addition or shading (i.e., control). Starting DOC concentrations for Experiment A were: 16.4, 12.0, 16.4 and 12.0 mg $L^{-1}$ for the reverse osmosis, shading, DOC-shading and control treatments, respectively.

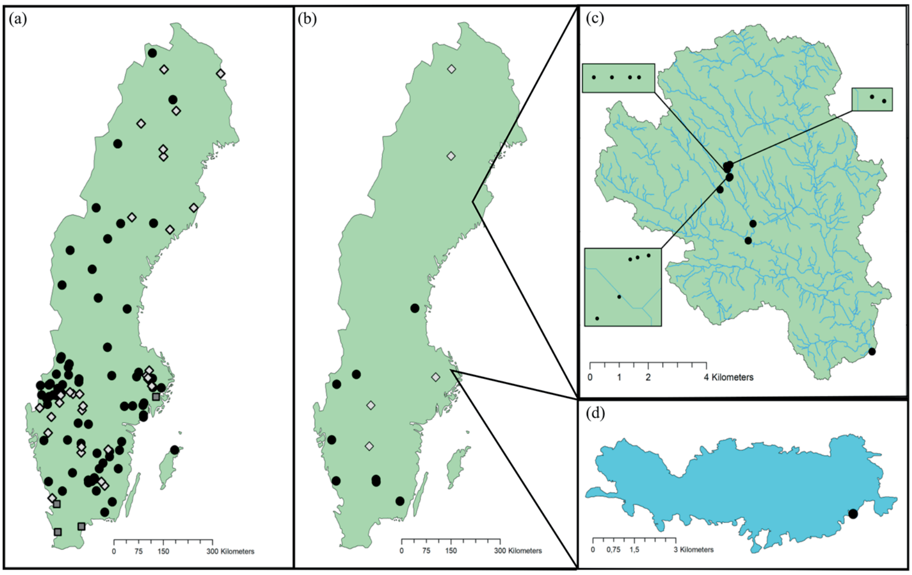

*Figure 2* Study sites for each individual paper of this thesis; location of Swedish study lakes (circles, *n*=71), streams (diamonds, *n*=30), and river mouths (squares, *n*=4) (Paper I) (a), location of Swedish study lakes (circles, *n*=8), and streams (diamonds, *n*=5) (Paper IV) (b), location of groundwater wells in the Krycklan catchment in Northern Sweden (Paper III) (c), and location of the mesocosm experiment in Lake Erken (Paper II) (d).

*Figure 3* Aerial photograph (photo credit: Erik Sahlée) of the mesocosm experiment in Lake Erken (a), sampling of the mesocosms (b), and groundwater sampling in the Krycklan catchment (c, d).

## Mesocosm and field sampling

Water sampling of the mesocosms were conducted weekly starting on the first day of the experiment and then four more times including the final sampling at the end of the experiment (Figure 3b) (Paper II). Sampling of $p\mathrm{CO}_2$ was performed first at each sampling occasion to avoid outgassing due to turbulence and disturbance from water sampling. Water samples for $p\mathrm{CO}_2$ analysis were taken with a syringe directly below the surface whereas water samples for all other analyses were collected using a tube sampler (1.5 m long, ~ 3 liter volume).

Sampling of groundwater was carried out in June (summer) and September (autumn) 2017 and in May (spring) 2018 (Figure 3c, d) (Paper III). Water was sampled from 16 groundwater wells with depths ranging from 3.4 to 19.5 m. The total volume of water in the well was determined before sampling and at least three times the volume of the well of water was removed prior to sampling to ensure collection of only the new infiltrating water. In a few wells the water refilled at such a slow rate that sampling took several days. Water temperature and $p\mathrm{CO}_2$ were measured directly in the field while samples for analysis of DOC, DIC, the stable carbon isotope of DIC ($\delta^{13}$C-DIC), $\mathrm{CH}_4$, pH and total nitrogen (N) were stored in the dark at 4°C for a maximum of seven days until analysis. Water samples collected for DIC and $\mathrm{CH}_4$ analysis were collected in the same vial and acidified directly using phosphoric acid ($\mathrm{H}_3\mathrm{PO}_4$).

## $p\mathrm{CO}_2$ analyses

All samples for $p\mathrm{CO}_2$ measurements collected for this thesis (Paper II and III) were analyzed directly in the field using the headspace equilibration method (described in Sobek *et al.* (2003)) as modified by Kokic *et al.* (2015). Bubble-free water samples of 30 mL were taken with a 60 mL polypropylene syringe equipped with a three-way stopcock. A 30 mL headspace of ambient air was then introduced and the syringe was vigorously shaken for one min to equilibrate the dissolved gas from the water into the headspace. The equilibrated air was then transferred into another syringe before being analyzed on a portable infrared gas analyzer (IRGA, EGM-4). All gas samples were analyzed within 5 min of sampling. The $p\mathrm{CO}_2$ of ambient air was also measured to correct for the $p\mathrm{CO}_2$ in the equilibrated air samples. The $p\mathrm{CO}_2$ was calculated according to Weiss (1974) using Henry's law constant, correcting for temperature, atmospheric pressure and added ambient air $\mathrm{CO}_2$.

For Paper I and IV, $\mathrm{CO}_2$ was calculated based on water temperature, alkalinity and pH according to Weyhenmeyer *et al.* (2012). From this, $p\mathrm{CO}_2$ was calculated using Henry's law corrected for temperature and atmospheric pressure (Weiss 1974).

## Additional chemical analyses

All chemical analyses for Paper I and IV were performed in an accredited laboratory at the Swedish University of Agricultural Sciences (SLU) following standard limnological procedures. Analytical methods are published online on SLU's website. TOC concentrations were considered equivalent to DOC concentrations since the particulate fraction of organic carbon generally is less than 1% in boreal inland waters (Laudon *et al.* 2011).

For the mesocosm experiment (Paper II), additional chemical analyses were performed by members of the KAWater team. Filtered water samples were analyzed on a Sievers M9 TOC analyzer for DOC concentrations while DIC concentrations were measured on a Sievers 900 TOC analyzer. Bacterial carbon production (BCP) was analyzed using the 3H-leucine incorporation method by Smith & Azam (1992). Ethanol extractions were performed with subsequent spectrophotometry analysis to establish chlorophyll *a* (chl *a*) concentrations following standard techniques (Kutser *et al.* 2005). The pH was measured directly in the mesocosms using a YSI multiprobe. Light was measured at seven depths in each mesocosm with a light meter. Total N and total phosphorus (P) were analyzed on unfiltered samples on a SEAL AutoAnalyzer. Water color was measured as absorbance at 420 nm ($abs_{420}$) using a Lambda 40 UV/VIS spectrophotometer (Paper II).

Additional groundwater chemical analyses were performed by laboratory staff at the Department of Forest Ecology and Management at SLU (Paper III). The DOC was measured as TOC on a Shimadzu TOC-VCPH. For DIC and $CH_4$ determination, a headspace method was used and headspace $CO_2$ and $CH_4$ concentrations were analyzed by gas chromatography (GC) (Wallin *et al.* 2010; Wallin *et al.* 2014; Åberg & Wallin 2014). Concentrations of DIC and partial pressure of $CH_4$ ($pCH_4$) were then calculated using temperature-dependent equations for the carbonate equilibrium (Gelbrecht *et al.* 1998) and Henry's Law (Weiss 1974). The $\delta^{13}$C-DIC was analyzed from the $CO_2$ headspace using an isotope ratio mass spectrometer. Total N was measured on a Shimadzu TNM1 whereas the pH was measured with a Mettler Toledo Digi117-water combined pH meter.

## Catchment hydrology

In addition to the water chemical data acquired from the Swedish national freshwater monitoring program, hydrological data downloaded from the Swedish Meteorological and Hydrological Institute (SMHI)'s website was used for Paper IV. Data acquired were monthly average precipitation, discharge and groundwater for the catchments of the study lakes and streams.

## Statistics

In this thesis a variety of different statistical approaches were used, i.e., Mann Kendall trend tests, one way analyses of variances (ANOVAs), repeated measures ANOVAs, linear regressions and partial least squares regressions (PLS). Assumptions of normality and heteroscedasticity were tested prior to statistical analyses. If necessary, transformations of data or non-parametrical methods were applied in order to test significance. To identify long-term changes, non-parametric Mann Kendall trend tests were performed on yearly median water chemical and hydrological values (Paper I and IV). To find influential variables on lake and stream surface water $p\mathrm{CO}_2$ PLS were used (Paper IV). One way ANOVAs were used to evaluate the direct effects of DOC additions in the mesocosm experiments whereas repeated measures ANOVAs, with mesocosm as a random effect, were performed to test for differences in $p\mathrm{CO}_2$ and $p\mathrm{CO}_2$ drivers between mesocosm treatments (Paper II). One way ANOVAs were used to test for differences in groundwater chemistry between seasons (Paper III). Linear regressions were performed on the mean of the values measured at the different seasons to investigate relationships between groundwater chemistry and depth as well as between groundwater $p\mathrm{CO}_2$ and other groundwater carbon species (Paper III).

# Results and Discussion

## Long-term effects of increased DOC on surface water $pCO_2$

Over the past 20 years, DOC concentrations have been increasing in surface waters across the boreal region (Evans *et al.* 2005; Filella & Rodriguez-Murillo 2014; Monteith *et al.* 2007). On a spatial scale, high DOC concentrations have been shown to correspond to high levels of surface water $pCO_2$. However, the effect of a long-term DOC increase on $pCO_2$ in inland waters are still unknown. In Paper I, the long-term relationship between DOC and $pCO_2$ in boreal inland waters was investigated. In more than half of the 105 study waters, DOC was found to have increased significantly during the period 1997 to 2013, however, only a few of those showing a DOC increase also increased in $pCO_2$ (Figure 4). Overall, long-term water chemistry trends in 71 lakes, 30 streams and four river mouth systems were investigated. Out of the 39 lakes that had increased in surface water DOC concentrations during the 17-year study period, merely four had also increased in $pCO_2$. Half of the 30 streams had increased in DOC and of those 15 streams, only two had also increased in $pCO_2$. While all four river mouths had increased in DOC during the period 1997 to 2013, none of them showed an increase in $pCO_2$. These results relate to a previous study of long-term $pCO_2$ trends in lakes of the Adirondack park in northeastern U.S, where only six out of 31 lakes had increased in $pCO_2$ (Seekell & Gudasz 2016). Contradicting the results of this thesis, Seekell & Gudasz (2016) did, however, also observe a significant increase in DOC in the six lakes with enhanced $pCO_2$. Due to the established spatial relationship between surface water DOC and $pCO_2$ and the results from the six study lakes of Seekell & Gudasz (2016), enhanced $pCO_2$ levels were expected in most of the waters that had increased in DOC. It was expected that either in situ $CO_2$ production through bacterial mineralization of allochthonous DOC would have increased or the inflow of catchment produced $CO_2$ would have increased in conjunction with the increased DOC input. However, this was not the case in the majority of the study waters in Paper I. Both in situ $CO_2$ production and $CO_2$ input from the catchment may indeed have increased, however other processes, that at the same time draw down the $pCO_2$, must have been stimulated to a greater extent. The lack of long-term DOC-$pCO_2$ relationship could potentially have been due to a change in hydrological patterns. Although precipitation has decreased in some areas of Sweden, in many areas precipitation, and therefore also runoff, have increased since the 1990's (Bengtsson & Rana 2014; Chen *et al.* 2015; Weyhenmeyer *et al.* 2016). This increase in precipitation could have resulted in a dilution effect of the $CO_2$-rich groundwater input, leading to the observed uncoupling of DOC-$p$$CO_2$ trends on a temporal scale. In addition to hydrological changes, the lack of DOC-$p$$CO_2$ relationships could potentially be explained by a change in alkalinity or DOC quality for some of the systems (Kothawala *et al.* 2014; Seekell & Gudasz 2016).

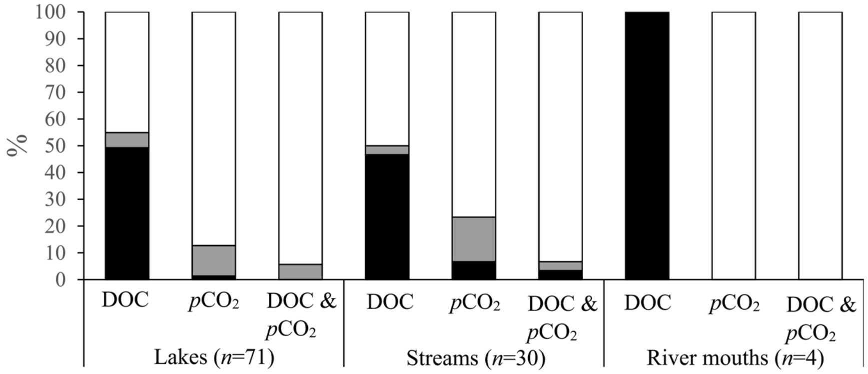

*Figure 4* Percentage of surface waters of Swedish boreal lakes (*n*=71), streams (*n*=30) and river mouths (*n*=4) that had increased significantly in dissolved organic carbon (DOC), partial pressure of carbon dioxide ($pCO_2$) and both DOC and $pCO_2$. The black and grey parts of the bars combined refer to the systems investigated in Paper I with significant increases during the period 1997 to 2013. The grey part of the bar alone refers to the systems investigated in Paper IV during the period 1997 to 2017.

## Short-term effects of increased DOC on lake water $pCO_2$

After discovering that DOC and $pCO_2$ were uncoupled through time, I wanted to investigate the short-term effect of increased DOC on surface water $pCO_2$. The effect of enhanced allochthonous DOC input could be three-fold (Figure 1). Firstly, allochthonous DOC may function as a carbon source to heterotrophs, stimulating $CO_2$ production. Secondly, DOC could contribute to water color, hence increasing light attenuation and potentially hampering $CO_2$ consumption by primary producers. Thirdly, DOC may have an acidifying effect, which can lead to decreased pH with a subsequent increase in $CO_2$ as the distribution within the carbonate system shifts. In Paper II, two mesocosm experiments were performed in a meso-eutrophic lake. In the first mesocosm experiment, DOC concentrations were manipulated, whereas in the second experiment both DOC concentrations and light climate were manipulated. During the four-week durations of the experiments, a significant $p\mathrm{CO_2}$ increase was observed in all treatments relative to the control (Figure 5). Hence, there was a clear short-term response of $p\mathrm{CO_2}$ to the allochthonous DOC input. Additionally, $p\mathrm{CO_2}$ also increased during the four-week experiment due to a change in light climate. Generally, the $p\mathrm{CO_2}$ increases were most pronounced during the first one to two weeks of the experiments, further emphasizing how quick the responses to DOC additions were. Also, the first $p\mathrm{CO_2}$ measurements were performed about 18 hours after DOC additions, and already then, a clear increase in $p\mathrm{CO_2}$ could be seen in the majority of the treatments. The lack of long-term $p\mathrm{CO_2}$-DOC relationship and the clear short-term $p\mathrm{CO_2}$-DOC relationship could be related to the quality of the DOC. This has previously been shown in a study comparing short-term and long-term bacterial carbon consumption (Guillemette & del Giorgio 2011). The labile carbon is quickly consumed leaving behind the more recalcitrant carbon, hence the microbial response to enhanced allochthonous DOC input might be more pronounced in the short-term. However, there was no difference in bacterial production between any of the treatments in either of our mesocosm experiments despite the increased $p\mathrm{CO_2}$. Lennon (2004) performed a mesocosm experiment with DOC additions where he measured the $\mathrm{CO_2}$ response and, similar to our mesocosm studies, found a significant increase in $\mathrm{CO_2}$ in response to enhanced DOC concentrations. However, contradicting to the results of this thesis, Lennon (2004) also found a significant increase in bacterial productivity. Two of the key differences between ours and Lennon's (2014) mesocosm experiments are the length and sampling strategy. Our experiments were performed for four weeks, sampling on a weekly basis whereas Lennon's (2014) experiment was performed for 10 days with daily sampling. The short-term effect of DOC on bacterial production may indeed be short, and the increase of the bacterial activities during the first week in response to the added allochthonous DOC might have been missed. However, there are several mechanisms other than bacterial mineralization affecting $p\mathrm{CO_2}$ in inland waters, some closely related to DOC, others independent of DOC.

## Internal drivers of surface water $p\mathrm{CO_2}$

Our mesocosm experiments (Paper II) allowed me to get a better mechanistic understanding of the effect increased DOC input could have on lake water $p\mathrm{CO_2}$ and to further explore the internal drivers of surface water $p\mathrm{CO_2}$. To test the results on a broader scale, a study which included field observations was performed. In Paper IV, long-term monitoring data were used to investigate reasons behind significantly increasing $p\mathrm{CO_2}$ in eight lakes and five streams, distributed all across Sweden that had increased in $p\mathrm{CO_2}$ during the 21-year period of 1997 to 2017. In the mesocosm experiments (Paper II), the DOC additions as well as the shading had a strong effect on the light climate decreasing the amount of light available for photosynthesis. There was a negative relationship between $p\mathrm{CO}_2$ and light climate (Figure 6a, b). The increased light attenuation must have had a negative impact on primary production, decreasing $\mathrm{CO}_2$ bio-uptake and subsequently increasing the $p\mathrm{CO}_2$ in the mesocosms. In Paper IV, there was also a potential negative effect of shading through darkening of the waters on primary production. Of the eight study lakes, four had increased in water color (i.e., absorbance at 420 nm), which was used as a proxy indicator for primary production, and this could have been a reason for the observed $p\mathrm{CO}_2$ increase. These results relate to previous studies showing a negative effect of shading on primary production caused by allochthonous DOC (Ask *et al.* 2009; Carpenter *et al.* 1998). The large natural variation in water color due to differences in DOC concentrations and DOC sources could potentially be a reason for variation in primary production (Carpenter *et al.* 1998). In the mesocosms, *chl a* had increased in all treatments, which was a bit surprising as *chl a* often has been used as a proxy indicator for primary production (Huot *et al.* 2007; Michelutti *et al.* 2005; Roehm *et al.* 2009), and should thus have decreased with increased shading. However, primary producers are able to produce more chlorophyll to compensate for the lack of light (Richardson *et al.* 1983), hence water color may be a more suitable proxy indicator for primary production in this context.

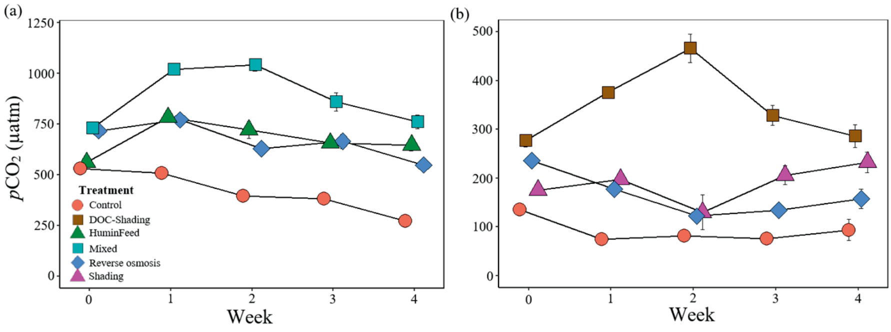

*Figure 5* Weekly variations in the partial pressure of carbon dioxide ($p\mathrm{CO}_2$) under four treatments (see legends in figure) during two four-week mesocosm experiments, Experiment A (a) and Experiment B (b), with dissolved organic carbon (DOC) additions and/or changed light climate through shading. Values are mean (±SE, n=5). Error bars are sometimes so small that they are hidden within the symbols.

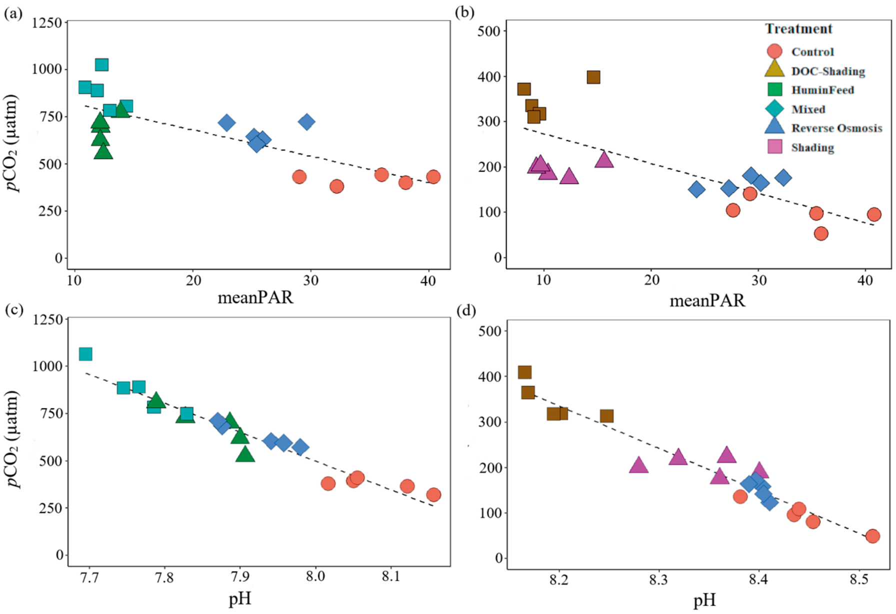

*Figure 6* Relationships between partial pressure of carbon dioxide ($pCO_2$) and average photosynthetically active radiation (meanPAR) throughout the entire water column in the mesocosm (a, b) and time-weighted averaged pH (c, d) from two four-week mesocosm experiments with dissolved organic carbon (DOC) additions and/or changed light climate through shading (see legend in figure for treatments). The left panel refer to result from the first experiment and the right panel refer to the second experiment.

Nutrient limitation can also be an important factor controlling primary production and thus regulating $pCO_2$ (Figure 1). In Paper II, the DOC additions led to higher concentrations of total P, which is another proxy indicator of primary production since P often is the limiting nutrient for primary production in inland waters. If the primary producers in the mesocosms were nutrient limited, this enhanced total P should have led to increased primary production, and consequently decreased $pCO_2$. However, the opposite pattern was observed. In Paper IV, total P was used as a proxy indicator for primary production, in addition to light climate, and no evidence for nutrient-limited primary production being the reason for the observed $pCO_2$ increase in the lakes during the period 1997 to 2013 was found. In regards to the streams in Paper IV, three of the five study streams had decreased significantly in total P during the 21-year study period and it is possible that this hampered primary production, which resulted in the $pCO_2$ increase. Overall, there were high variations in the relationships between changes in $pCO_2$ and total P, suggesting that in some waters nutrient limited primary production could be driving factor for $pCO_2$, whereas in other waters this process would be less important.

In the mesocosm experiments (Paper II), there was also a strong relationship between $pCO_2$ and pH (Figure 6c, d). Allochthonous DOC can be quite acidic due to humic acids. In the first experiment there were two different sources of DOC, and one of these, which was extracted from humic stream water (i.e., the reverse osmosis treatment) had a pH of 3.4. This reverse osmosis DOC was also used in the second experiment. All reverse osmosis DOC addition treatments showed a significant increase in $p\mathrm{CO}_2$ already on the day after DOC additions. This acidic DOC input probably resulted in an instant shift in the carbonate system with decreased pH and a subsequent increase in $p\mathrm{CO}_2$. The reduced primary production due to changes in the light climate could also have led to a reduced pH, adding on to the observed negative relationship between $p\mathrm{CO}_2$ and pH. This was particularly apparent in the second experiment in the treatment with added DOC and changed light climate due to shading as this treatment had a much higher $p\mathrm{CO}_2$ than the treatment with only DOC addition, despite having the same DOC concentrations. In Paper IV, two of the five streams decreased in pH, hence it is possible that the observed long-term $p\mathrm{CO}_2$ increase was due to distribution changes within the carbonate system in these two waters. The importance of the carbonate system on surface water $p\mathrm{CO}_2$ has recently been demonstrated (Marce *et al.* 2015; Stets *et al.* 2017) and the results from this thesis further emphasize the need of acknowledging the carbonate system in research of $\mathrm{CO}_2$ dynamics in inland waters and subsequent gas exchange with the atmosphere.

Paper IV also allowed us to explore the factors affecting DOC mineralization within lakes and streams, such as DOC concentration, water temperature and DOC quality (Figure 1). Of the eight lakes and five streams, four lakes and two streams had increased significantly in DOC during the 21-year study period (Figure 4). Despite the general uncoupling of long-term DOC-$p\mathrm{CO}_2$, it is still possible that in these four lakes and two streams, the long-term $p\mathrm{CO}_2$ increase was due to enhanced microbial mineralization resulting from increased DOC concentrations. Temperature can also affect microbial mineralization of DOC as enhanced temperatures have been shown to lead to higher mineralization rates in lake sediments (Gudasz *et al.* 2010). Increased temperature was found in only one of the 13 study waters in Paper IV, hence there was no strong trend suggesting temperature would be a key internal driver of surface water $p\mathrm{CO}_2$. The quality of DOC could also be an important driver of surface water $p\mathrm{CO}_2$ (Bodmer *et al.* 2016) and in five of the eight lakes and in three of the five streams changes in at least one of the DOC quality proxy indicators, $\mathrm{abs}_{420}$/DOC and C:N (i.e., the ratio between DOC and dissolved organic N) were observed. The most pronounced DOC quality change was a significant increase in C:N, which occurred in five lakes and two streams. An increased C:N suggests that the proportion of humic-like DOC has increased (Kothawala *et al.* 2014). There is however, some disagreement regarding the reactivity of humic-like DOC. While it has been argued in some studies that humic-like DOC can be considered recalcitrant (Hosen *et al.* 2014; Miller & McKnight 2010), in other studies, bioreactivity of DOC has been found to increase with increased proportions of humic-like DOC (Amon & Benner

1996; Bodmer *et al.* 2016). Larger and more complex organic compounds have also been shown to be associated with elevated $CO_2$ levels (D'Amario & Xenopoulos 2015). The results of this thesis are in line with the findings of Amon & Benner (1996), Bodmer *et al.* (2016) and D'Amario & Xenopoulos (2015), however, more research into the reactivity of DOC of different quality and how this changes through time is needed.

## External drivers of surface water $p\mathrm{CO}_2$

External (i.e., catchment processes) drivers can be as, or even more, important as internal drivers for regulating inland surface water $p\mathrm{CO}_2$ (Figure 1) and this was further explored in Paper III and Paper IV. In Paper III, dissolved and gaseous carbon species in groundwater at 3 to 20 m depth in a boreal catchment were quantified. The aim was to get a better understanding of the carbon biogeochemistry of intermediate groundwater since this water can be an important contributor to surface water chemistry in downstream rivers, particularly during base flow, and in lakes (Hagedorn *et al.* 2000; Hood *et al.* 2005; Maberly *et al.* 2013). The groundwater sampled from all 16 wells was supersaturated with $CO_2$ relative to the atmosphere. This finding is in agreement with previous studies investigating $CO_2$ concentrations in groundwater in the U.S (Boerner & Gates 2015; Macpherson 2009). A relationship between $p\mathrm{CO}_2$ and depth of the well, with decreasing $p\mathrm{CO}_2$ with depth, was also observed (Figure 7a). It is quite possible that soil respiration decreases further down the soil profile and this could be the reason for the negative $p\mathrm{CO}_2$-depth relationship. There was also a negative relationship between $p\mathrm{CO}_2$ and pH (Figure 7c) as well as a positive relationship between pH and depth (Figure 7b). The negative relationship between $p\mathrm{CO}_2$ and pH was not a surprise, as they are tightly linked and can control each other. Consequently, the negative $p\mathrm{CO}_2$-depth relationship could be a result of the positive pH-depth relationship, which could be due to increased silicate weathering with depth. Higher silicate weathering and subsequent increased pH in deeper groundwater has previously been shown for groundwater down to 4.3 m depth (Klaminder *et al.* 2011). Whether the $p\mathrm{CO}_2$ and pH relationships with depth are due to changes in soil respiration or silicate weathering cannot for certain be determined from the results of this thesis. Both processes are most probably very important in the soils of this catchment and it is likely that they change with depth. Hence, the observed $p\mathrm{CO}_2$ and pH relationships with depth are likely due to a combination of both processes. Due to the tight link between $p\mathrm{CO}_2$ and soil respiration as well as silicate weathering, these processes can be important external drivers for surface water $p\mathrm{CO}_2$.

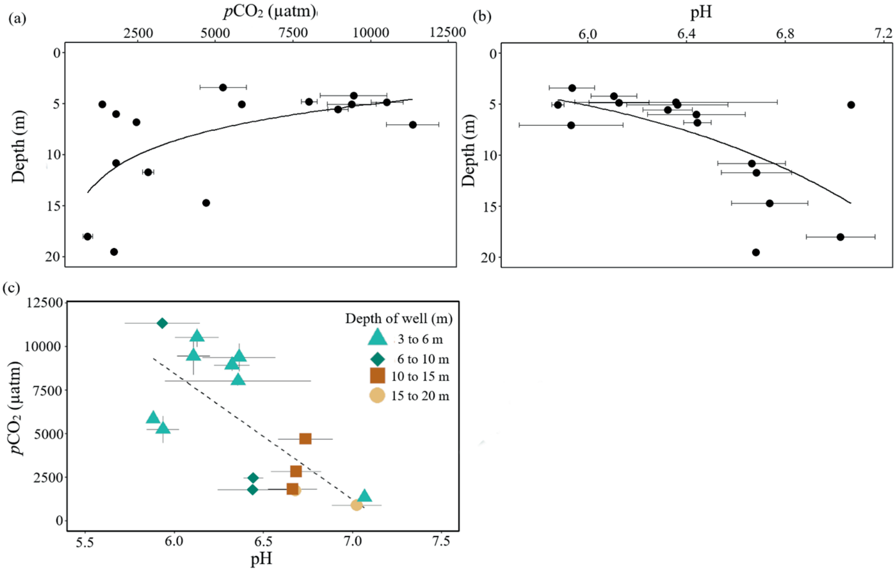

*Figure 7* The partial pressure of carbon dioxide ($pCO_2$) (a) and pH (b) in groundwater wells at different depths and the relationship between $pCO_2$ and pH (c) in the groundwater of the Krycklan catchment. The symbols in panel c are color coded according to the depth of the sampled well. For all variables, data are presented as mean values ± standard error ($n$=3, SE).

Although it was hypothesized that the observed long-term $pCO_2$ increase could have been due to increased groundwater level, since groundwater can be an important source of $CO_2$ to surface water, no support for this hypothesis was found in Paper IV. During the 21-year study period from 1997 to 2017, groundwater levels had remained the same in all 13 study catchments, except the catchment of the stream Bergmyrbäcken, where groundwater levels had decreased. Since precipitation can affect the ratio of surface runoff to groundwater input to lakes and streams, and thus surface water $pCO_2$, long-term trends in precipitation were also investigated. As with groundwater, very little change had occurred in precipitation during the period 1997 to 2017 in the study catchments. Precipitation had remained the same in all study catchments, except the catchment of Fräcksjön where precipitation had decreased. Since a decrease in precipitation could lead to lower surface runoff to groundwater input, and thus more $CO_2$-rich groundwater entering the lake, the observed decrease in precipitation could potentially be the reason to the observed $pCO_2$ increase in Fräcksjön. Overall, very little long-term changes in the hydrological processes in the study catchments were found. Likewise, the conditions were stable across seasons in all measured organic and inorganic carbon species in the study catchment investigated in Paper III. This suggests that below ground conditions are generally stable through time and although groundwater can be an important regulator of surface water chemistry, internal processes appeared to more strongly regulate $p$CO$_2$ of the study waters of Paper IV than external processes.

# Conclusions and Future Perspectives

The role of inland waters in the carbon cycle, particularly the transport and transformation of carbon along the land to ocean aquatic continuum, through river systems, lakes, estuaries and coastal waters, to the open ocean must be considered in climate change mitigation schemes. In the most recent report of the Intergovernmental Panel on Climate change (IPCC), alarming information on accelerated global warming can be found (IPCC 2014). Global warming is to a large extent induced by greenhouse gas emissions, and more than one quarter of the total annual $CO_2$ emissions is emitted from inland waters (Drake *et al.* 2018; IPCC 2014). Consequently, inland waters play an important role for greenhouse gas emissions, hence future estimates of $CO_2$ emissions from inland waters are needed. Additionally, understanding the turnover of carbon in inland water ecosystems and integrating these fluxes into the global carbon cycle are important steps toward appropriate $CO_2$ management. One important step towards better predictions of future $CO_2$ emissions from inland waters and better management practices towards $CO_2$ mitigation is to better understand the biogeochemical and hydrological mechanisms regulating inland water $CO_2$ concentrations, which was the main goal of this thesis. Overall, the complexity of the drivers of $CO_2$ dynamics in inland waters is demonstrated and several mechanisms that are at play concomitantly are highlighted (Table 1, Figure 8). On a spatial scale, there is a positive DOC-$p$CO$_2$ relationship and therefore DOC concentrations are often used to predict $p$CO$_2$ and thereby $CO_2$ emissions from inland waters. Consequently, it was expected that the observed increase in surface water DOC concentration across the boreal region over the past 20 years had resulted in an increase in $p$CO$_2$, however a positive relationship on a temporal scale could not be established. Furthermore, in our mesocosm study no support for the hypothesis that increased allochthonous DOC input stimulates bacterial activities was found. Instead, addition of DOC to lake water in our mesocosm experiments resulted in a reduction of light available for photosynthesis thus reducing the photosynthesis to respiration ratio, which turned out to be the main driver of the increased $p$CO$_2$. Another key driver of the increased $p$CO$_2$ was an increase in pH due to decreased photosynthesis as well as addition of acidic DOC. If the input of colored DOC to inland waters increase in a warmer and wetter climate, the light conditions may change sufficiently to suppress primary production and lead to enhanced $CO_2$ emissions. In the fourth paper, it was shown that reasons behind $p$CO$_2$ increases are rather site-specific with complex interactions of drivers that clearly differed between lakes and streams. The dominating mechanism in lakes was stimulation of microbial mineralization by increased DOC and/or changed DOC quality or suppressed primary production due to light limitation by increased water color. In streams, the dominating mechanism was either carbonate system distribution changes due to decreased pH or a possible decreased primary production due to nutrient limitation. Such site-specific patterns show the importance of including several $pCO_2$ driving mechanisms, and of recognizing the difference between lakes and streams, when aiming to predict future $CO_2$ emissions from inland waters. Consequently, simulations of future $CO_2$ emissions from inland waters need to consider the findings of this thesis.

*Table 1.* Summary of the key hydrological and biogeochemical surface water $CO_2$ driving mechanisms found in the studies of this thesis.

| *System* | Mechanism |
|---|---|
| *Lakes* | |
| Internal | Light limitation/decreased photosynthesis (Paper II, IV) |
| | Increased acidity/decreased pH (Paper II) |
| | Increased DOC concentration (Paper IV) |
| | Changed DOC quality (Paper IV) |
| *Streams* | |
| Internal | Increased acidity/decreased pH (Paper IV) |
| | Nutrient limitation/decreased photosynthesis (Paper IV) |
| Lakes & Streams | |
| External | Surface runoff (Paper I) |
| | Groundwater input (Paper I, III) |
| | • Soil respiration (Paper III) |
| | • Weathering/pH (Paper III) |

For future studies one important topic to investigate is the relationship between DOC quality and $pCO_2$. Both the quality of DOC within inland waters and in the catchment soils as both could have an effect on surface water $pCO_2$. Moreover, food web processes were not covered in this thesis, but could potentially also be strong regulators of $CO_2$ emissions from inland waters and should therefore be considered in future studies. Furthermore, in Paper III, concentrations of both organic and inorganic carbon species in groundwater were shown to vary greatly on a spatial scale. This information is critical when aiming to upscale carbon concentrations in groundwater to regional or larger scale since it shows the importance of multiple sampling locations as well as sampling at different depths. Groundwater can be an indirect source of $CO_2$ emissions due to the influence of groundwater on surface water chemistry, therefore more studies into the carbon dynamics of groundwater are needed.

It could be particularly useful to model groundwater movement and investigate the connectivity between groundwater at different depths and surface water.

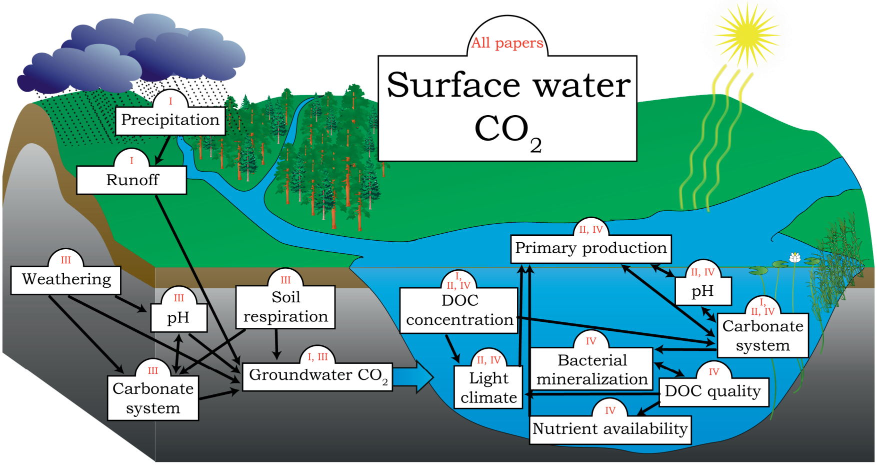

*Figure 8* Conceptual figure showing the key biogeochemical and hydrological mechanisms driving $CO_2$ concentrations in surface waters of boreal lakes and streams, which are discussed in this thesis. The mechanisms that are shown in figure 1, but not in figure 8 were found not to be important for $CO_2$ concentrations in the boreal lakes and streams included in this thesis. Photochemical oxidation was however removed as it was not investigated in the studies of this thesis. The different roman numbers refers to the papers of this thesis where the processes were found to be dominating.

# Sammanfattning på svenska

Inlandsvatten är en essentiell resurs för liv på jorden. Förutom det självklara att inlandsvatten används som dricksvatten, förser inlandsvatten mänskligheten med många viktiga funktioner som produktion av mat och energi, transport och rekreation. Trots att inlandsvatten endast täcker ungefär 1% av jordens yta så har de stor betydelse i jordens kretslopp av kol. Kol är en av livets byggstenar och förekommer i alla levande organismer. Kol förekommer som organiska och oorganiska föreningar och kan genom ett antal olika biokemiska processer lätt omvandlas från den ena föreningen till den andra. Omvandlingen av organiskt kol till oorganiskt kol sker genom respiration och nedbrytning medan omvandling av oorganiskt kol till organisk kol sker via fotosyntes. Dessa processer definierar huvudsakligen levande organismer och spelar därför en grundläggande roll för livet på jorden. Kol ingår i växthusgasen koldioxid som har ökat markant i atmosfären genom mänsklig påverkan och lett till påtagliga klimatförändringar.

Inlandsvatten kan innehålla stora mängder kol och koldioxid. Mycket av kolet i inlandsvatten kommer från omgivande skog och mark och tidigare trodde forskare att inlandsvatten endast transporterar kol vidare till haven. Numera vet man att en stor del av kolet som kommer från omkringliggande mark omvandlas i sjöar och vattendrag till koldioxid genom mikrobiella processer. Grundvatten kan genom nedbrytningsprocesser i jorden också bestå av höga mängder koldioxid. En stor del av koldioxiden i sjöar och vattendrag har producerats direkt i marken och tillförs till vattnet via grundvatten. Det finns även andra viktiga processer som kan leda till ändrade koldioxidhalter i inlandsvatten, som bland annat fotosyntes av växtplankton och alger och förändringar i pH genom t.ex. försurning. Fotosyntes av växtalger leder också till att organiskt kol produceras inom sjöar och vattendrag. Organiskt kol från terrestra miljöer består till stor del av humus, vilket övervägande utgörs av nedbrutna växtdelar, och är brunsvart till färgen. Detta gör att inlandsvatten med höga mängder organiskt kol är brunaktigt. Ökad brunhet i vatten på grund av höga mängder organiskt kol kan leda till minskad produktion av växtalger eftersom den bruna färgen på vattnet gör att mindre ljus finns tillgängligt för fotosyntes. Humus har också lågt pH, vilket gör att en ökad mängd organiskt kol kan leda till försurning. Organiskt kol kan vara av olika kvalité, dels på grund av var det har producerats och dels på hur det har bearbetats, vilket gör det lättare eller svårare för bakterier att bryta ner det och omvandla det till koldioxid.

Genom alla de processer som kan leda till ökade koldioxidhalter i inlandsvatten är många av jordens sjöar och vattendrag övermättade i koldioxid. Detta gör att många inlandsvatten avger koldioxid till atmosfären. Trots svårigheten att få fram exakta siffror på hur mycket koldioxid som avges från jordens alla sjöar och vattendrag så visar ny forskning tydligt på att det handlar om mer koldioxid än alla terrestra ekosystem tillsammans tar upp ifrån atmosfären av de utsläpp som människan orsakar. För att kunna förstå och förutspå hur koldioxidnivåer i sjöar och vattendrag ändras i förhållande till miljöförändringar är det viktigt att förstå processerna som reglerar koldioxidhalterna i inlandsvatten. Det främsta syftet med denna avhandling var därför att undersöka både de koldioxidreglerande processer som verkar inom sjöar och vattendrag och de koldioxidreglerande processer som verkar i marken som omgärdar våra inlandsvatten. Det andra syftet med denna avhandling var att studera de lång- och kortsiktiga effekter ökad organisk kolmängd har på koldioxidhalter i inlandsvatten.

I den första studien undersöktes vattenkemin i över hundra Svenska sjöar, vattendrag och flodmynningar för att identifiera hur koldioxidhalterna påverkats av ökad tillförsel av organiskt kol under en 17-årsperiod. I den andra studien undersökte vi hur en ökad tillförsel av organiskt kol kortsiktigt påverkar koldioxidhalten i sjövatten. Detta gjordes genom två mesokosm experiment i sjön Erken i östra Sverige där vi manipulerade mängden organiskt kol samt ljusklimatet. I det tredje projektet studerade vi mängden organiskt och oorganiskt kol i grundvattnet i ett avrinningsområde i norra Sverige. Vi tog prover från 16 grundvattenbrunnar som var mellan 3 och 20 m djupa. Detta gjordes på våren, sommaren och hösten för att se om det var någon skillnad mellan de olika säsongerna. I den fjärde studien återgick vi till de sjöar och vattendrag från första studien som hade ökat i koldioxidhalt för att undersöka orsaken till ökningen.

Eftersom bakterier bryter ner organiskt kol och då bildar koldioxid förväntade vi oss att den ökade mängden organiskt kol i ytvattnet skulle leda till ökade koldioxidhalter i vår första studie. Dock visade våra resultat att de flesta vatten som visade på ökade mängder organiskt kol inte hade fått högre koldioxidhalter under de 17 år som studien täckte. Även om den ökade mängden organiskt kol skulle ha lett till en ökad bakteriell nedbrytning så märktes ej detta på koldioxidhalterna. Det kan vara så att den ökade nederbörd som observerats över stora delar av Sverige de senaste 20 åren har lett till att en större del av vattenkemin i sjöar och vattendrag påverkas av ytavrinning än av grundvattentillförsel. Vatten som tillförs till inlandsvatten genom ytavrinning består av större mängder organiskt kol och mindre mängder koldioxid, medan förhållandena är det motsatta för grundvatten. Att grundvatten kan ha höga halter av koldioxid men små mängder organiskt kol var något vi visade i den tredje studien. Den ökade nederbörden kan därför leda till en ökad mängd organiskt kol i ytvatten utan att koldioxidhalten höjs. Samtidigt kan ökad nederbörd resultera i brunare vatten, vilket i sin tur leder till ett ändrat ljusklimat som kan påverka växtplankton.

Resultaten från de två mesokosmexperimenten visade tydligt att den ökade mängden organiskt kol ledde till en ökad koldioxidhalt, och detta var till följd av minskad fotosyntes av växtalgerna på grund av det ändrade ljusklimat som det organiska kolet medförde. Tillsättning av det organiska kolet gjorde att mängden ljus i vattnet minskade markant, vilket medförde att växtalgerna tog upp mindre koldioxid eftersom det fanns mindre ljus tillgängligt för fotosyntes, vilket ledde till ökade koldioxidhalter. Tillsättningen av organiskt kol ledde också till en försurning som gjorde att koldioxidhalterna ökade. Resultat från den fjärde studien, där vi undersökte orsaken till varför koldioxidhalten i några svenska sjöar och vattendrag hade ökat markant under en 21-års period, visar också på hur ändrat ljusklimat och försurning kan leda till högre koldioxidhalter i inlandsvatten. Resultaten från den fjärde studien visade också på att kvalitén på det organiska kolet kan ha stor betydelse för hur mycket som omvandlas till koldioxid.

Eftersom processer i omgivande mark också kan ha stor betydelse för koldioxidhalter i ytvatten undersökte vi hur koldioxidhalterna i grundvatten kan variera på olika djup i marken. Genomgående fann vi höga mängder koldioxid på alla uppmätta djup över hela avrinningsområdet vi studerade. Överlag så minskade koldioxidmängden med djup vilket tyder på att de bakteriella aktiviteterna också minskade med djup. Samtidigt kan silikatvittringen ha ökat på djupare nivåer, vilket också leder till lägre koldioxidhalt.

Sammantaget visar resultaten från denna avhandling att det är stor variation i vilken koldioxidreglerande process som har störst påverkan på koldioxidhalten i inlandsvatten. Vi fann inget samband mellan koldioxidhalt och mikrobiell aktivitet, varken på kort eller på lång sikt. Istället visade våra resultat att brunare vatten på grund av ökat organiskt material gjorde att växtplankton och alger hade mindre ljus tillgängligt för fotosyntes vilket ledde till minskat upptag av koldioxid och därmed högre koldioxidhalt i vattnet. Förändringar i pH på grund av försurning från organiskt material och från minskad fotosyntes gjorde också att koldioxidhalten höjdes. Vilka koldioxidreglerande processer som är viktigast kan skilja mellan sjöar och vattendrag, vilket våra resultat tydligt visade. Mängden organiskt material och ljusklimatet visades vara av stor vikt i sjöar, medan i vattendrag så var surhetsgrad och mängden näring för växtplankton och alger viktiga koldioxidreglerande processer. Tidsperspektivet kan också ha väldigt stor betydelse. Därför är det viktigt att undersöka processer både på kort och på lång sikt. En stor del av jordens sjöar och vattendrag kommer fortsätta vara koldioxidkällor i ett ändrat klimat. Ett ändrat klimat kommer sannolikt att påverka våra inlandsvatten, men hur de påverkas kan vara väldigt individuellt.

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# Paper I

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Global Biogeochemical Cycles

RESEARCH ARTICLE

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# No long-term trends in $pCO_2$ despite increasing organic carbon concentrations in boreal lakes, streams, and rivers

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**Key Points:**

- $pCO_2$ remained rather constant in boreal inland waters over the past two decades
- Long-term trends in DOC concentration and $pCO_2$ in inland waters are uncoupled
- Changes in runoff patterns most likely explain the observed uncoupling between DOC and $pCO_2$

**Supporting Information:**

- Supporting Information S1
- Table S1

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**Abstract** Concentrations of dissolved organic carbon (DOC) from terrestrial sources have been increasing in freshwaters across large parts of the boreal region. According to results from large-scale field and detailed laboratory studies, such a DOC increase could potentially stimulate carbon dioxide ($CO_2$) production, subsequently increasing the partial pressure of $CO_2$ ($pCO_2$) in freshwaters. However, the response of $pCO_2$ to the presently observed long-term increase in DOC in freshwaters is still unknown. Here we tested whether the commonly found spatial DOC-$pCO_2$ relationship is also valid on a temporal scale. Analyzing time series of water chemical data from 71 lakes, 30 streams, and 4 river mouths distributed across all of Sweden over a 17 year period, we observed significant DOC concentration increases in 39 lakes, 15 streams, and 4 river mouths. Significant $pCO_2$ increases were, however, only observed in six of these 58 waters, indicating that long-term DOC increases in Swedish waters are disconnected from temporal $pCO_2$ trends. We suggest that the uncoupling of trends in DOC concentration and $pCO_2$ are a result of increased surface water runoff. When surface water runoff increases, there is likely less $CO_2$ relative to DOC imported from soils into waters due to a changed balance between surface and groundwater flow. Additionally, increased surface water runoff causes faster water flushing through the landscape giving less time for in situ $CO_2$ production in freshwaters. We conclude that $pCO_2$ is presently not following DOC concentration trends, which has important implications for modeling future $CO_2$ emissions from boreal waters.

## 1. Introduction

Over the last two decades increasing dissolved organic carbon (DOC) concentrations, mostly derived from the terrestrial environment, have been observed in surface waters across the Northern Hemisphere and especially in the boreal region [*Evans et al.*, 2005; *Filella and Rodriguez-Murillo*, 2014; *Monteith et al.*, 2007]. Some argue that DOC trends are consistent with changes in temperature and hydrology [*Eimers et al.*, 2008; *Worrall and Burt*, 2007], while others propose that increasing DOC trends result from a reduction of anthropogenic sulfur in the atmosphere with a subsequent decrease in acid deposition [*Evans et al.*, 2006; *Monteith et al.*, 2007; *Roulet and Moore*, 2006; *Vuorenmaa et al.*, 2006]. The key drivers behind increasing DOC concentrations in Swedish surface waters during the past decades have been suggested to be changes in runoff combined with a decline in sulfate ($SO_4^{2-}$) deposition [*Erlandsson et al.*, 2008].

Consequences of increasing DOC concentrations can be many, ranging from enhanced metal concentrations to decreased water transparency and changes in pH and alkalinity [*Effler et al.*, 2010; *Kopacek et al.*, 2003; *Lead et al.*, 1999]. Even elevated surface water partial pressure of carbon dioxide ($pCO_2$) has been related to higher levels of DOC concentrations across temperate and boreal lakes [*Lapierre and del Giorgio*, 2012; *Sobek et al.*, 2005, 2003]. Biological and photochemical degradation of DOC has been suggested as an important factor underlying this positive relationship between DOC and carbon dioxide ($CO_2$) concentrations [*Graneli et al.*, 1996; *Lapierre et al.*, 2013]. Photochemical oxidation can directly transform allochthonous DOC to $CO_2$ as well as produce lower molecular weight fractions of DOC available for bacterial mineralization [*Tranvik and Bertilsson*, 2001]. Allochthonous DOC may also be directly degraded by biological processes and subsequently converted to $CO_2$ in freshwaters [*Guillemette et al.*, 2013; *McCallister and del Giorgio*, 2012]. Increases in DOC concentrations usually have an immediate effect on lake internal $CO_2$ production according to incubation experiments. For boreal lake waters it has, for example, been observed that each 1 mg $L^{-1}$ DOC concentration increase results in a lake internal $CO_2$ production flux increase by about 28 mg C $m^{-2}$ $d^{-1}$ [*Algesten et al.*, 2005].

Lake internal biological mineralization of DOC has traditionally been suggested to account for most of the widespread $CO_2$ supersaturation of lakes [*Cole et al*., 2000; *del Giorgio and Peters*, 1994; *Jonsson et al*., 2001]. However, over the past years a number of studies have suggested that the observed $CO_2$ supersaturation could not be explained merely by net heterotrophy but that an additional, sometimes even dominant, fraction of external $CO_2$ input from the catchment via surface, subsurface, and groundwater flow is necessary to retain the high in-lake $CO_2$ concentrations [*Maberly et al*., 2013; *McDonald et al*., 2013; *Stets et al*., 2009; *Weyhenmeyer et al*., 2015b]. Whether the $CO_2$ is produced in the catchment and delivered via inflowing waters or produced within the water column through in situ carbon transformation processes could potentially be distinguished through the use of carbon stable isotopes ($\delta^{13}C$) of dissolved inorganic carbon (DIC) [*Aravena et al*., 1992; *Atekwana and Krishnamurthy*, 1998], but these data are not avaliable on larger scales. However, despite debates on the sources of $CO_2$ in waters, there is more agreement on the sources of DOC. In boreal lakes, usually more than 90% of the mineralized DOC is of allochthonous origin [*Jonsson et al*., 2001].

Although a positive relationship between DOC and surface water $pCO_2$ has frequently been reported [*Lapierre and del Giorgio*, 2012; *Sobek et al*., 2005, 2003], the effect of a long-term DOC increase on $pCO_2$ in freshwaters is still unknown. There are two possible scenarios that both would result in a concurrent DOC and $pCO_2$ increase: $CO_2$ is produced through mineralization of allochthonous DOC, with higher production rates at higher DOC concentrations, or $CO_2$ concentrations simply covary with DOC concentrations; i.e., both DOC and $CO_2$ originate from the same terrestrial source without further major transformation. The second scenario has recently been described by *Seekell and Gudasz* [2016]. They suggested that DOC and $pCO_2$ can covary due to recovery from acidification. Both DOC and $pCO_2$ are highly sensitive to acidification-induced changes in ionic strength of soils [*Evans et al*., 2005; *Lozanovska et al*., 2016]. In Sweden, recovery from acidification is also apparent, seen by decreasing $SO_4^{2-}$ concentrations in waters [*Weyhenmeyer*, 2008] and increasing alkalinity [*Futter et al*., 2014]. However, during the past years recovery from acidification has leveled out and instead climate change effects, in particular precipitation and runoff changes, seem to drive DOC concentration increases [*Weyhenmeyer et al*., 2014].

Irrespective of whether DOC and $pCO_2$ covary or if $CO_2$ is mainly produced within the water column through mineralization of DOC, we expect $pCO_2$ to increase when DOC concentrations increase. We therefore hypothesize that Swedish freshwaters with a significant DOC increase over time also show a significant $pCO_2$ increase. We further hypothesize that DOC to $pCO_2$ relations are different between lakes, streams, and river mouths. In lakes we expect that in situ mineralization of DOC is the more important process driving $CO_2$ production, this becoming more apparent in lakes with longer water residence time since in these waters there is generally more time available to efficiently mineralize DOC to $CO_2$ [*Algesten et al*., 2004]. In streams and river mouths water flows faster; hence, there is less time for in situ carbon transformation processes. Consequently, in streams and river mouths we expect that trends in DOC and $pCO_2$ are mainly driven by catchment processes; i.e., they strongly covary.

## 2. Materials and Methods

### 2.1. Lake, Stream, and River Mouth Data

In this study we used water chemical data from 178 lakes, 86 streams, and 42 river mouths distributed across all of Sweden, covering both the boreal and hemiboreal regions (Figure 1). The water chemistry data were acquired from the Swedish national freshwater monitoring program [*Folster et al*., 2014]. Data acquired were: total organic carbon (TOC), conductivity, pH, alkalinity, total phosphorous (total P), and $SO_4^{2-}$. All water samples were collected at 0.5 m depths, except in more shallow streams where samples were taken closer to the surface. Many surface waters in Sweden have been limed since the late 1970s to counter surface water acidification caused by acid deposition [*Henrikson et al*., 1995]; however, none of the limed waters were included in this study.

All chemical analyses were performed at the SWEDAC (Swedish Board for Accreditation and Conformity) accredited laboratory at the Swedish University of Agricultural Sciences following standard limnological procedures. Analytical methods used can be found at http://www.slu.se/en/departments/aquatic-sciences-assessment/laboratories/geochemical-laboratory/water-chemical-analyses/. The data are made freely available by the Swedish University of Agricultural Sciences and can be downloaded from http://www.slu.se/vatten-miljo. Total organic carbon concentrations were used as a proxy for DOC concentrations as the particulate fraction of organic carbon in boreal and hemiboreal freshwaters generally is less than 1% [*Laudon et al.*, 2011]. All analyses considered in this study were made on unfiltered water.

From the collected data, concentrations of $CO_2$ were calculated using water temperature, alkalinity (only positive values were selected), and pH (only values >5.4 were used to minimize incorrect estimates of $CO_2$ concentrations; see below) according to *Weyhenmeyer et al.* [2012]. Subsequently, $pCO_2$ (in microatmospheres) were determined based on calculated $CO_2$ concentrations and Henry's constant according to Henry's law and adjusted for atmospheric pressure at sample site elevation [*Weyhenmeyer et al.*, 2012]. Calculation of $pCO_2$ from alkalinity and pH may result in overestimated values, and $CO_2$ cannot be calculated from pH values of less than 5.2 [*Abril et al.*, 2015; *Hunt et al.*, 2011; *Raymond et al.*, 2013]. Since also pH values slightly above 5.2 provide very uncertain estimates of $pCO_2$ [*Raymond et al.*, 2013; *Wallin et al.*, 2014], we minimized the uncertainty in the calculation of $pCO_2$ by discarding all pH values of less than 5.4. Calculations of $pCO_2$ are also uncertain in organic-rich waters. We removed the effect of organic acids on the total alkalinity by applying the triprotic model by *Hruska et al.* [2003] to estimate the dissociation of organic acid anions ($RCOO^-$) from measured pH and TOC. The calculated organic alkalinity was subtracted from the measured total alkalinity, and this new value for alkalinity was used to calculate $pCO_2$ according to *Weyhenmeyer et al.* [2012] followed by analyses for long-term trends in $pCO_2$. Finally, we minimized uncertainties in $pCO_2$ calculations by using median values rather than means to avoid the impact of outliers.

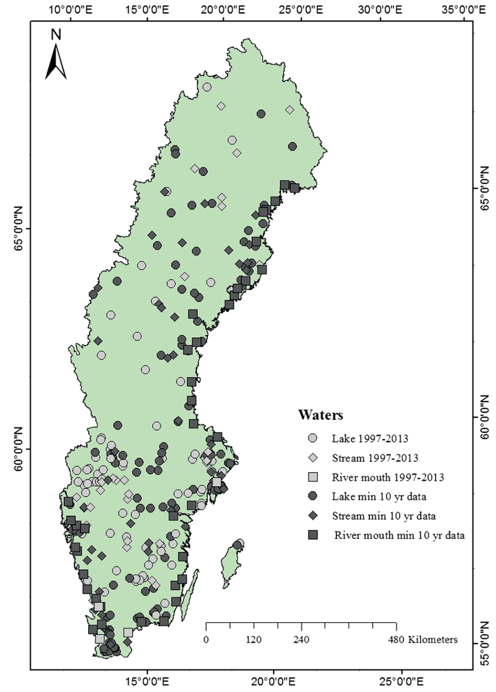

**Figure 1.** Location of the Swedish study lakes (circles), streams (diamonds), and river mouths (squares). Grey symbols show waters with complete time series during 1997–2013; black symbols show waters with shorter time series but which have at least 10 years of data.

### 2.2. Data Analyses

For the data analyses we used two databases. Initial analyses were performed with the entire data set, i.e., waters for which there was a minimum of 10 years of data available (=database 1). The database comprised 178 lakes, 86 streams, and 42 river mouths distributed across all of Sweden (Figure 1). The lakes included in the analysis were mostly small, with a median surface area of 0.73 $km^2$, and shallow, having a median mean depth of 4.4 m. The lakes had been sampled between 10 and 28 years, while the longest sampling period for the streams was from 1959 to 2013, although some years were missing. Of the 86 streams, 11 had been sampled for 46 years. For the river mouths, samples had been collected for up to 27 years (Table 1).

As there was a wide range of sampling periods as well as some missing years of sampling for many waters, we used a subset of database 1 with complete data during 1997 to 2013 (= database 2). To make comparisons between sites, freshwaters with a minimum of four samples for each year were included. This resulted in a

**Table 1.** Number of Years Sampled, Longest Sampling Period, Years Missing in Longest Sampling Period, and Median Sampling Period for Lakes, Streams, and River Mouths Included in the Initial Analysis

| | Number of Years Sampled | Longest Sampling Period | Years Missing in Longest Sampling Period | Median Sampling Period (Years) |
|---|---|---|---|---|
| Lakes | 10 to 28 | 1983 to 2013 | 1985 to 1987 | 18 |
| Streams | 10 to 52 | 1959 to 2013 | 1961 to 1964 | 16 |
| River mouths | 10 to 27 | 1987 to 2013 | - | 13 |

total of 71 lakes, 30 streams, and 4 river mouths to be analyzed for long-term trends in DOC and $p\mathrm{CO_2}$ (Figure 1). The lakes included in the second analysis were distributed across all of Sweden; however, there were more lakes in the southern parts (Figure 1). Lakes included in the second analysis were generally small, having a median surface area of 0.99 $\mathrm{km^2}$, and shallow, with a median mean depth of 4.8 m. About two thirds of the streams included in the second analysis were distributed in the southern parts of Sweden. The four river mouths included in the analysis were all located in southern Sweden (Figure 1).

### 2.3. Statistics

All analyses were performed on yearly median values. Prior to analysis, data were tested for normality using the Shapiro-Wilk *W* test. Due to the nonnormal distribution of $p\mathrm{CO_2}$, we chose the nonparametric Mann-Kendall trend test as it does not require normally distributed data. A Mann-Kendall trend test shows if there is a monotonic upward or downward trend of the variable of interest over time. We used an Excel macro for the Mann-Kendall trend test (Microsoft Office 2015) and the software package JMP version 11.0.0 (SAS Institute Inc. 2013) for all other calculations and statistical analyses. For all tests we set the significance at an alpha level of 0.05. When we determined a significant trend, the results were referred to as increase or decrease, whereas when we did not find any significant change, the results were referred to as no change.

## 3. Results

### 3.1. Overall Trends in DOC and $p\mathrm{CO_2}$ in Lakes, Streams, and River Mouths

Of the 178 lakes included in the initial analysis, which encompassed all waters with a minimum of 10 years of data, there was a significant DOC concentration increase in 101 (i.e., 57%) lakes, while in the remaining 77 lakes, DOC concentrations did not show a significant trend over time. Of the 101 lakes with increasing DOC concentrations, 22 (i.e., 22%) also increased significantly in $p\mathrm{CO_2}$ (Mann-Kendall trend test results: $p < 0.05$).

The long-term DOC patterns in streams were similar to lakes, i.e., in 42% of the 86 streams DOC concentrations had increased significantly (Mann-Kendall test: $p < 0.05$). Eight out of the 36 streams with increasing DOC concentrations (i.e., 22%) also showed a significant increase in $p\mathrm{CO_2}$ (Mann-Kendall test: $p < 0.05$).

River mouths showed similar long-term DOC patterns with a significant increase in DOC concentration in 17 (40%) of the 42 river mouths. However, there was no significant increase over time in $p\mathrm{CO_2}$ in any of the river mouths (Mann-Kendall test results $p > 0.05$).

### 3.2. Trends in Lakes During 1997 to 2013

During the period 1997 to 2013, lake DOC concentrations ranged between 0.6 and 25.1 mg $\mathrm{L^{-1}}$ across all lakes, with the lowest and highest values both occurring in 2005. More than half (39 out of 71) of the lakes demonstrated a significant increase in DOC concentrations, and four of these also showed a significant increase in $p\mathrm{CO_2}$ (Mann-Kendall test: $p < 0.05$) (Figures 2 and 3 and Table S1).

Among the variables which potentially can have a strong influence on $p\mathrm{CO_2}$ in waters, i.e., alkalinity, pH, $\mathrm{SO_4^{2-}}$, and primary production (here we used total P concentrations as a proxy according to *Wetzel* [1992]) we found significantly increasing trends over time in alkalinity, pH, and total P concentrations in 13, 8, and 6 lakes, respectively, of the 39 lakes with significantly increasing DOC concentrations (Figure 3 and Table S1). In the majority (97%) of lakes with increasing DOC concentrations, $\mathrm{SO_4^{2-}}$ concentrations had decreased significantly (Mann-Kendall test: $p < 0.05$).

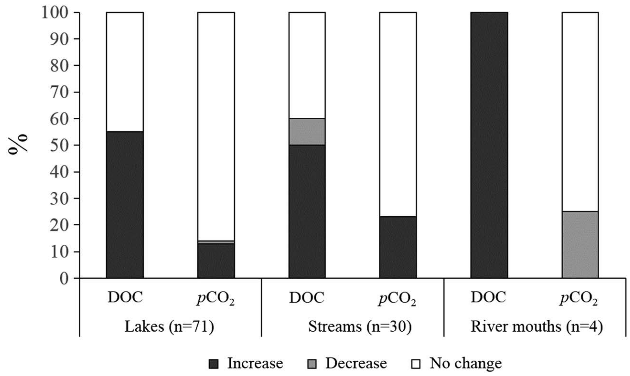

**Figure 2.** Percentage of surface waters of Swedish boreal lakes (*n* = 71), streams (*n* = 30), and river mouths (*n* = 4) with significant increase, decrease, or with no change in dissolved organic carbon (DOC) or partial pressure of carbon dioxide ($pCO_2$) during the period 1997 to 2013.

### 3.3. Trends in Streams During 1997 to 2013

In the study streams included in the second analysis, DOC concentrations ranged between 1.0 and 28.4 mg $L^{-1}$ throughout 1997 to 2013. The highest stream DOC concentration occurred in 2009 and the lowest in 2013. Half of the streams (15 out of 30) showed a significant increase in DOC concentrations, and of these, only two demonstrated a significant increase in $pCO_2$ (Mann-Kendall test: $p < 0.05$; Figures 2 and 3 and Table S2).

Of the 15 streams with increasing DOC, alkalinity had increased significantly in five streams, pH in one stream, and total P in two streams (Figure 3 and Table S2). As seen in the lakes, most streams with increased DOC also showed a decrease in $SO_4^{2-}$, i.e., in 11 of the 15 streams.

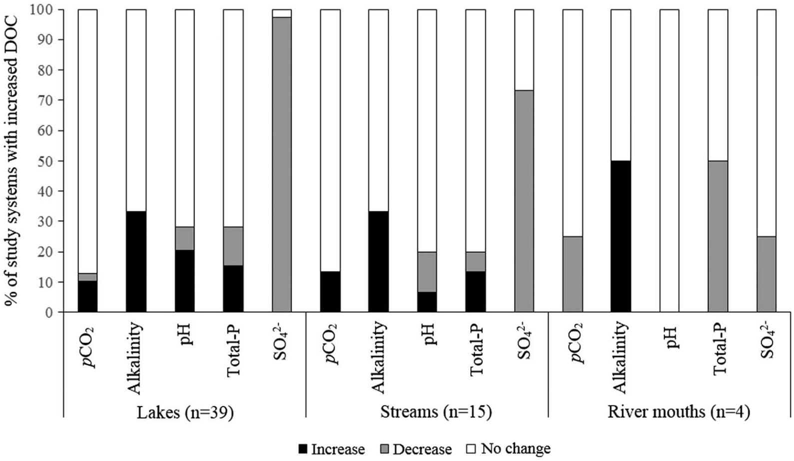

**Figure 3.** Percentage of Swedish boreal lake waters (*n* = 39), streams (*n* = 15), and river mouths (*n* = 4) with significant increase, decrease, or with no change in partial pressure of carbon dioxide ($pCO_2$), alkalinity, pH, total phosphorous (total P), and sulfate ($SO_4^{2-}$) concentrations during the period 1997 to 2013. All waters had increased significantly in dissolved organic carbon concentration (DOC) during this period.

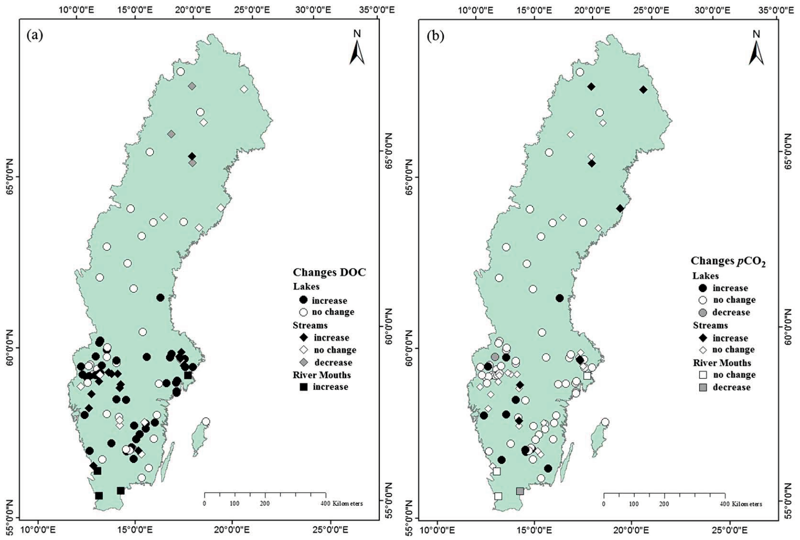

**Figure 4.** Location of Swedish study lakes (circles, *n* = 71), streams (diamonds, *n* = 30), and river mouth (squares, *n* = 4) and illustration of changes in (a) dissolved organic carbon (DOC) and (b) partial pressure of carbon dioxide ($pCO_2$) in the surface waters (black = significant increase, white = no change, grey = significant decrease) during the period 1997 to 2013.

### 3.4. Trends in River Mouths During 1997 to 2013

During the period 1997 to 2013 river mouth DOC concentrations ranged from 6.9 to 22.7 mg $L^{-1}$, with the lowest and highest measured concentrations occurring in 1998 and 2011, respectively. All four river mouths showed significant DOC concentration increases, but none showed a significant increase in $pCO_2$ (Figure 2).

Alkalinity had increased in two of the four river mouths from 1997 to 2013, pH had increased in none, total P concentrations had decreased in two of the river mouths, and $SO_4^{2-}$ had significantly decreased in one river mouth (Figure 3 and Table S3).

### 3.5. Spatial Variability in Trends Over Time

For all waters (lakes, streams, and river mouths) included in the second analysis, i.e., from 1997 to 2013, increases in DOC concentrations were mostly occurring in the southern parts of Sweden (Figure 4a). No obvious spatial pattern was observed for changes in $pCO_2$ due to very few waters having significantly altered $pCO_2$. However, most of the lakes with increased $pCO_2$ were located in southern Sweden, whereas the streams with enhanced $pCO_2$ were found both in the south and in the north of Sweden (Figure 4b).

## 4. Discussion

Our study clearly demonstrates that in the majority of Swedish freshwaters $pCO_2$ has not changed significantly over the past decades despite significantly increasing DOC concentrations. These findings apply to lakes, streams, and river mouths. Thus, our hypothesis of concurrent DOC and $pCO_2$ increases over time was supported in only six out of 58 freshwaters that had shown a significant increase in DOC concentrations. Consequently, trends over time in DOC and $pCO_2$ are generally uncoupled across all types of Swedish freshwaters.

Originally, we had expected that increasing DOC concentrations go along with increasing $pCO_2$ either due to increased lake internal $CO_2$ production or due to increased lake external $CO_2$ inflow together with the DOC

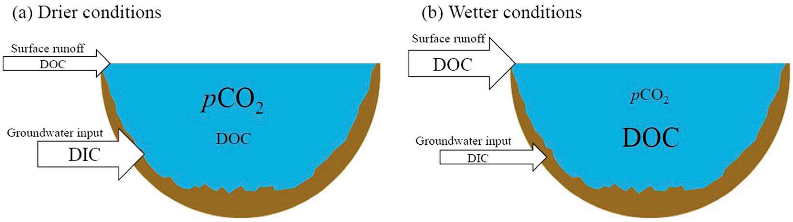

**Figure 5.** Conceptual model of the effect of hydrological processes on partial pressure of carbon dioxide ($pCO_2$) in freshwater. (a) During drier conditions freshwaters receive proportionally less shallow groundwater compared to dissolved inorganic carbon (DIC)-rich deep groundwater with subsequently higher $pCO_2$. (b) During more wet conditions shallower groundwater gets flushed into freshwaters diluting the DIC-rich deep groundwater such that an increase in dissolved organic carbon (DOC) does not result in increased $pCO_2$.

inflow. However, although we cannot rule out that more $CO_2$ is produced within the water column or that lake external $CO_2$ inputs through recovery from acidification or runoff increases have increased, there must be other overriding factors that result in relatively constant $pCO_2$ levels over time.

The lack of long-term trends in $pCO_2$ despite increasing DOC concentrations might be caused by an accelerated $CO_2$ loss from the water column. Such losses are, for example, possible when primary production increases. We found, however, decreasing rather than increasing total P concentrations (Figure 3), indicating that the $CO_2$ loss by primary production most likely has not substantially increased during the past years. In addition, primary production in the boreal waters has usually only a minor impact on the aquatic carbon cycling [*Algesten et al.*, 2004], another reason why it is rather unlikely that $CO_2$ in the boreal waters has increasingly been lost over time due to increased primary production.

An alternative and more likely explanation to the lack of change in $pCO_2$ is a change in hydrology. Hydrological processes affect $pCO_2$ in freshwater in a number of ways. First, changes in runoff patterns alter the ratio between shallow and deep groundwater flow into lakes and streams [*Laudon et al.*, 2007]. Streams typically have a strong hydrochemical connectivity with the catchment soils [*Hope et al.*, 2004; *Laudon et al.*, 2011]. Consequently, much of the $CO_2$ in streams comes from direct inputs of DIC, fixed and mineralized in the catchment and delivered via the groundwater [*Leith et al.*, 2015; *Öquist et al.*, 2009; *Winterdahl et al.*, 2016]. A recent study demonstrated that in situ DOC mineralization was a minor source of $CO_2$ in small boreal headwater streams and that the main source of stream $CO_2$ was $CO_2$-rich groundwater [*Winterdahl et al.*, 2016]. Likewise, *Weyhenmeyer et al.* [2015b] showed that direct inputs of DIC from the terrestrial surroundings of a lake have a stronger influence on $CO_2$ concentrations in lake water than do lake internal $CO_2$ production. As precipitation and runoff have shown an overall increase across Sweden over the past few decades, in particular during the 1990s and 2000s [*Bengtsson and Rana*, 2014; *Lindström and Bergström*, 2004; *Weyhenmeyer et al.*, 2014, 2015a], we suggest that most waters now receive proportionally more shallow groundwater compared to deep groundwater [*Laudon et al.*, 2007] (Figure 5). Such an increase most likely results in a DOC concentration increase in surface waters as DOC concentration in soil profiles tends to increase toward the top soil layers [*Grabs et al.*, 2012; *Kaiser and Kalbitz*, 2012]. Although the literature is scarce, similar soil profiles of $CO_2$ concentrations do not show the same pattern due to soil $CO_2$ having a soil-atmospheric exchange resulting in higher $CO_2$ at deeper soil horizons [*Öquist et al.*, 2009; *Winterdahl et al.*, 2016]. Consequently, there would be a dilution effect on the DIC input from the terrestrial surroundings which could explain the lack of change in $pCO_2$ in response to enhanced DOC concentrations.

Hydrological processes also affect water retention time, both in the landscape and within water systems. The lake and stream internal $CO_2$ production shows a dependency on landscape water retention time with more efficient production in waters with a long water retention time [*Catalán et al.*, 2016; *Hanson et al.*, 2011]. Since precipitation and water flushing through the landscape, in particular through lakes, has generally increased across Sweden over the past decades [*Weyhenmeyer et al.*, 2014, 2015a], we suggest that there could be less time available for mineralization of DOC in lakes, making DOC mineralization less efficient and resulting in a weaker direct coupling between DOC and $p\mathrm{CO_2}$. Rather inefficient lake internal DOC mineralization in comparison to other processes might further be supported by our observations that DOC-$p\mathrm{CO_2}$ long-term relationships were rather similar across lakes and streams.

All river mouths showed significantly increasing DOC concentrations, while none had an increase in $p\mathrm{CO_2}$. We suggest that the DOC to $\mathrm{CO_2}$ transformation in waters becomes less efficient during transport from headwaters down to the sea, as a response to faster water flushing through the landscape. Alternatively, the labile fractions of DOC are rapidly lost upstream, while the more recalcitrant DOC is transported downstream along the land to ocean continuum [*Creed et al*., 2015]. Consequently, when reaching the river mouth most of the DOC may consist of recalcitrant carbon which cannot be utilized by microbes.

The lack of change in $p\mathrm{CO_2}$ despite increasing DOC concentrations could potentially also be a matter of DOC quality change as this affects the reactivity of DOC and thus its potential for mineralization to $\mathrm{CO_2}$ [*Mostovaya et al*., 2016]. In general, DOC can be more humic or more protein-like [*Kothawala et al*., 2014]. Protein-like DOC is generally derived from autochthonous algae and microbes; it is often labile and biologically reactive and is both produced and degraded over time within aquatic systems [*Guillemette and del Giorgio*, 2011]. Humic-like DOC is generally derived from the terrestrial environment and is susceptible to within-lake processes and may be rapidly lost from the water column by mineralization, flocculation, or transformation to other DOC by-products [*Kothawala et al*., 2014]. Flocculation, in particular, is an important transformation process for humic-like DOC components as it has been shown that in lakes, up to 22% of terrestrially derived DOC can be lost from the water column merely from flocculation and subsequent sedimentation [*Einarsdottir et al*., 2017; *von Wachenfeldt and Tranvik*, 2008]. The observed increase in DOC in freshwaters in the present study is probably mostly of terrestrial origin, hence consisting of humic-like components [*Kothawala et al*., 2014]. Consequently, in the lakes, a significant amount of DOC may flocculate resulting in less carbon being available for $\mathrm{CO_2}$ production. Flocculation may, however, not be as important in streams and river mouths as faster flowing water limit the period of time for flocculation and settling of organic matter [*von Wachenfeldt and Tranvik*, 2008]. Nonetheless, further studies are needed where the quality of the DOC is investigated to elucidate whether DOC quality plays an important role in $p\mathrm{CO_2}$ changes through time.

Apart from biogeochemical processes, also uncertainties in our $p\mathrm{CO_2}$ calculations might have caused some bias in the $p\mathrm{CO_2}$ trends over time. Estimating $p\mathrm{CO_2}$ from pH and alkalinity is commonly used in the literature [*Raymond et al*., 2013; *Sobek et al*., 2005; *Weyhenmeyer et al*., 2012] but has been criticized for being an uncertain method especially in acidic organic-rich waters with low alkalinity [*Abril et al*., 2015; *Hunt et al*., 2011; *Wallin et al*., 2014]. In the absence of long-term data sets of directly measured $p\mathrm{CO_2}$, we, however, needed to rely on calculated $p\mathrm{CO_2}$ in this study. Many of the waters in our study are organic-rich (median DOC = 8.8 mg $\mathrm{L^{-1}}$) with low alkalinity (median alkalinity = 0.141 meq $\mathrm{L^{-1}}$). We minimized uncertainties in the $p\mathrm{CO_2}$ calculations by removing the effect of organic acids, excluding highly acidic waters, and performing all statistics on median values. Additionally, we used nonparametric methods where we considered relative rather than absolute $p\mathrm{CO_2}$ values.

Still, $p\mathrm{CO_2}$ trends over time might be affected by temporal changes in alkalinity and pH. Alkalinity has increased in more than half of the waters in our study during the period 1997 to 2013, whereas pH has generally remained constant. Alkalinity is a measure of the capacity of an aqueous solution to neutralize acids; hence, increased alkalinity is an indication of recovery from acidification. Consequently, the increased alkalinity observed in our study systems could reflect that Swedish freshwater systems have become less acidified [*Skjelkvale et al*., 2001]. According to *Seekell and Gudasz* [2016], recovery from acidification should result in increased $p\mathrm{CO_2}$ in our lake types and not in constant or decreased $p\mathrm{CO_2}$ as observed in this study. Increased alkalinity also implies that inorganic carbon concentrations have increased; however, as we do not see an increase in $p\mathrm{CO_2}$, much of this carbon may exist as carbonates and bicarbonates. *Stets et al*. [2017] highlighted the importance of carbonate buffering for understanding $\mathrm{CO_2}$ dynamics in freshwaters as $\mathrm{CO_2}$ concentrations can be buffered despite large changes in the DIC pool. However, this effect is greatest in waters with high alkalinity and high pH. The systems in the study by *Stets et al*. [2017] had a median alkalinity of 2.78 meq $\mathrm{L^{-1}}$ whereas the majority (89%) of waters in our study had an alkalinity of less than 1.0 meq $\mathrm{L^{-1}}$. Furthermore, only six of the waters in our study with alkalinity over 1.0 meq $\mathrm{L^{-1}}$ had increased in DOC, and of these only one stream and one river mouth had increased in alkalinity. Consequently, changes in alkalinity could potentially explain the lack of change in $p\mathrm{CO}_2$ in these two waters. However, in the remaining 56 waters this is likely not the case due to the low alkalinity. *Stets et al.* [2017] suggested that in low alkalinity waters ($< 1.0$ meq L$^{-1}$) the pool of ionized $\mathrm{CO}_2$ is small. Therefore, although alkalinity increased in many of our study waters, alkalinity is still low in the majority of the waters; hence, an increased buffering capacity could generally not explain the lack of change in $p\mathrm{CO}_2$. Thus, the overall pattern of uncoupled DOC and $p\mathrm{CO}_2$ long-term trends seems robust.

## 5. Conclusion

Although a positive relationship between DOC and $p\mathrm{CO}_2$ has often been observed on a spatial scale, we were unable to establish a positive relationship on a temporal scale. Our results show that DOC concentrations and $p\mathrm{CO}_2$ trends in lakes, streams, and river mouths through time are uncoupled and that changes in surface water runoff may explain this uncoupling. However, other processes, such as changes in alkalinity or DOC quality, may also be important for the fate of $p\mathrm{CO}_2$, at least in some of the waters. To disentangle the relative importance of all these processes, additional detailed site-specific research is needed. It is striking, however, that there were no overall long-term $p\mathrm{CO}_2$ trends despite increasing DOC concentrations. Since DOC concentrations are often used to predict $p\mathrm{CO}_2$ and thereby $\mathrm{CO}_2$ emissions from inland waters [e.g., *Raymond et al*., 2013; *Sobek et al*., 2003], predictions of future $\mathrm{CO}_2$ emissions from inland waters need to consider the findings of this study. Although precipitation is predicted to further increase in the boreal region as a response to climate change [*Chen et al*., 2015; *Teutschbein et al*., 2015], presumably resulting in more DOC being flushed into inland waters, it is unlikely that $p\mathrm{CO}_2$ will follow this increase.

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# Supplementary information

# No long-term trends in $p\mathrm{CO_2}$ despite increasing organic carbon concentrations in boreal lakes, streams and rivers

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*Table S1* Median values of dissolved organic carbon (DOC), partial pressure of $CO_2$ ($pCO_2$), alkalinity (Alk.), pH, total phosphorous (Tot-P), and sulfate ($SO_4^{2-}$) and results from Mann Kendall Tests for Alk., pH, Tot-P and $SO_4$ for all lakes included in database 2 (minimum four samples per year for water chemistry parameters during the period 1997 to 2013). A plus refers to a significant increase ($p < 0.05$), minus refers to a significant decrease ($p < 0.05$) and "nc" refers to no change ($p > 0.05$).

| *Change*<br>**Lake** | **DOC ($mgL^{-1}$)** | **$pCO_2$ (µatm)** | **Alk. ($mekvL^{-1}$)** | **pH** | **Tot-P (µg/l)** | **$SO_4^{2-}$ ($mekvL^{-1}$)** | **Alk. (MK)** | **pH (MK)** | **Tot-P (MK)** | **$SO_4^{2-}$ (MK)** |
|---|---|---|---|---|---|---|---|---|---|---|
| *Increase DOC and $pCO_2$* | | | | | | | | | | |
| Fiolen | 7.6 | 951 | 0.059 | 6.7 | 11.0 | 0.119 | + | + | + | - |
| Fräcksjön | 9.9 | 1381 | 0.057 | 6.5 | 9.0 | 0.098 | + | nc | nc | - |
| Stensjön | 7.0 | 1097 | 0.038 | 6.4 | 6.0 | 0.034 | nc | nc | nc | - |
| Ymsen | 10.5 | 1411 | 0.636 | 7.4 | 55.0 | 0.140 | nc | + | nc | - |
| *Increase DOC. no change $pCO_2$* | | | | | | | | | | |
| Botungen | 9.9 | 1649 | 0.060 | 6.4 | 15.0 | 0.076 | + | nc | + | - |
| Edasjön | 14.4 | 2719 | 0.982 | 7.3 | 34.0 | 0.143 | nc | nc | nc | - |
| Sandsjön | 13.4 | 2273 | 0.070 | 6.2 | 18.0 | 0.056 | nc | nc | + | - |
| Siggeforasjön | 17.1 | 1695 | 0.109 | 6.6 | 11.0 | 0.085 | nc | nc | nc | - |
| Allgjuttern | 7.4 | 939 | 0.061 | 6.7 | 5.0 | 0.154 | + | nc | - | - |
| Björken | 8.4 | 1222 | 0.225 | 7.0 | 9.0 | 0.180 | + | nc | nc | - |
| Björklången | 12.2 | 1767 | 0.084 | 6.5 | 8.0 | 0.044 | nc | nc | nc | - |
| Bodasjön | 7.95 | 1414 | 0.051 | 6.4 | 6.0 | 0.086 | nc | nc | nc | - |
| Bosjön | 11.8 | 1471 | 0.088 | 6.6 | 7.0 | 0.046 | nc | nc | nc | - |
| Bysjön | 7.6 | 1641 | 0.078 | 6.5 | 10.0 | 0.077 | nc | nc | nc | - |
| Dagarn | 7.4 | 1350 | 0.109 | 6.6 | 6.0 | 0.082 | + | nc | nc | - |
| Ekholmssjön | 10.0 | 1896 | 0.352 | 7.0 | 18.0 | 0.153 | nc | nc | nc | - |
| Fjärasjö | 10.5 | 1271 | 0.106 | 6.7 | 9.0 | 0.160 | nc | - | nc | - |
| Fysingen | 9.2 | 1266 | 1.979 | 7.9 | 25.0 | 2.160 | + | nc | - | - |
| Försjön | 10.6 | 1104 | 0.083 | 6.7 | 7.0 | 0.139 | nc | - | - | - |
| Glimmingen | 6.7 | 1009 | 0.196 | 7.1 | 5.0 | 0.160 | nc | nc | nc | - |
| Hagasjön | 9.3 | 1669 | 0.065 | 6.4 | 8.0 | 0.095 | + | nc | nc | - |
| Hinnasjön | 13.0 | 1884 | 0.034 | 6.1 | 13.0 | 0.160 | + | nc | + | - |
| Holmehultasjön | 10.3 | 1448 | 0.146 | 6.8 | 10.0 | 0.130 | nc | nc | nc | - |
| Humsjön | 7.8 | 1338 | 0.122 | 6.8 | 11.0 | 0.065 | nc | nc | nc | - |
| Lillsjön | 16.5 | 2652 | 0.417 | 7.0 | 65.0 | 0.186 | nc | nc | + | nc |
| Mögesjön | 9.3 | 1609 | 0.055 | 6.4 | 8.0 | 0.063 | nc | nc | nc | - |
| Rattsjön | 0.02 | 1308 | 0.030 | 6.2 | 6.0 | 0.042 | nc | nc | nc | - |
| Rinnen | 11.6 | 1918 | 0.059 | 6.3 | 9.0 | 0.056 | + | + | - | - |
| Rundbosjön | 10.9 | 2020 | 0.369 | 7.1 | 49.0 | 0.326 | + | nc | nc | - |
| Skårdalsvattnet | 11.1 | 1728 | 0.024 | 6.0 | 25.0 | 0.072 | + | - | nc | - |
| Skärgölen | 7.7 | 1644 | 0.141 | 6.7 | 8.0 | 0.138 | nc | + | nc | - |
| Skärlen | 4.1 | 826 | 0.030 | 6.3 | 6.0 | 0.146 | + | nc | - | - |
| Stora Envättern | 10.4 | 986 | 0.039 | 6.5 | 7.0 | 0.115 | nc | + | nc | - |
| Stor-Hässlingen | 9.9 | 1539 | 0.113 | 6.7 | 7.0 | 0.041 | nc | nc | nc | - |
| Tångerdasjön | 11.0 | 1819 | 0.321 | 7.0 | 37.5 | 0.103 | nc | + | nc | - |
| Tärnan | 10.8 | 1443 | 0.301 | 7.1 | 11.5 | 0.127 | nc | nc | nc | - |
| Yngern | 6.2 | 955 | 0.241 | 7.2 | 12.0 | 0.220 | + | nc | nc | - |
| Överudssjön | 11.0 | 2007 | 0.158 | 6.7 | 42.5 | 0.093 | nc | + | + | - |
| *No change DOC, increase $pCO_2$* | | | | | | | | | | |
| Brunnsjön | 15.9 | 1462 | 0.010 | 5.9 | 12.0 | 0.178 | + | nc | nc | - |
| Svartsjön | 18.8 | 1830 | 0.172 | 6.8 | 20.0 | 0.030 | nc | nc | nc | - |
| Ulvsjön | 8.8 | 1456 | 0.027 | 6.0 | 7.0 | 0.066 | + | nc | nc | - |
| Översjön | 6.5 | 1026 | 0.013 | 5.8 | 6.0 | 0.065 | + | nc | nc | - |
| Hojagöl | 7.5 | 1980 | 0.043 | 6.1 | 9.0 | 0.165 | + | nc | nc | - |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Skärsjön | 4.6 | 762 | 0.094 | 6.9 | 9.0 | 0.195 | + | + | - | - |
| Stora Skärsjön | 4.9 | 1062 | 0.113 | 6.8 | 8.0 | 0.101 | nc | nc | nc | - |
| *No change DOC, decrease $p\mathrm{CO}_2$* | | | | | | | | | | |
| Trehörningen | 6.8 | 1269 | 0.106 | 6.7 | 4.0 | 0.066 | nc | + | nc | - |
| *No change DOC or $p\mathrm{CO}_2$* | | | | | | | | | | |
| Abiskojaure | 1.3 | 818 | 0.201 | 7.1 | 4.0 | 0.086 | nc | nc | nc | nc |
| Alstern | 7.4 | 1189 | 0.136 | 6.8 | 5.0 | 0.067 | nc | nc | nc | - |
| Billingen | 8.7 | 1771 | 0.152 | 6.7 | 19.5 | 0.081 | + | nc | nc | - |
| Björnklammen | 7.5 | 1476 | 0.096 | 6.6 | 5.0 | 0.065 | nc | nc | nc | - |
| Bästeträsk | 11.5 | 718 | 2.095 | 8.2 | 5.0 | 0.152 | nc | nc | nc | - |
| Degervattnet | 8.1 | 974 | 0.169 | 7.0 | 5.0 | 0.043 | nc | nc | nc | - |
| Dunnervattnet | 5.6 | 767 | 0.079 | 6.8 | 4.0 | 0.026 | + | nc | nc | - |
| Fyrsjön | 6.8 | 1233 | 0.715 | 7.5 | 6.0 | 0.059 | nc | nc | nc | nc |
| Gröcken | 9.2 | 1297 | 0.076 | 6.6 | 5.0 | 0.037 | nc | nc | - | - |
| Jutsajaure | 6.0 | 912 | 0.080 | 6.7 | 8.0 | 0.021 | nc | + | nc | - |
| Krutejaure | 1.1 | 528 | 0.018 | 6.3 | 2.0 | 0.014 | + | nc | - | - |
| Norra Örsjön | 6.3 | 1289 | 0.117 | 6.7 | 4.0 | 0.069 | nc | nc | nc | - |
| Remmarsjön | 9.5 | 1538 | 0.047 | 6.3 | 9.0 | 0.028 | + | nc | - | - |
| Sangen | 6.8 | 1083 | 0.096 | 6.7 | 9.0 | 0.027 | nc | nc | nc | - |
| Spjutsjön | 4.6 | 1200 | 0.084 | 6.6 | 4.0 | 0.103 | + | nc | nc | - |
| Stora Lummersjön | 7.6 | 2232 | 0.111 | 6.5 | 12.0 | 0.081 | nc | nc | nc | - |
| Stor-Backsjön | 12.1 | 1230 | 0.152 | 6.9 | 11.0 | 0.041 | nc | nc | nc | - |
| Stor-Tjulträsket | 2.15 | 937 | 0.326 | 7.3 | 4.0 | 0.046 | + | nc | - | nc |
| Sännen | 8.2 | 1111 | 0.020 | 6.1 | 11.0 | 0.243 | + | - | nc | - |
| Tängersjö | 10.9 | 1283 | 0.072 | 6.6 | 8.0 | 0.087 | nc | nc | nc | - |
| Västra Helgtjärnen | 7.8 | 838 | 0.623 | 7.6 | 5.0 | 0.029 | + | nc | - | - |
| Västra Solsjön | 5.1 | 974 | 0.121 | 6.9 | 4.0 | 0.084 | + | nc | nc | - |
| Älgsjön | 18.5 | 2187 | 0.173 | 6.7 | 21.0 | 0.124 | nc | nc | nc | - |
| Öjsjön | 6.8 | 1059 | 0.103 | 6.8 | 6.0 | 0.138 | nc | nc | nc | - |
| Övre Fjätsjön | 4.8 | 996 | 0.076 | 6.7 | 7.0 | 0.018 | + | nc | nc | - |

*Table S2* Median values of dissolved organic carbon (DOC), partial pressure of $\mathrm{CO}_2$ ($p\mathrm{CO}_2$), alkalinity (Alk.), pH, total phosphorous (Tot-P), and sulfate ($\mathrm{SO}_4^{2-}$) and results from Mann Kendall tests for Alk., pH, Tot-P and $\mathrm{SO}_4$ for all streams included in database 2 (minimum four samples per year for water chemistry parameters during the period 1997 to 2013). A plus refers to a significant increase ($p < 0.05$), minus refers to a significant decrease ($p < 0.05$) and "nc" refers to no change ($p > 0.05$).

| *Change*<br>**Stream** | **DOC (mgL$^{-1}$)** | **$p\mathrm{CO}_2$ (µatm)** | **Alk. (mekvL$^{-1}$)** | **pH** | **Tot-P (µg L$^{-1}$)** | **$\mathrm{SO}_4^{2-}$ (mekv L$^{-1}$)** | **Alk. (MK)** | **pH (MK)** | **Tot-P (MK)** | **$\mathrm{SO}_4^{2-}$ (MK)** |
|---|---|---|---|---|---|---|---|---|---|---|
| *Increase DOC and $p\mathrm{CO}_2$* | | | | | | | | | | |
| Vindbron | 15.1 | 4171 | 2.940 | 7.6 | 57.0 | 0.766 | nc | - | nc | nc |
| Visman Nybbe | 17.3 | 3079 | 0.145 | 6.5 | 43.0 | 0.995 | nc | - | nc | nc |
| *Increase DOC, no change $p\mathrm{CO}_2$* | | | | | | | | | | |
| Alsterälven Alster | 10.2 | 1757 | 0.164 | 6.7 | 37.0 | 0.087 | + | nc | nc | - |
| Byälven Säffle V | 8.4 | 1164 | 0.121 | 6.8 | 16.0 | 0.079 | nc | nc | nc | - |
| Fyrisån Klastorp | 16.0 | 3415 | 2.917 | 7.7 | 45.0 | 0.707 | nc | nc | nc | nc |
| Gullspångsälven | 7.6 | 874 | 0.146 | 7.0 | 10.0 | 0.103 | nc | nc | nc | - |
| Göta Älv Vargön | 4.6 | 763 | 0.277 | 7.3 | 10.0 | 0.220 | + | nc | nc | - |
| Klarälven Almar | 8.0 | 1272 | 0.106 | 6.7 | 10.0 | 0.037 | nc | nc | nc | - |
| Laxtjärnsbäcken | 3.4 | 904 | 0.143 | 6.9 | 4.0 | 0.027 | nc | nc | - | - |
| Norrhultsbäcken | 10.6 | 2753 | 0.051 | 6.0 | 11.0 | 0.152 | + | + | nc | - |
| Norsälven Norsbron | 9.0 | 1150 | 0.103 | 6.7 | 14.0 | 0.087 | + | nc | + | - |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Oxundaån Rosendal | 10.3 | 2823 | 2.295 | 7.7 | 64.0 | 1.267 | + | nc | nc | - |
| Upperudsälv. Köpmannebron | 5.5 | 1044 | 0.152 | 6.9 | 10.0 | 0.120 | nc | nc | nc | - |
| Vattholma N Bron | 19.9 | 6450 | 2.312 | 7.3 | 26.0 | 0.645 | nc | nc | + | nc |
| Ölman Hult | 4.4 | 2331 | 0.246 | 6.8 | 69.0 | 0.075 | nc | nc | nc | - |
| *Decrease DOC, increase $pCO_2$* | | | | | | | | | | |
| Akkarjåkkå | 2.0 | 856 | 0.217 | 7.1 | 7.0 | 0.034 | nc | nc | nc | nc |
| Bergmyrbäcken | 6.8 | 1004 | 0.104 | 6.8 | 6.0 | 0.024 | + | + | - | nc |
| *Decrease DOC, no change $pCO_2$* | | | | | | | | | | |
| Viepsajåkkå | 2.6 | 917 | 0.153 | 7.0 | 4.0 | 0.036 | + | nc | - | nc |
| *No change DOC, Increase $pCO_2$* | | | | | | | | | | |
| Dammån | 11.7 | 1496 | 0.021 | 5.8 | 8.0 | 0.098 | nc | nc | nc | - |
| Fyrisån Flottsund | 15.1 | 4011 | 2.563 | 7.5 | 63.0 | 0.874 | nc | - | nc | nc |
| Svedån Sved | 7.8 | 1062 | 0.150 | 6.9 | 9.0 | 0.116 | nc | nc | nc | - |
| Yl Kihlankijoki | 5.1 | 1399 | 0.110 | 6.6 | 13.0 | 0.028 | nc | nc | nc | nc |
| *No change DOC or $pCO_2$* | | | | | | | | | | |
| Borgviksån Borgvik | 7.6 | 1215 | 0.131 | 6.8 | 10.0 | 0.084 | + | nc | nc | - |
| Domneån Utl. Vättern | 16.1 | 2105 | 0.224 | 6.8 | 30.5 | 0.135 | nc | nc | nc | nc |
| Höjdabäcken | 8.9 | 1450 | 0.034 | 6.1 | 7.0 | 0.025 | nc | nc | nc | - |
| Lena Kyrka | 14.3 | 3820 | 3.342 | 7.7 | 47.0 | 0.874 | nc | nc | nc | nc |
| Muddusälven | 5.4 | 896 | 0.189 | 7.0 | 6.0 | 0.031 | nc | nc | - | nc |
| Raurejukke | 2.6 | 844 | 0.211 | 7.2 | 5.0 | 0.043 | nc | nc | - | nc |
| Rokån | 14.7 | 974 | 0.031 | 5.5 | 16.0 | 0.032 | + | nc | nc | - |
| Sävjaån Kuggebro | 16.0 | 4603 | 2.257 | 7.5 | 61.0 | 1.183 | nc | nc | + | nc |

*Table S3.* Median values of dissolved organic carbon (DOC), partial pressure of $CO_2$ ($pCO_2$), alkalinity (Alk.), pH, total phosphorous (Tot-P), and sulfate ($SO_4^{2-}$) and results from Mann Kendall tests for Alk., pH, Tot-P and $SO_4$ for all river mouths included in database 2 (minimum four samples per year for water chemistry parameters during the period 1997 to 2013). A plus refers to a significant increase ($p < 0.05$), minus refers to a significant decrease ($p < 0.05$) and “nc” refers to no change ($p > 0.05$).

| ***Change***<br>River mouth | **DOC (mg L$^{-1}$)** | **$pCO_2$ (µatm)** | **Alk. (mekvL$^{-1}$)** | **pH** | **Tot-P (µg L$^{-1}$)** | **$SO_4^{2-}$ (mekv L$^{-1}$)** | **Alk. (MK)** | **pH. (MK)** | **Tot-P (MK)** | **$SO_4^{2-}$ (MK)** |
|---|---|---|---|---|---|---|---|---|---|---|
| *Increase DOC, no change $pCO_2$* | | | | | | | | | | |
| Kävlingeån Högsmölla | 8.3 | 2652 | 3.238 | 7.8 | 74.0 | 0.322 | + | nc | - | nc |
| Smedjeån V. Mellby | 13.4 | 2671 | 0.353 | 6.9 | 48.5 | 0.872 | nc | nc | nc | nc |
| Stockholm Centralbron | 8.1 | 1130 | 0.930 | 7.6 | 28.0 | 0.345 | + | nc | - | - |
| *Increase DOC, decrease* $_{pCO2}$ | | | | | | | | | | |
| Helgeån Hammarsjön | 16.1 | 1562 | 0.466 | 7.2 | 33.0 | 0.489 | nc | nc | nc | nc |

# Paper II

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# Colored organic matter increases $CO_2$ in meso-eutrophic lake water through altered light climate and acidity

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## Abstract

Many surface waters across the boreal region are browning due to increased concentrations of colored allochthonous dissolved organic carbon (DOC). Browning may stimulate heterotrophic metabolism, may have a shading effect constraining primary production, and may acidify the water leading to decreased pH with a subsequent shift in the carbonate system. All these effects are expected to result in increased lake water carbon dioxide ($CO_2$) concentrations. We tested here these expectations by assessing the effects of both altered allochthonous DOC input and light conditions through shading on lake water $CO_2$ concentrations. We used two mesocosm experiments with water from the meso-eutrophic Lake Erken, Sweden, to determine the relative importance of bacterial activities, primary production, and shifts in the carbonate system on $CO_2$ concentrations. We found that DOC addition and shading resulted in a significant increase in partial pressure of $CO_2$ ($p\text{CO}_2$) in all mesocosms. Surprisingly, there was no relationship between bacterial activities and $p\text{CO}_2$. Instead the experimental reduction of light by DOC and/or shading decreased the photosynthesis to respiration ratio leading to increased $p\text{CO}_2$. Another driving force behind the observed $p\text{CO}_2$ increase was a significant decrease in pH, caused by a decline in photosynthesis and the input of acidic DOC. Considering that colored allochthonous DOC may increase in a warmer and wetter climate, our results could also apply for whole lake ecosystems and $p\text{CO}_2$ may increase in many lakes through a reduction in the rate of photosynthesis and decreased pH.

Lakes play an essential role in the global carbon cycle as they are active sites for carbon transformations (Cole et al. 2007; Battin et al. 2009; Tranvik et al. 2009). Much of the organic and inorganic carbon processed in lakes originates from the surrounding terrestrial ecosystems (i.e., allochthonous). Dissolved organic carbon (DOC) and surface water partial pressure of carbon dioxide ($p\text{CO}_2$) are, on a spatial scale, positively correlated, which has been suggested to be due to in-lake mineralization of DOC (Hope et al. 1996; Sobek et al. 2003; Lapierre and del Giorgio 2012). The external carbon inputs and their mineralization in lakes largely contribute to the widespread supersaturation of carbon dioxide ($CO_2$) in lake surface waters and to its subsequent evasion to the atmosphere (Jonsson et al. 2007; Lapierre and del Giorgio 2012). However, there are also other processes, such as import of inorganic carbon (Weyhenmeyer et al. 2015), primary production (Balmer and Downing 2011), and distributions within the carbonate system (Lazzarino et al. 2009), which can affect $CO_2$ concentrations in surface waters.

Over the past two decades, increasing DOC concentrations, mostly derived from the terrestrial environment, have been observed in surface waters across large parts of the boreal region (Evans et al. 2005; Monteith et al. 2007; Filella and Rodriguez-Murillo 2014). Increasing DOC inputs can have, at least, three effects on $p\text{CO}_2$ (Fig. 1). First, allochthonous DOC may be readily available and degraded by microorganisms and subsequently converted into $CO_2$ in freshwaters. Hence, increased DOC input may stimulate $CO_2$ production by heterotrophs (Lennon 2004; McCallister and del Giorgio 2012; Guillemette et al. 2013). Indeed, studies have shown that bacterial respiration of allochthonous DOC is one of the key drivers of net heterotrophy in high-DOC lakes (Tranvik 1992; del Giorgio and Peters 1994). Also, heterotrophic bacteria may be more efficient at taking up nutrients than phytoplankton under high-DOC conditions leading to repressed phytoplankton production and subsequent decrease in phytoplankton $CO_2$ uptake (Jansson et al. 2007; Ask et al. 2009).

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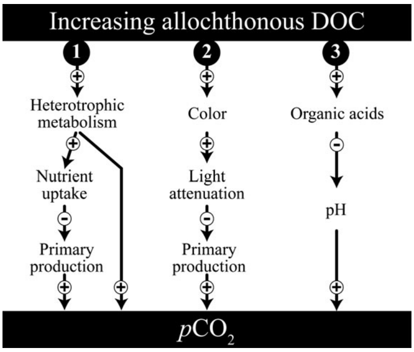

**Fig. 1.** Conceptual model of three possible main effects of increased allochthonous DOC on $pCO_2$ concentrations in lake water discussed in this study. Plus indicates a positive effect, and minus indicates a negative effect.

Second, allochthonous DOC generally contains large proportions of humic-like components with high amounts of aromatic structures, which give water a brownish color. These chromophoric aromatic structures are effective at absorbing photosynthetically active radiation (PAR); hence, allochthonous DOC may have a strong positive effect on light attenuation (Jones 1992; Pace and Cole 2002). This increased light attenuation can constrain primary production as a large fraction of PAR is absorbed by the DOC rather than by the photoautotrophs (Jones 1992). For instance, Thrane et al. (2014) found that chromophoric DOC absorbed, on average, more than 50% of PAR in the majority of their 75 Northern European study lakes, which spanned a large DOC range. Consequently, shading may further enhance net heterotrophy of high-DOC lakes (Cole et al. 2000).

Third, allochthonous DOC is partly composed of organic acids; hence, DOC can have an acidifying effect and lower the pH. A decreased pH could subsequently lead to an increase in free $CO_2$ as the distribution within the carbonate system shifts and the proportion among free $CO_2$, bicarbonate ($HCO_3^-$), and carbonate ($CO_3^{2-}$) changes. To underline the importance of DOC as a regulator of pH, increased DOC is one of the major factors contributing to spring flood pH decline in boreal streams of northern Sweden (Laudon and Bishop 1999; Laudon et al. 2001). Conversely, elevated $pCO_2$ levels due to enhanced bacterial respiration or decreased primary production could also lead to decreased pH. Decreasing pH trends were observed in clear water lakes in northern Wisconsin during winter, when primary production was low, and it was suggested that this was due to build-up of under ice $pCO_2$ levels (Kratz et al. 1987).

All three effects of increasing allochthonous DOC inputs could result in increased $CO_2$ concentrations (Fig. 1), yet the relative importance of these three effects are unknown. The knowledge gap is particularly apparent for eutrophic lakes, as most studies on carbon processing in inland waters have been performed in boreal oligotrophic lakes (Sobek et al. 2003; Alin and Johnson 2007; Ask et al. 2009). However, in eutrophic lakes, $CO_2$ sources may be outweighed by fixation of $CO_2$ by primary production, making them net autotrophic and $CO_2$ sinks, particularly during summer (Balmer and Downing 2011). Increased DOC input could potentially switch a eutrophic lake from being a net sink to become a net source of atmospheric $CO_2$. In oligotrophic lakes, it has been shown that moderate DOC input can have a positive effect on primary production due to enhanced nutrient availability (Seekell et al. 2015*a*). However, since eutrophic lakes are not as nutrient limited, the negative effect of allochthonous DOC input on biological $CO_2$ uptake due to increased light attenuation may be greater than the potential positive effect due to increased nutrient availability in eutrophic lakes, warranting further investigations in those ecosystems. Here, we used two mesocosm experiments with water from the meso-eutrophic Lake Erken, Sweden, to test the effect of allochthonous DOC input and altered light conditions through shading on $CO_2$ production. Furthermore, we aimed to determine the relative importance of bacterial activities, primary production, and shifts in the carbonate system on lake water $CO_2$ concentrations.

We propose that increased allochthonous DOC input will lead to enhanced $CO_2$ concentrations, which, in meso-eutrophic lakes, will result in a reversal from net uptake to net release of $CO_2$ (Fig. 1). We tested three hypotheses: (1) allochthonous DOC input stimulates bacterial activities, thus resulting in increased $CO_2$ concentrations; (2) increased light attenuation by allochthonous DOC hampers $CO_2$ uptake by autotrophs; and (3) an increase in allochthonous DOC decreases pH, causing a shift in the carbonate system, leading to increased $CO_2$ concentrations.

## Methods

### Field site and experimental mesocosms

We conducted two mesocosm experiments with water from Lake Erken (59°51′N, 18°36′E), a meso-eutrophic dimictic lake in eastern Sweden, with a lake surface area of 24.2 $km^2$, a mean depth of 9 m, and maximum depth of 21 m (Pettersson 1990). The mesocosms consisted of high-density polyethylene, white opaque, open top cylinders, 2 m deep with a diameter of 0.92–1.01 m. A total of 20 mesocosms were held on a fixed and floating wooden jetty, and positioned 10–20 m from the shore sitting approximately 0.6 m above the lake bottom (Fig. 2).

### Experimental designs

The mesocosm experiments were part of a larger study which aimed to investigate the influence of addition of allochthonous DOC and shading on biogeochemical processes and the food web. Two crossed full factorial design experiments

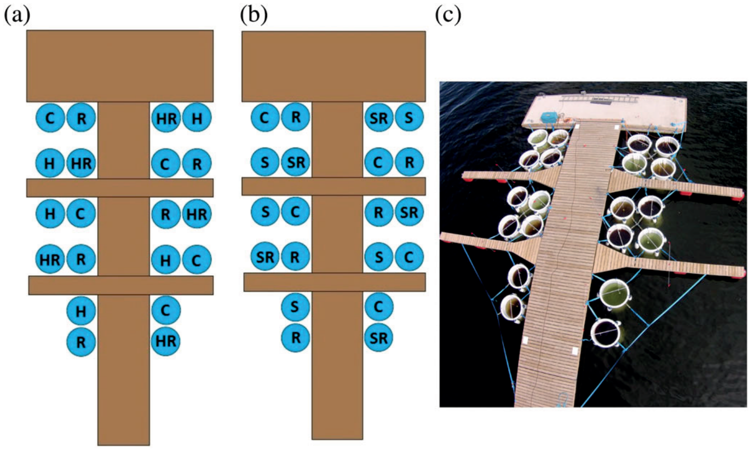

**Fig. 2.** Schematic of the experimental setup for **(a)** Experiment A and **(b)** Experiment B and **(c)** an aerial photograph of the mesocosm Experiment A. The circles in **(a)** and **(b)** represent the mesocosms and illustrate the different treatments. Treatments for Experiment A: (1) addition of reverse osmosis concentrate of DOC from humic stream water (R); (2) DOC from HuminFeed® (H); (3) a mix of DOC from reverse osmosis and HuminFeed (HR); and (4) no addition of DOC (C). Treatments for Experiment B: (1) addition of reverse osmosis concentrate of DOC from humic stream water (R); (2) covering of the outside of the mesocosms using black polyethylene film and on top using black nylon chiffon fabric, to generate a shading effect (S); (3) addition of DOC and shading (SR); and (4) no addition of DOC or shading (C).

with five replicates of four treatments were run between 15 June and 13 July (Experiment A) and between 10 August and 07 September (Experiment B) 2016. The mesocosms were filled up, at the commencement of the experimental periods (i.e., Day 0), to 1.65 m with filtered (through 200 $\mu$m to remove large plankton, algal colonies, and fish) lake water, leading to volumes between 1000 and 1300 liters. The mesocosms were cleaned between the two experiments and refilled with lake water for Experiment B. Additional zooplankton were added from assembled open-water zooplankton tows collected in Lake Erken for controlled studies of the effect of DOC addition on the food web (separate study). In the first experiment (A), we added approximately 14 individuals of large zooplankton (large Cladocerans including Daphnia and Copepods) from natural zooplankton communities of Lake Erken per liter of lake water to all mesocosms. In the second experiment (B), approximately 34 individuals of large zooplankton (Cyclopids and Cladocerans) per liter were added. The difference in zooplankton addition was due to the difference in zooplankton catch for the same effort, thus the increased zooplankton density in Experiment B mimics natural lake conditions later in summer. During the period between the two experiments, there was also high phytoplankton production in Lake Erken, likely due to increased water temperature, leading to lower $p\text{CO}_2$ levels and higher chlorophyll *a* (Chl *a*) concentrations at the commencement of Experiment B. All mesocosms were mixed manually twice daily throughout the duration of both experiments to avoid stratification, using a disc mounted on a shaft, a method shown to minimize impact on plankton biomass (Striebel et al. 2013).

In Experiment A, we manipulated the amount of allochthonous DOC. Four treatments were set up: (1) addition of DOC concentrated from a humic stream draining a forested wetland (59°92′N, 17°34′E), with a DOC concentration of 37.7 (± 0.49 SE) mg $L^{-1}$; the stream water was filtered (0.2 $\mu$m) with a submersible pump through 10-in. filter cartridges, and DOC was concentrated by reverse osmosis using a Real Soft PROS/2S unit as described by Serkiz and Perdue (1990), to a final concentration of approximately 800 mg $L^{-1}$ (i.e., reverse osmosis); (2) DOC from HuminFeed® (Humintech, GmbH), an alkaline extract of Leonardite, which has previously been used as a humic matter source in aquatic studies (Heinze et al. 2012; Rasconi et al. 2015) (i.e., HuminFeed); (3) a mix of reverse osmosis concentrate and HuminFeed adding equal amounts of DOC from the two sources (i.e., mixed); and (4) no addition of DOC (i.e., control) (Fig. 2a). For initial DOC concentrations after DOC manipulations, see Table 1. The reverse osmosis concentrate was stored at dark at 4°C until the beginning of the experiment (for 21–77 d). The reverse osmosis concentrate and the HuminFeed (dissolved in MilliQ before addition on site) were added to the mesocosms to increase the in situ DOC concentration by about 5 mg $L^{-1}$ in the reverse osmosis and the HuminFeed treatments and by about 10 mg $L^{-1}$ in the mixed treatment. These DOC concentrations were chosen to represent natural DOC levels in boreal lakes, while also have a notable effect on $p\text{CO}_2$ (Sobek et al. 2003) as well as being

**Table 1.** Concentrations of DOC, BCP, Chl *a*, pH, total nitrogen (N), and total phosphorous (P) on the first sampling day (the day after DOC additions) of two mesocosm experiments with DOC additions and/or changed light climate through shading. For all variables, data are presented as mean values ± standard error (SE). Treatments for Experiment A were: no addition of DOC (control), addition of DOC from concentrated humic stream water using reverse osmosis (reverse osmosis), addition of DOC from HuminFeed (HuminFeed), and a mix of DOC from reverse osmosis concentrate and HuminFeed (mixed). Treatments for Experiment B were: no addition of DOC (control), addition of DOC from concentrated humic stream water using reverse osmosis (reverse osmosis), increased light attenuation via shading using shading cloth (shading), and both DOC addition from reverse osmosis concentrate and shading (DOC-shading).

| | **Treatments** | | | |
|---|---|---|---|---|
| **Experiment A** | **Control** | **Reverse osmosis** | **HuminFeed** | **Mixed** |
| DOC (mg $L^{-1}$) | 13.0±0.05 | 18.1±0.10 | 18.4±0.06 | 23.5±0.05 |
| BCP ($\mu$g C $L^{-1}$ $h^{-1}$) | 0.50±0.08 | 0.52±0.05 | 0.48±0.09 | 0.47±0.07 |
| Chl *a* ($\mu$g $L^{-1}$) | 2.21±0.82 | 2.45±1.56 | 1.83±0.97 | 3.06±1.14 |
| pH | 8.28±0.07 | 8.18±0.07 | 8.29±0.03 | 8.17±0.06 |
| Total N ($\mu$g $L^{-1}$) | 613±11.80 | 743±7.70 | 860±90.90 | 905±31.80 |
| Total P ($\mu$g $L^{-1}$) | 14.5±0.51 | 15.5±0.46 | 17.1±1.88 | 17.2±0.76 |
| **Experiment B** | **Control** | **Reverse osmosis** | **Shading** | **DOC-shading** |
| DOC (mg $L^{-1}$) | 12.0±0.01 | 16.4±0.08 | 12.0±0.05 | 16.4±0.04 |
| BCP ($\mu$g C $L^{-1}$ $h^{-1}$) | 0.92±0.13 | 1.13±0.13 | 0.95±0.15 | 0.93±0.13 |
| Chl *a* ($\mu$g $L^{-1}$) | 13.7±4.68 | 15.6±2.89 | 14.5±2.69 | 14.6±2.58 |
| pH | 8.69±0.05 | 8.59±0.04 | 8.64±0.02 | 8.53±0.05 |
| Total N ($\mu$g $L^{-1}$) | 711±35.26 | 811±4.86 | 692±4.94 | 798±28.60 |
| Total P ($\mu$g $L^{-1}$) | 21.5±1.14 | 22.9±1.20 | 22.8±1.26 | 22.4±1.02 |

logistically feasible. We expected a subsidy effect (in terms of energy release through oxidation of terrestrial organic material) from the reverse osmosis concentrate whereas the HuminFeed was assumed to predominantly affect the light climate. HuminFeed has previously been used to reduce light climate in a mesocosm experiment testing the effect of changed light climate on community resilience and stability (Hillebrand et al. 2017). The reverse osmosis, HuminFeed, and mixed treatments increased water color by 300%, 1400%, and 1700%, respectively, compared to the control. Water color was measured as absorbance at 420 nm in a 5-cm quartz cuvette using a Lambda 40 UV/VIS spectrophotometer (Perkin Elmer) after filtering through a glass microfiber filter (approximately 1.2$\mu$m pore size, Grade GF/C, Whatman$^{TF}$, GE Healthcare).

In Experiment B, we again manipulated the DOC input by addition of reverse osmosis concentrate. However, we covered the top of mesocosms with a black mesh and the outside with black plastic to alter and maintain natural DOC concentrations, rather than using HuminFeed. Again, four treatments were set up (for initial DOC concentrations after DOC manipulations see Table 1): (1) addition of reverse osmosis concentrate of DOC from the same humic stream water as in Experiment A (i.e., reverse osmosis); (2) covering of the outside of the mesocosms using black polyethylene film and on top using black nylon chiffon fabric, to generate a shading effect (i.e., shading); (3) addition of reverse osmosis concentrate of DOC and shading (i.e., DOC-shading); and (4) no DOC addition or shading (i.e., control) (Fig. 2b). In the treatments with added DOC, that is, the reverse osmosis and DOC-shading treatments, water color increased by 220% and 210%, respectively, compared to the control. For both experiments, DOC was added only once, at Day 0, and the first measurements were conducted the following day. The black nylon chiffon fabric reduced PAR at the water surface by 60.7% (± 0.8 SE). After 1 week, three young-of-the-year perch (*Perca fluviatilis L.*) of approximately the same biomass (4.37 g ± 0.14 SE) were added to each of the mesocosms in Experiment B, to investigate the effect of DOC addition on the food web (separate study). The fish were caught by seine netting from Lake Erken (permit C59/15, authorized by the Uppsala board of animal ethics).

Starting DOC concentrations ranged between 13.0 and 23.5 mg $L^{-1}$ for Experiment A and between 12.0 and 16.4 mg $L^{-1}$ for Experiment B (Table 1). Chemical conditions for the lake water observed through the monitoring in Lake Erken are equivalent to conditions in the control treatment at the commencement of the experiments (as reported in Table 1).

## Measurements

Manual sampling of $pCO_2$ was conducted weekly, between 10:00 and 14:00 (Central European Summer Time [CEST]), using the headspace equilibrium method (described in Sobek et al. [2003]) as modified by Kokic et al. (2015). From each mesocosm, 30 mL of water was taken with a syringe right below the surface followed by adding 30 mL of ambient air to create a headspace. Initially, triplicates were taken to test the reproducibility of the measurements. They varied on average by 1.5%; hence, single sample was sufficient to provide a good estimate of the $pCO_2$ in the surface water of each mesocosm. Equilibrated gas samples were analyzed on a portable infrared gas analyzer (IRGA, EGM-4) within 5 min of sampling. The $pCO_2$ was calculated according to Weiss (1974) using the appropriate Henry's constant after correcting for temperature, atmospheric pressure, and the amount of ambient air $CO_2$ added. Sampling for $pCO_2$ was performed first at each sampling occasion to avoid outgassing due to turbulence and disturbance from water sampling. Water samples for dissolved inorganic carbon (DIC) analyses were taken directly after sampling for $pCO_2$ at each sampling occasion. Aliquots of 17 mL were injected into gas tight glass vials leaving no headspace and later analyzed on a Sievers 900 TOC analyzer (GE Analytical Instruments).

Water samples of 15–18 liters per mesocosm were collected weekly, between 11:00 and 15:00 (CEST), using a tube sampler (1.5 m long, ~ 3 liter volume). Water was sampled from five to six different places throughout the water column in the mesocosms and pooled for subsampling to minimize stochasticity. Aliquots of 50 mL were filtered (approximately 0.7 $\mu$m effective pore size, grade GF/F, Whatman$^{TF}$, GE Healthcare) and analyzed for DOC concentration using a Sievers M9 TOC analyzer (GE Analytical Instruments).

Four aliquots of 1.7 mL (three replicates and one blank) of pooled water from each mesocosm were used to determine bacterial carbon production (BCP) via incorporation of $^3$H-leucine into the protein fraction using the protocol of Smith and Azam (1992). The samples were incubated at in situ temperatures at a final leucine concentration of 100 $\mu$mol L$^{-1}$ for 1 h in the dark. Additionally, 580 mL of water was taken from the pooled samples for Chl *a* analysis. Samples were vacuum filtrated through a glass microfiber filter (approximately 1.2 $\mu$m effective pore size, Grade GF/C, Whatman$^{TF}$, GE Healthcare), frozen at −20°C in the dark until further analysis. Following ethanol extraction (95%), samples were analyzed on a Lambda 40 UV/VIS spectrophotometer (Perkin Elmer) in a 1-cm cuvette at the wavelengths of 665 and 750 nm following the ISO 10260 standard technique (e.g., Strombeck and Pierson 2001; Kutser et al. 2005). Furthermore, total nitrogen (N) and total phosphorous (P) were analyzed on unfiltered pooled water samples on a SEAL AutoAnalyzer 3HR (Seal Analytical).

We measured pH between 15:00 and 17:00 (CEST) directly in the mesocosms on a weekly basis using a YSI multiprobe (EXO2 Multiparameter Sonde, YSI). As it is difficult to disentangle whether pH drives $CO_2$ or $CO_2$ drives pH, we quantified a $CO_2$ effect on pH by accounting for potential pH changes through bacterial mineralization and primary production. In addition to pH, light was measured weekly at seven depths in each mesocosm using a handheld light meter (Li-Cor LI-A, LI-COR) equipped with a light sensor (Li-Cor, LI-192 SA Underwater Quantum, LI-COR). Based on the light measurements, we calculated the vertical light attenuation coefficient for PAR ($K_d$) for each mesocosm. Using $K_d$, we could calculate the average light availability (meanPAR) throughout the entire 1.65 m water column for each mesocosm using an equation modified from Minor et al. (2016)

$$\text{meanPAR} = \left(\text{PAR}_{(Z=0)}\right)\left(K_d z\right)^{-1}\left(1 - e^{-K_d z}\right)$$

where $\text{PAR}_{(Z=0)}$ is the light intensity immediately at the surface at the depth of 0 m, $K_d$ is the vertical light attenuation coefficient for PAR and $z$ is the depth of the mesocosms (i.e., 1.65 m). The $\text{PAR}_{(Z=0)}$ was set to 100% for all mesocosms without shading cloth, whereas for the shading and DOC-shading treatments $\text{PAR}_{(Z=0)}$ was set to 39.3% to account for the reduction of incoming PAR at the water surface due to the black fabric covering those treatments.

### Statistics

We performed the mixed-effect model repeated measures analyses of variances (RM-ANOVA) to test for differences in $pCO_2$, BCP, Chl *a*, pH, and light climate between treatments, with mesocosm ID as a random factor. Additionally, we performed one-way ANOVAs on the measurements of $pCO_2$, BCP, Chl *a*, and pH taken on the first day of the experiments to evaluate the direct effect of DOC on these parameters. Where significant differences were detected, multiple comparisons of means within treatment groups were performed using the post hoc Tukey Test. Statistical analyses were performed in the software package JMP version 13.0.0 (SAS Institute 2013) or R Version 1.0.136 (R-Development-Core-Team 2010). Significance was set at an alpha level of 0.05 for all tests. Data were tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Bartlett's test.

## *Results*

### Changes in light climate

In Experiment A, additions of DOC significantly reduced the light throughout the water column in the mesocosms, with the DOC from HuminFeed having a stronger effect on light attenuation than DOC from the reverse osmosis concentrate (Table 2). However, there was no significant difference in $K_d$ or meanPAR between the HuminFeed and mixed treatments (Table 2). Approximately 35% of incoming light was available for photosynthesis throughout the water columns of the control treatments, whereas in the HuminFeed and mixed treatments, the light availability was reduced to merely 12% of incoming PAR (Table 2).

In Experiment B, addition of black mesh fabric on top of the mesocosms and black plastic around the outside resulted in significantly less light being available for photosynthesis throughout the water column compared to the control and the reverse osmosis treatments (Table 2). Only 10% and 11%

**Table 2.** Vertical light attenuation coefficient for PAR ($K_d$) ± SE and the average PAR (meanPAR) ± SE throughout the water columns in two mesocosm experiments. Letters in parentheses refer to results from Tukey's post hoc test following one-way ANOVAs. Treatments not connected by the same letter are significant different at 0.05. See legend of Table 1 for explanation of treatments.

| Treatments | $K_d$ ($m^{-1}$) | MeanPAR ($\mu$mol photons $m^{-2}$ $s^{-1}$) |
|---|---|---|
| *Experiment A* | | |
| Control | 1.68±0.09 (A) | 35.1±1.49 (A) |
| Reverse osmosis | 2.40±0.11 (B) | 25.8±1.07 (B) |
| HuminFeed | 4.90±0.12 (C) | 12.54±0.31 (C) |
| Mixed | 4.99±0.15 (C) | 12.46±0.45 (C) |
| *Experiment B* | | |
| Control | 1.70±0.10 (A) | 35.9±2.75 (A) |
| Reverse osmosis | 2.15±0.11 (AB) | 28.6±1.35 (A) |
| Shading | 2.20±0.13 (AB) | 11.4±0.62 (B) |
| DOC-shading | 2.56±0.15 (B) | 10.0±0.67 (B) |

of incoming PAR was available to phytoplankton in the shading and DOC-shading treatments, respectively (Table 2). Contrary to what was observed in Experiment A, there was no difference in meanPAR between the control and the reverse osmosis treatments in Experiment B (Table 2).

### Effects of DOC input and changed light climate on $pCO_2$

In Experiment A, addition of DOC from reverse osmosis concentrate resulted in a rapid increase in $pCO_2$ with significantly higher $pCO_2$ in the reverse osmosis and mixed treatments than in the control and HuminFeed treatments ($F_{3,16} = 29.6$, $p < 0.0001$). In the control, $pCO_2$ appeared to steadily decrease throughout the experiment, and by Week 2, the system had switched from being oversaturated to being undersaturated in $CO_2$ relative to the atmosphere (Fig. 3a). In the mixed treatment, $pCO_2$ appeared to increase considerably during the first week, but after 2 weeks, $pCO_2$ started to decline (Fig. 3a). From Week 1 onward, the highest $pCO_2$ was observed when both HuminFeed and reverse osmosis concentrate were added (mixed treatment), while there was no difference in $pCO_2$ between the reverse osmosis and HuminFeed treatments (Fig. 3a; Tables 3, 4).

On Day 1 in Experiment B, there were already significantly higher $pCO_2$ in the DOC addition treatments (i.e., reverse osmosis and DOC-shading) relative to the control ($F_{3,16} = 13.6$, $p = 0.0001$). During the first 2 weeks of the experiment, $pCO_2$ in the DOC-shading treatment appeared to increase steadily, and after 2 weeks, the system had switched from being a sink of $CO_2$ to being a source of $CO_2$ (Fig. 3b). However, by Week 3, $pCO_2$ levels were again below atmospheric concentrations, while all other treatments were $CO_2$ sinks during the entire Experiment B. Throughout the experiment, highest $pCO_2$ was observed in the DOC-shading combined treatment, which had significantly higher $pCO_2$ than all other treatments (Tables 3, 4). No difference in $pCO_2$ was observed between the reverse osmosis and the shading treatments; however, these treatments had significantly higher $pCO_2$ than the control (Tables 3, 4).

### Effects of DOC input and changed light climate on BCP, Chl *a*, and pH

In Experiment A, BCP ranged between 0.50 and 0.96 $\mu$g C $L^{-1}$ $h^{-1}$; however, there was no significant difference between treatments on Day 1 ($F_{3,16} = 0.48$, $p = 0.703$) or throughout the duration of the experiment (Fig. 3c; Table 3). As with BCP, there was no difference in Chl *a* between treatments on Day 1 ($F_{3,16} = 0.99$, $p = 0.423$). Throughout the duration of the experiment, we observed significant differences in Chl *a* between treatments, although this was not affected by time (Tables 3, 4). Chl *a* concentrations varied between 0.7 and 3.5 $\mu$g $L^{-1}$ and were significantly higher in the HuminFeed and mixed treatments than in the control and reverse osmosis treatments (Fig. 3e; Table 4). On Day 1, there was already significantly lower pH in the reverse osmosis and mixed treatments compared to the control and HuminFeed treatments ($F_{3,16} = 4.04$, $p = 0.026$). The pH ranged from 8.0 to 8.4 throughout the experiment and differed significantly between treatments; however, this was dependent on time (Fig. 3g; Table 3). The control treatment had significantly higher pH than all other treatments and lowest pH was observed in the mixed treatment (Tables 3, 4).

In Experiment B, there was no difference in BCP between treatments on Day 1 ($F_{3,16} = 2.64$, $p = 0.085$). BCP ranged from 0.67 to 0.88 $\mu$g C $L^{-1}$ $h^{-1}$, and as in Experiment A, there was no significant difference in BCP between treatments (Fig. 3d; Table 3). Again, there was no difference in Chl *a* between treatments on Day 1 ($F_{3,16} = 0.26$, $p = 0.854$); however, throughout the experiment, significant differences in Chl *a* between treatments were observed (Table 3). Chl *a* concentrations ranged between 5.7 and 20.0 $\mu$g $L^{-1}$, and contrary to our expectations, we found highest Chl *a* in the DOC-shading treatment (Fig. 3f; Table 4). As in Experiment A, there was a significant difference in pH already on Day 1 in Experiment B with significantly lower pH in the reverse osmosis and the DOC-shading treatments (i.e., all treatments with reverse osmosis DOC addition) compared to the other two treatments ($F_{3,16} = 13.3$, $p = 0.0001$). The pH was generally higher in Experiment B, ranging from 8.4 to 8.9 (Fig. 3h). There was a significant difference in pH between treatments with the lowest pH observed in the DOC-shading treatment throughout the experiment (Tables 3, 4).

## Discussion

Our study demonstrates that browning due to increased input of colored allochthonous DOC can increase $CO_2$

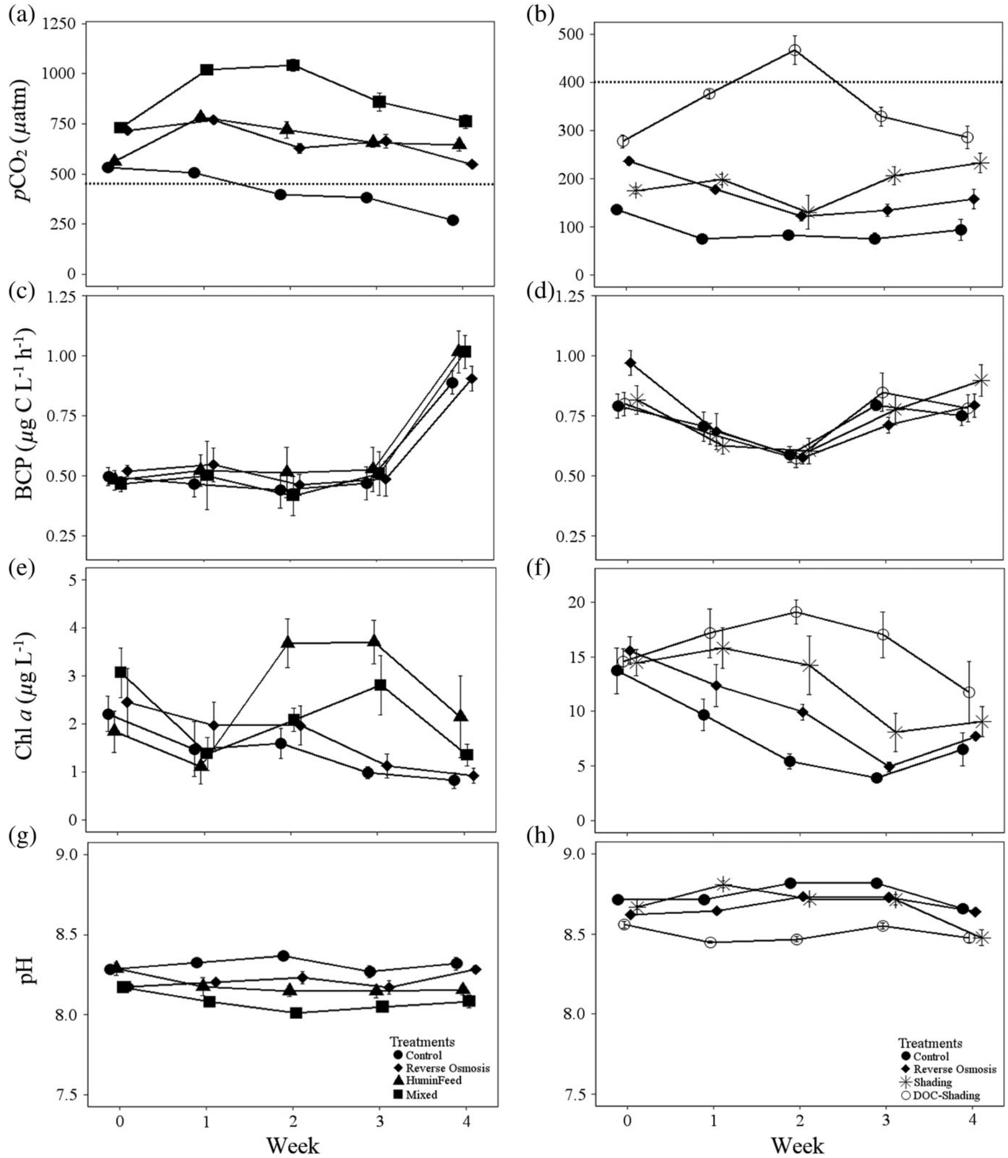

**Fig. 3.** Weekly variations in water chemistry under four treatments (see legends in figure) during two 4-week mesocosm experiments with DOC additions and/or changed light climate through shading. The left panel shows the results of Experiment A and the right panel of Experiment B. Panels **(a and b)** refers to the $pCO_2$, panels **(c and d)** to the BCP, panels **(e and f)** to Chl *a*, and panels **(g and h)** to pH. Values are mean (± SE, $n = 5$). Error bars are sometimes so small that they are hidden within the symbols. The dotted line in panels **(a and b)** represents the partitioning of oversaturation and undersaturation of $CO_2$ relative to the atmosphere.

**Table 3.** F-statistics and *p* values from mixed-effect model RM-ANOVA to test for differences in $pCO_2$, BCP, Chl *a*, and pH observed in two 4-week mesocosm experiments.

| | F | | | | *p* | | | |
|---|---|---|---|---|---|---|---|---|
| | $pCO_2$ | BCP | Chl *a* | pH | $pCO_2$ | BCP | Chl *a* | pH |
| *Experiment A* | | | | | | | | |
| Treatment | 36.2 | 0.2 | 5.5 | 32.0 | <0.0001* | 0.8906 | 0.0086* | <0.0001* |
| Time | 17.7 | 125.3 | 3.1 | 0.5 | 0.0007* | <0.0001* | 0.0965 | 0.5013 |
| Treatment × time | 5.5 | 1.5 | 3.0 | 25.8 | 0.0087* | 0.2573 | 0.0606 | <0.0001* |
| *Experiment B* | | | | | | | | |
| Treatment | 80.1 | 0.2 | 20.0 | 35.6 | 0.8311 | <0.0001* | <0.0001* | <0.0001* |
| Time | 2.2 | 0.1 | 39.6 | 1.3 | <0.0001* | <0.0001* | 0.0009* | <0.0001* |
| Treatment × time | 3.8 | 1.4 | 2.0 | 2.7 | 0.2521 | 0.0049 | 0.0477 | 0.0016 |

*Significant at $p > 0.05$.

**Table 4.** Results from Tukey's post hoc analyses following mixed-effect model RM-ANOVA, where significant differences were detected. Treatments not connected by the same letter are significantly different at a significance level of 0.05. See legend of Table 1 for explanation of treatments.

| | $pCO_2$ | Chl *a* | pH |
|---|---|---|---|
| *Experiment A* | | | |
| Control | A | A | A |
| Reverse osmosis | B | AB | B |
| HuminFeed | B | B | B |
| Mixed | C | B | C |
| *Experiment B* | | | |
| Control | A | A | A |
| Reverse osmosis | B | AB | A |
| Shading | B | B | A |
| DOC-shading | C | C | B |

concentrations in lake water, which is in agreement with previous research (Lennon 2004; Guillemette et al. 2013). Surprisingly, there was no difference in BCP between treatments in either of the experiments; hence, we found no support for our Hypothesis 1 (Fig. 1) that increased allochthonous DOC input stimulates bacterial activities (Table 3; Fig. 3). In Experiment A, average BCP was 0.55 and 0.58 $\mu$g C $L^{-1}$ $h^{-1}$ for the control and the mixed treatment, respectively. This is equivalent to an average of 0.42 to 0.45 g carbon assimilated by bacteria per mesocosm in the control and mixed treatments, respectively, for the entire experiment (based on mean values for each treatment multiplied by experimental time and volume of mesocosms). A bacterial growth efficiency of 30% would correspond to a bacterial mineralization of, on average, 1.84 $\mu$g C $L^{-1}$ $h^{-1}$ for the control treatment and 1.94 $\mu$g C $L^{-1}$ $h^{-1}$ for the mixed treatment. We calculated a bacterial carbon respiration of 1.00 and 1.05 g per mesocosm, for the control and mixed treatments, respectively, for the entire experiment. Corresponding bacterial respiration for Experiment B were 1.31 and 1.33 g carbon per mesocosm for the control and DOC-shading treatments, respectively. However, in Experiment A, we saw a loss in DOC of 0.52 g in the control and of 2.36 g in the mixed treatment, while in Experiment B, DOC loss was 0.46 and 1.00 g carbon per mesocosm in the control and DOC-shading treatments, respectively. Although a part of the added DOC may have been consumed by the bacteria and accounted for some of the observed $pCO_2$ increase, carbon consumptions were equal in all treatments. Consequently, part of the carbon consumed by bacteria must have been sustained by autochthonous sources (i.e., primary production).

Closely related to our study, Lennon (2004) demonstrated in a mesocosm experiment that bacterial production increased significantly with DOC enrichment and argued that microbial metabolism of the terrestrial subsidy was responsible for the observed increase in $CO_2$. Although inorganic nutrients were positively correlated with subsidy supply in the study by Lennon (2004), they were not responsible for the increased $CO_2$ as inputs of N and P alone did not account for increasing $CO_2$, suggesting that $CO_2$ was less responsive to inorganic nutrients than organic material. Lennon (2004) did, however, increase the DOC concentration in the treatments by 153%, whereas we increased the DOC concentrations by 39–80% in Experiment A and by 36% in Experiment B. Perhaps we would have seen a response in bacterial production if we had added more DOC or if the experiment had been performed in a lake with lower initial DOC concentrations. Also, the mesocosm experiment by Lennon (2004) was only running for 10 d and we cannot rule out that we might have seen a change in bacterial activities if we had measured the bacterial activity more often during the first week. It has been shown that labile carbon can be quickly consumed by bacteria during the first week, resulting in an increase in $pCO_2$, and leaving the more recalcitrant DOC which in another experiment had caused $pCO_2$ to stabilize or decrease (Guillemette and del Giorgio 2011). Our reverse osmosis concentrate was stored for up to 77 d before being used for the experiments, and although it was stored cold and dark, the most labile carbon may have already been consumed when the DOC was added to the mesocosms. Furthermore, Lapierre et al. (2013) showed that there was no relationship between colored organic matter and the bioavailability of DOC in their study waters as the DOC pool in browner waters were as biologically available as the DOC in clear water. Addition of reverse osmosis DOC in our study may not have increased the amount of bioavailable DOC enough in the mesocosms to show a response in bacterial activities. The lack of relationship between bacterial production and $p\text{CO}_2$ in our experiments suggests that there were other factors than heterotrophic respiration that mainly controlled $CO_2$ concentrations.

Another factor driving $p\text{CO}_2$ in our experiments might be primary production. We expected to see a negative relationship between Chl *a* and $p\text{CO}_2$, but surprisingly, in both experiments we found the highest Chl *a* concentrations in the darkest treatments, that is, mixed and DOC-shading. A possible explanation for the high Chl *a* concentrations in the darker treatments could be that the photoautotrophs produced more chlorophyll to compensate for the decreased light availability (Richardson et al. 1983). Phytoplankton increase their Chl *a* to biomass ratio when light availability decreases (Enberg et al. 2015). Accordingly, we found the highest Chl *a* per phytoplankton individual and highest Chl *a* per phytoplankton biovolume in the DOC-shading treatment (Supporting Information Fig. S4; Supporting Information Tables S1, S2). Additionally, in Experiment A, we found no difference in phytoplankton biovolume between treatments, and in Experiment B, biovolume was significantly lower in the DOC-shading than all other treatments (Supporting Information Fig. S4; Supporting Information Tables S1, S2). The reduction of light availability in the darker treatments (i.e., HuminFeed, mixed, shading, and DOC-shading) may have led to a reduction in the $CO_2$ bio-uptake by the phytoplankton which subsequently led to increased $p\text{CO}_2$ in these treatments, thus supporting our Hypothesis 2 (Fig. 1).

Increasing DOC may to some extent stimulate primary production due to nutrients associated with the allochthonous organic matter, but at higher concentrations, the shading effect of colored organic matter has been shown to dominate (Seekell et al. 2015*b*). Likewise, Kelly et al. (2018) generated a model to assess how simultaneous changes in DOC and nutrients could impact lake primary production and found that both gross primary production and algal biomass increased with increasing DOC up to a threshold. Upon addition of reverse osmosis concentrate, the total P and total N increased by 6% and 21%, respectively, in Experiment A. Corresponding numbers for HuminFeed were 17% and 40%, respectively. Nutrient enhancement due to DOC additions was almost twice as high in the HuminFeed compared to the reverse osmosis treatment, but this difference was not significant (Supporting Information Fig. S1; Supporting Information Table S1). This could potentially explain the higher Chl *a* concentrations in the HuminFeed treatment, yet this was not reflected in the $p\text{CO}_2$. Hence, a change in the light climate is a more likely explanation to the increased Chl *a* concentrations. Addition of reverse osmosis concentrate in Experiment B led to increased total P and total N concentrations by 7% and 14%, respectively. However, Chl *a* was higher in the shaded treatment where no DOC, hence no nutrients, had been added. Consequently, the DOC-mediated nutrient effect on primary production can be assumed to be of minor importance.

A third factor influencing $p\text{CO}_2$ in our experiments could be changes in pH. Overall, DOC addition decreased the pH, thus supporting our Hypothesis 3 (Fig. 1) that an acidifying effect of DOC can decrease pH and subsequently increase $p\text{CO}_2$. This pattern has been confirmed in a study investigating the $p\text{CO}_2$ in surface waters of more than 900 Florida lakes where pH was found to be the best predictor of $p\text{CO}_2$ (Lazzarino et al. 2009). The pH of surface waters is essentially controlled by the ratios of $CO_2 : HCO_3^- : CO_3^{2-}$, and if acids, such as the humic acids in DOC, are added to water, the equilibrium will be shifted leading to increased amounts of free $CO_2$ relative to $HCO_3^-$ and $CO_3^{2-}$ (Cole and Prairie 2009). There was a clear effect of the added DOC from the reverse osmosis concentrate, as all treatments with this DOC addition showed significantly lower pH on the first day of the experiments than the treatments without addition. The reverse osmosis concentrate had an initial pH of 3.4 and upon addition of this DOC, pH decreased with 0.1 unit in both experiments. This would theoretically, according to the carbonate equilibrium (Weiss 1974), correspond to an increase in $p\text{CO}_2$ for Experiment A of 177 $\mu$atm where pH decreased from 8.2 to 8.1. The theoretical value was close to the observed increase in $p\text{CO}_2$ of 186 $\mu$atm. The close correspondence between the theoretical and observed increase in $p\text{CO}_2$ for Experiment A together with the strong negative relationship between $p\text{CO}_2$ and pH (Supporting Information Fig. S2) suggests a carbonate equilibrium control of $CO_2$ concentrations. This is further supported as $p\text{CO}_2$ increased despite decreasing DIC concentrations in all DOC addition treatments (Supporting Information Fig. S1). The DIC pool in Lake Erken, and thus also in the mesocosms, is high ($> 20$ mg DIC $L^{-1}$). Although a small change in pH of just 0.1 unit would have a low effect on the relative redistribution within the carbonate system, it can cause a significant change in absolute $p\text{CO}_2$, given in general low $p\text{CO}_2$ being observed during the experiments. Furthermore, to change the pH by 0.1 unit merely through a change in $CO_2$ would require an increase of 87.3 and 32.7 $\mu$g C $L^{-1}$ on average in Experiment A and B, respectively. This would require respiration rates of 3.55 and 1.36 $\mu$g C $L^{-1}$ $h^{-1}$, whereas we only measured respiration rates of 1.09 and 2.18 $\mu$g C $L^{-1}$ $h^{-1}$ at the start of Experiments A and B, respectively. Consequently, production of $CO_2$ through bacterial mineralization of the added DOC could not have resulted in the decreased pH. The measured $p\text{CO}_2$ values for both experiments were well in agreement (± 40 $\mu$atm on average) with the $p\mathrm{CO}_2$ values calculated from measured pH and DIC according to Cai and Wang (1998), further confirming our findings (Supporting Information Fig. S3).

For Experiment B, the corresponding change in $p\mathrm{CO}_2$ should theoretically be an increase by 56 $\mu$atm (when pH decreased from 8.7 to 8.6). Although we observed an increase in $p\mathrm{CO}_2$ of 98 $\mu$atm, a strong negative relationship between pH and $p\mathrm{CO}_2$ was still observed in Experiment B (Supporting Information Fig. S2). This increased $p\mathrm{CO}_2$ and the observed decrease in pH in the shading treatment could potentially be due to a decrease in the photosynthesis to respiration ratio resulting from the increased light attenuation (del Giorgio and Peters 1994). Consequently, in Experiment B, the instant increase in $p\mathrm{CO}_2$ in the treatments with added DOC could be explained by the acidifying effect of the reverse osmosis concentrate. Later in the experiment, biotic factors became more important and decreasing photosynthetic rates due to limited light availability in the shaded treatments could explain the increased $p\mathrm{CO}_2$ and subsequently decreased pH. Due to the initial acidifying effect of the reverse osmosis concentrate followed by a reduction in photosynthesis the highest $p\mathrm{CO}_2$ was observed in the DOC-shaded treatments. This is further emphasized in the treatments receiving HuminFeed in Experiment A. Addition of DOC from HuminFeed did not result in an initial drop in pH or an increase in $p\mathrm{CO}_2$; however, after 1 week, pH had decreased and $p\mathrm{CO}_2$ had increased. Again, the limited light availability led to decreased photosynthesis which increased $p\mathrm{CO}_2$ and subsequently decreased pH. Similar to the DOC-shading, we found the highest response in $p\mathrm{CO}_2$ in the mixed treatment in Experiment A.

The acidifying effect of the reverse osmosis DOC was a short-term driver of $p\mathrm{CO}_2$, whereas changes in primary production due to altered light climate occurred over time in our mesocosms. However, the acidifying effect of DOC on lake water could potentially be more important in a natural system with continuous input of DOC. Lake Erken is alkaline during most of the year and is in that regard not a typical Swedish lake as the majority of lakes are nonalkaline boreal lakes with a pH < 7. The carbonate system may play a larger role in controlling $CO_2$ dynamics in more acidic oligotrophic lakes, commonly found in Sweden, as a change in pH in acidic water would have a greater effect on $p\mathrm{CO}_2$ than in alkaline water. Due to the low alkalinity in these waters, addition of the acidic reverse osmosis concentrate would likely decrease the pH by more than 0.1, subsequently increasing the $p\mathrm{CO}_2$ by more than what was seen in our study. Conversely, atmospheric emissions of sulfur dioxide ($SO_2$) have decreased considerably in the northern hemisphere since the 1970s (Vuorenmaa et al. 2006). Decreasing trends in $SO_2$ emissions have been suggested as an underlying driver for increased DOC concentrations in freshwater systems (Evans et al. 2005; Vuorenmaa et al. 2006). Recovery from acidification increases soil water pH and would, in theory, therefore increase pH of lake water. This could potentially be one explanation as to why there is lacking evidence of long-term trends in $p\mathrm{CO}_2$, despite increasing surface water DOC concentrations (Seekell and Gudasz 2016; Nydahl et al. 2017). Perhaps these two processes, recovery from acidification and the acidifying effect of DOC, may to some extent balance each other out.

Another possible source of $CO_2$ is photochemical mineralization of DOC (Graneli et al. 1996). The monthly average photochemical production of $CO_2$ in Swedish lakes during the months of our experiments (June–September) was 686 mg C $m^{-2}$ $month^{-1}$ (Koehler et al. 2014), which corresponds to 14.3 $\mu$g C $L^{-1}$ $d^{-1}$ in our mesocosms. This is in the range of the observed DOC loss and could potentially explain some of the observed $CO_2$ production. However, we would expect lower photochemical mineralization in the shading treatment compared to the control in Experiment B, yet we have higher $p\mathrm{CO}_2$ in the shading treatment. Although we cannot rule out the effect of photochemical mineralization, it would not be high enough to explain the large increase in $p\mathrm{CO}_2$ with allochthonous DOC addition.

Overall, only moderate effects of DOC additions and increased light attenuation on $CO_2$ dynamics were observed. The control treatments were undersaturated halfway through Experiment A and highly undersaturated at the start of Experiment B. Many temperate zone eutrophic lakes are undersaturated with $CO_2$, at least during the summer (Balmer and Downing 2011). Increased DOC input to these lakes could potentially switch these systems from being sinks to acting as sources of $CO_2$ to the atmosphere, particularly if the lakes are close to equilibrium with the atmospheric $CO_2$. This highlights the importance of considering trophic states and other lake characteristics in assessments of the contribution of inland waters to atmospheric $CO_2$. Accordingly, in a comparison of DOC budgets of 82 different water bodies, it was demonstrated that mesotrophic and eutrophic waters frequently accumulate rather than lose DOC over time, which may be an effect of DOC generation via indigenous primary production being higher than mineralization of DOC (Evans et al. 2017). It is likely that these ecosystems are also net sinks of $CO_2$. Agricultural eutrophication, a highly significant environmental problem (Carpenter et al. 1998; Charlton et al. 2018), is likely to continue to increase as the need for food production rises with global population. However, increased eutrophication may also result in an opposite switch where oligotrophic lakes become sinks of $CO_2$ rather than sources due to increased atmospheric carbon sequestration as sediment and DOC, further emphasizing the importance of eutrophic lake ecosystem research (Pacheco et al. 2013).

In conclusion, we found that increased allochthonous DOC input leads to enhanced $CO_2$ concentrations in meso-eutrophic lake water, which we attributed to a decreased photosynthesis to respiration ratio resulting from reduced light availability as well as to altered acidity. The allochthonous organic subsidy for bacterial mineralization was found to be low and this could perhaps be due to poor bioavailability of the DOC. Input of allochthonous DOC from the reverse osmosis concentrate, which to a substantial extent contains organic acids, appeared to have shifted the carbonate system leading to a rapid decrease in pH and subsequently increased $CO_2$. However, this acidifying effect of DOC was more pronounced in the early stage of the experiments. Later, the change in light climate appeared to play the key role in controlling $p$$CO_2$. Decreased light availability may have led to an increased respiration to photosynthesis ratio, resulting in increased $p$$CO_2$ and a subsequent decrease in pH. Allochthonous DOC input resulted in a consistent increase in $p$$CO_2$ in treatments relative to the controls for both experiments. This may also be the case for whole lake ecosystems, particularly considering that changes in climate and land-use can affect the export of DOC and nutrients from terrestrial to aquatic ecosystems (Leavitt et al. 2009; Kritzberg et al. 2014). Given the paucity of studies of the $CO_2$ dynamics of mesotrophic and eutrophic lake ecosystems and the projected future increase in nutrient loads to lakes, the impact of increased allochthonous DOC input on $CO_2$ dynamics in eutrophic inland waters needs further attention.

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## Conflict of Interest

None declared.

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# Supplementary information

# Colored organic matter increases $CO_2$ in meso-eutrophic lake water through altered light climate and acidity

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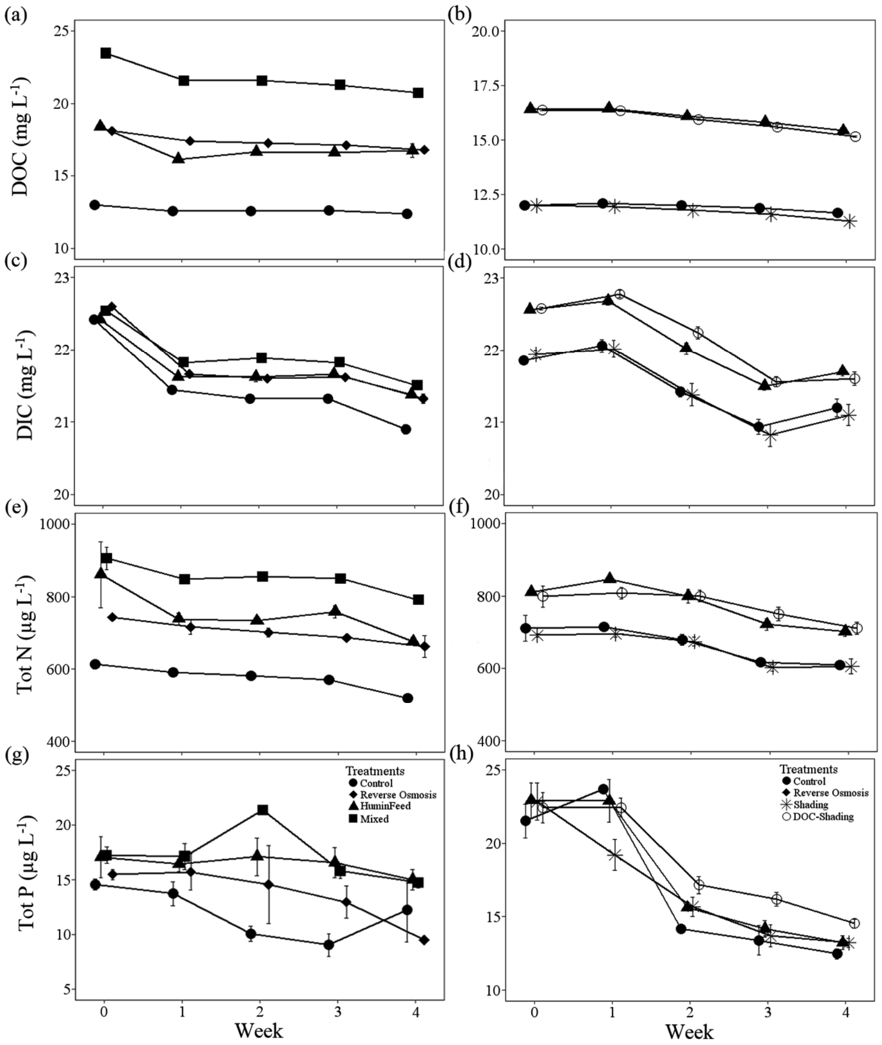

*Figure S1* Weekly (a, b) bacterial carbon production (BCP), (c, d) chlorophyll *a* (chl *a*), (e, f) pH and (g, h) dissolved inorganic carbon (DIC) observed in two four week mesocosm experiments. The first experiment (a, c, e, g) investigated the effect of two sources of allochthonous dissolved organic carbon (DOC) input on partial pressure of carbon dioxide ($p\text{CO}_2$) in lake water whereas the second experiment (b, d, f, h) investigated the effect of DOC addition and/or changed light climate through shading on $p\text{CO}_2$ in lake water. Treatments for experiment A (a) were: no addition of DOC (C), addition of DOC from concentrated humic stream water (R), addition of DOC from HuminFeed (H), and a mix of R and H (HR). Treatments for experiment B (b) were: no addition of DOC (C), addition of DOC from concentrated humic stream water (R), increased light attenuation via shading (S), and both DOC addition and shading (SR). Values are mean (±SE, n=5).

*Table S1* F-statistics and $p$ values from univariate split-plot repeated measure analyses of variances (RM-ANOVA) to test for differences in bacterial carbon production (BCP), chlorophyll *a* (chl *a*) and pH observed in two four week mesocosm experiments. The first experiment (A) investigated the effect of two sources of allochthonous dissolved organic (DOC) input on partial pressure of carbon dioxide ($p\mathrm{CO}_2$) in lake water whereas the second experiment (B) investigated the effect of DOC addition and/or changed light climate through shading on $p\mathrm{CO}_2$ in lake water.

| | **F** | | | | ***p*** | | | |
|---|---|---|---|---|---|---|---|---|
| | **BCP** | **chl *a*** | **pH** | **DIC** | **BCP** | **chl *a*** | **pH** | **DIC** |
| *Experiment A* | | | | | | | | |
| Treatment | $F_{3,16}$=0.21 | $F_{3,16}$=4.86 | $F_{3,16}$=32.0 | $F_{3,16}$=34.0 | 0.8906 | 0.0137 | <0.0001 | <0.0001 |
| Time | $F_{4,64}$=59.1 | $F_{4,64}$=6.26 | $F_{4,64}$=6.85 | $F_{4,64}$=817 | <0.0001 | 0.0003 | 0.0001 | <0.0001 |
| Treatment*Time | $F_{12,64}$=0.49 | $F_{12,64}$=2.95 | $F_{12,64}$=4.98 | $F_{4,64}$=817 | 0.9132 | 0.0025 | <0.0001 | <0.0001 |
| *Experiment B* | | | | | | | | |
| Treatment | $F_{3,16}$=0.291 | $F_{3,16}$=20.0 | $F_{3,16}$=35.6 | $F_{3,16}$=20.2 | 0.8311 | <0.0001 | <0.0001 | <0.0001 |
| Time | $F_{4,64}$=16.3 | $F_{4,64}$=12.4 | $F_{4,64}$=5.33 | $F_{4,64}$=555 | <0.0001 | <0.0001 | 0.0009 | <0.0001 |
| Treatment*Time | $F_{12,64}$=1.28 | $F_{12,64}$=2.73 | $F_{12,64}$=1.92 | $F_{12,64}$=3.11 | 0.2521 | 0.0049 | 0.0477 | 0.0016 |

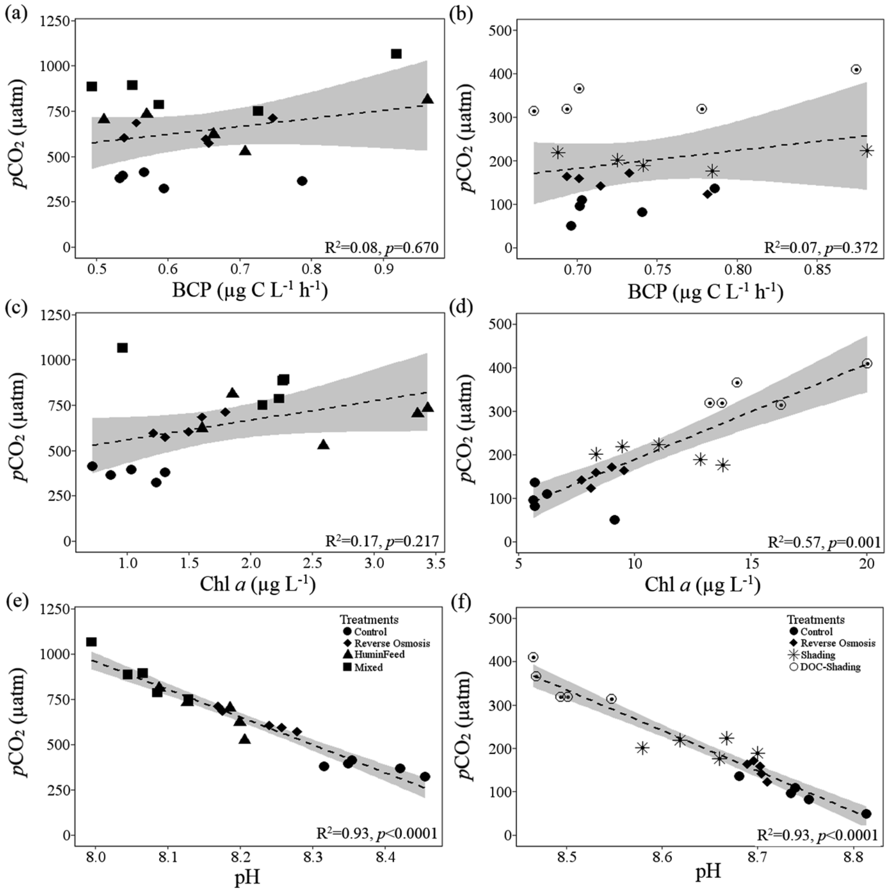

*Figure S2* Relationships between partial pressure of carbon dioxide ($p\mathrm{CO}_2$) and the variables (a, b) bacterial carbon production (BCP), (c, d) chlorophyll *a* (chl *a*) and (e, f) pH from two four week mesocosm experiments with dissolved organic carbon (DOC) additions and/or changed light climate through shading (see legend in figure for treatments). The left panel shows the results of Experiment A and the right panel of Experiment B. All values are time-weighted averages. The grey area represents the 95% confidence limit. $R^2$ and $p$ values from stepwise forward multiple linear regression analyses. We used time-weighted averages to avoid multiple comparisons and they were calculated by multiplying the values measured weekly, i.e., at each sampling time point, and dividing them by the sum of week numbers ($[(1\mathrm{x}V_1) + (2\mathrm{x}V_2) + (3\mathrm{x}V_3) + (4\mathrm{x}V_4)$ $(5\mathrm{x}V_5)]/(1+2+3+4+5)$, where V with subscript is any variable on sampling weeks 1-5). The forward stepwise regression analysis of $p\mathrm{CO}_2$ had an adjusted $R^2 = 0.94$, F = 91.5, $p < 0.0001$ and number of data points = 80 for Experiment A and an adjusted $R^2 = 0.96$, F = 167.9, $p < 0.0001$ and number of data points = 80 for Experiment B.

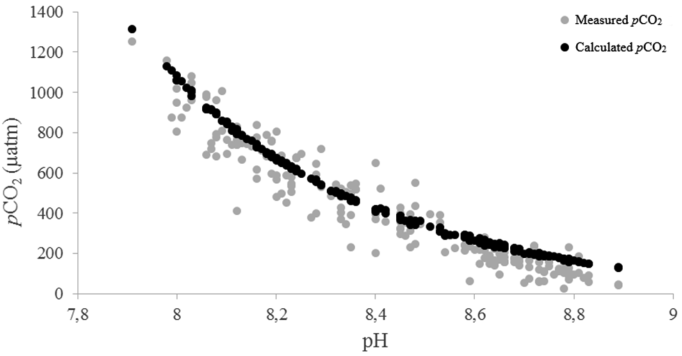

*Figure S3* Relationship between measured partial pressure of carbon dioxide ($pCO_2$) and pH (grey dots) and between calculated $pCO_2$ and pH (black dots). Calculated $pCO_2$ was calculated based on measured dissolved inorganic carbon (DIC) and pH (following methods outlined in Cai and Wang 1998).

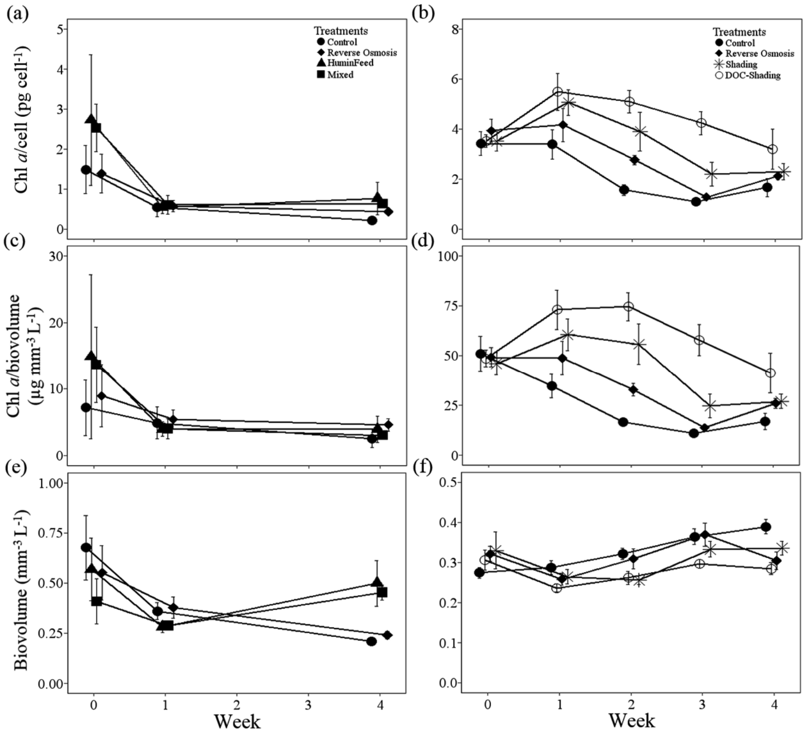

*Figure S4* Weekly (a, b) chlorophyll *a* per phytoplankton cell (chl *a*/cell) (c, d) chlorophyll *a* per phytoplankton biovolume (chl *a*/biovolume) and (e, f) phytoplankton biovolume (biovolume) observed in two four week mesocosm experiments (see legend in figure for treatments). Phytoplankton biovolume was estimated by matching size class of known volumes (Nordic Microalgae database; www.nordicmicroalgae.com) with measured dimensions and multiplying the density with volume from the database. Unfortunately, phytoplankton were not sampled for biovolume at two occasions during Experiment A, namely week two and week three.

# Paper III

# Groundwater carbon within a boreal catchment – spatiotemporal variability of a hidden aquatic carbon pool

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**Abstract** Groundwater is an essential resource providing water for societies and sustaining surface waters. Although groundwater at intermediate depth (between 3 and 20 m) could be highly influential at regulating lake and river surface water chemistry, studies quantifying organic and inorganic carbon (C) species in intermediate depth groundwater are still rare. Here, we quantified dissolved and gaseous C species in the groundwater of a boreal catchment at 3 to 20 m depth. We found that the partial pressure of carbon dioxide ($p\mathrm{CO}_2$), the stable carbon isotopic composition of dissolved inorganic carbon ($\delta^{13}$C-DIC) and pH showed a dependency with depth. Along the depth profile, a negative relationship was observed between $p\mathrm{CO}_2$ and $\delta^{13}$C-DIC, and between $p\mathrm{CO}_2$ and pH. We attribute the negative $p\mathrm{CO}_2$-pH relationship along the depth gradient to increased silicate weathering and decreased soil respiration with depth. Silicate weathering consumes carbon dioxide ($\mathrm{CO}_2$) and releases base cations, leading to increased pH and decreased $p\mathrm{CO}_2$. A negative relationship was also observed between $\delta^{13}$C-DIC and depth, potentially due to diffusion-related fractionation in addition to isotopic discrimination during soil respiration. Soil $\mathrm{CO}_2$ may diffuse downward when soil respiration is low, resulting in a fractionation of the $\delta^{13}$C-DIC. Additionally, the dissolved organic carbon (DOC) at greater depth may be recalcitrant consisting of old degraded material with a greater fraction of the heavier C isotope. Our study provides increased knowledge about the C biogeochemistry of groundwater at intermediate depth, which is important since these waters likely contribute to the widespread $\mathrm{CO}_2$ oversaturation in boreal surface waters.

*Keywords:* carbonate system, carbon dioxide, dissolved inorganic carbon, dissolved organic carbon, stable carbon isotopic composition of dissolved inorganic carbon

## Introduction

Groundwater is the second largest freshwater reservoir making up to 30% of all freshwater globally, to be compared with the surface water reservoir of approximately 0.3% (Shiklomanov, 2000). Groundwater is an integral part of a complex hydrologic cycle and plays a crucial role in sustaining surface water systems (streams, lakes and wetlands) with water (Alley, 2009). Despite the groundwater-surface water connectivity, the chemical composition of groundwater and the biogeochemical processes leading to changes in the chemical composition of groundwater have received relatively little attention in surface water studies (LaBaugh, 2009).

Small headwater streams are generally fed by shallow groundwater, hence the water chemistry in these systems closely reflect terrestrial processes such as those occurring in the riparian zone (Grabs et al., 2012; Ledesma et al., 2015). Consequently, the importance of catchment processes generally becomes prominent in small headwater streams, however, at larger scales stream water chemistry become regulated by factors other than shallow flow paths alone (Tiwari et al., 2014). As a consequence, as the catchment scales up from headwater to meso- and macro scale systems, there are increasing contributions from deeper groundwater with longer hydrological pathways, particularly during baseflow (Hood et al., 2006; Peralta-Tapia et al., 2015). Larger streams further down in a catchment are fed by both shallow and deep groundwater as well as by surface water from upstream. The combination of these sources, in addition to in-stream processes, regulates the downstream water chemistry (Hagedorn et al., 2000; Klaminder et al., 2011).

Groundwater usually contains high concentrations of dissolved inorganic carbon (DIC) (Boerner & Gates, 2015; Macpherson, 2009) and has been recognized as an important source of carbon dioxide ($CO_2$) in riverine systems, particularly in small headwater streams (Hotchkiss et al., 2015; Winterdahl et al., 2016). Direct inputs of $CO_2$ produced in catchment soils and delivered via subsurface and shallow groundwater flow to surface waters can result in high fluvial and in-lake $CO_2$ concentrations (Leith et al., 2015; Maberly et al., 2013; Weyhenmeyer et al., 2015). Despite this, groundwater has yet not sufficiently been considered in the global carbon (C) cycle, mainly due to the lack of empirical data.

Heterotrophic respiration of organic matter in soils and root respiration (i.e., soil respiration) are key sources of $CO_2$ in groundwater. Both processes occur with negligible isotopic discrimination between the organic matter substrate and the $CO_2$ produced (Lin & Ehleringer, 1997). Therefore, the stable C isotopic composition

($\delta^{13}C$) of $CO_2$ produced by respiration will be approximately equivalent to the $\delta^{13}C$ of the predominant organic matter (Doctor et al., 2008). Dissolution of soil $CO_2$ and mineral weathering are key sources of DIC in groundwater, making the $\delta^{13}C$ of DIC a useful tool for deciphering the DIC sources (Craft et al., 2002; Campeau et al., 2018; Deirmendjian & Abril, 2018). Carbonate weathering results in a $\delta^{13}C$-DIC of approximately 0‰, resulting in a less negative $\delta^{13}C$-DIC (Clark & Fritz, 1997), whereas silicate weathering results in a $\delta^{13}C$-DIC similar to soil respiration (Telmer & Veizer, 1999). The $\delta^{13}C$-DIC of soil respiration is close to that of the dominating plants in the catchments and ranges between -22 and -34‰ for $C_3$ plants (Vogel 1993). Mineral weathering can also lead to increased acid neutralizing capacity (ANC) and enhanced pH through the release of base cations, which could subsequently lead to a shift in the carbonate equilibrium towards a lower proportion $CO_2$ in comparison to other inorganic C species. Consequently, pH often increases with depth (Klaminder et al., 2011) which subsequently decrease the $CO_2$ proportion of the DIC pool. Additionally, the isotopic composition of soil $CO_2$ can become enriched in $\delta^{13}C$ by up to 5‰ relative to soil organic matter since the different isotopes diffuse against their own gradients through the soil pores (Amundson et al. 1998). Another important process that could lead to a change in isotopic composition of $CO_2$ is isotopic discrimination during microbial decomposition (Fromanek & Ambus, 2004).

A number of studies have shown groundwater to be supersaturated with methane ($CH_4$) relative to the atmosphere (Diermendjian et al., 2019; Jurado et al., 2018; Molofsky et al., 2016). Groundwater could act as a significant pathway for the indirect emission of both $CO_2$ and $CH_4$ (Minamikawa et al., 2010). In groundwater systems less than 400 m deep, $CH_4$ is produced solely through biogenic pathways (i.e., microbial mineralization) (Coleman et al., 1977). Biogenic $CH_4$ can be produced via $CO_2$ reduction or fermentation of acetate, in shallow groundwater has previously been assumed to be the dominating process (Chapelle, 2001). However, a previous study showed that in shallow groundwater in peat-rich areas, $CO_2$ reduction was the dominating pathway for $CH_4$ production (Campeau et al., 2018). Confined conditions where water circulation is absent or sluggish have been suggested to have highest reductive potential for $CH_4$ in groundwater (Gooddy & Darling, 2005).

Although groundwater may be a minor volumetric source of water to many lakes, the high concentrations of dissolved species could make groundwater a major driver of surface water chemistry (Einarsdottir et al., 2017; Schmidt et al., 2009; Shaw et al., 2013). Furthermore, deeper groundwater input could be highly influential regulating downstream river chemistry (Tiwari et al., 2017). Despite the importance of groundwater to surface water chemistry (Cook et al., 2003; Hotchkiss et al., 2015; Su et al., 2015), little is known about the groundwater chemical composition at intermediate depths (down to 20 m) and its influence on surface water chemistry. As groundwater at intermediate depth could potentially be a significant source to downstream surface waters in larger catchments (Sterte et al., 2018) it could also be an important source of various carbon species.

Here our goal was to overcome previous lack of empirical data and improve our understanding of the C biogeochemistry of groundwater at intermediate depths of the boreal landscape. Thereby providing better knowledge about its role in C dynamics of inland waters. To meet this goal, we quantified dissolved and gaseous C species in the groundwater across a mesoscale boreal catchment at depths between 3 and 20 m. By sampling at different depths, locations and seasons we assessed the spatial and temporal variability in groundwater C

## Materials and Methods

### *Study area*

The study was carried out within the 68 km$^2$ large Krycklan Catchment area (64°14′N, 19°46′E) located in the boreal region of northern Sweden (Figure 1; for further details, see Laudon et al., 2013). The catchment is composed of 15 monitored subcatchments 0.03 to 21.7 km$^2$ and has been intensively monitored for multidisciplinary research projects within the Krycklan Catchment Study (KCS) since 2002. The KCS also includes the Svartberget catchment where monitoring began already in 1981. Mean annual air temperature in the catchment is 1.8°C, and the annual precipitation is about 600 mm of which approximately 50% becomes runoff (Laudon et al., 2013). The average period of snow cover is 167 days, from late October to early May (Laudon & Löfvenius, 2016).

Forest covers most of the catchment (54-98%, of the subcatchments), with lower proportions of peatlands (0-44%) and agricultural land (0-3%). Surface waters (sum of lake and stream area) cover 0.1–4.7% of the subcatchments. The dominant tree type, particularly in the dry upslope areas, is Scots pine (*Pinus sylvestris*) (63%), followed by Norway spruce (*Picea abies*) (26%), which is found mostly in the wetter, lowland areas. The understory is dominated by ericaceous shrubs, mostly European blueberry (*Vaccinium myrtillus*) and lingonberry (*Vaccinium vitis-idaea*) on moss mats of *Hylocomium splendens* and *Pleurozium schreberi*. Deciduous shrubs and trees, primarily birch (*Betula* spp.) but also alder (*Alnus incana*) and

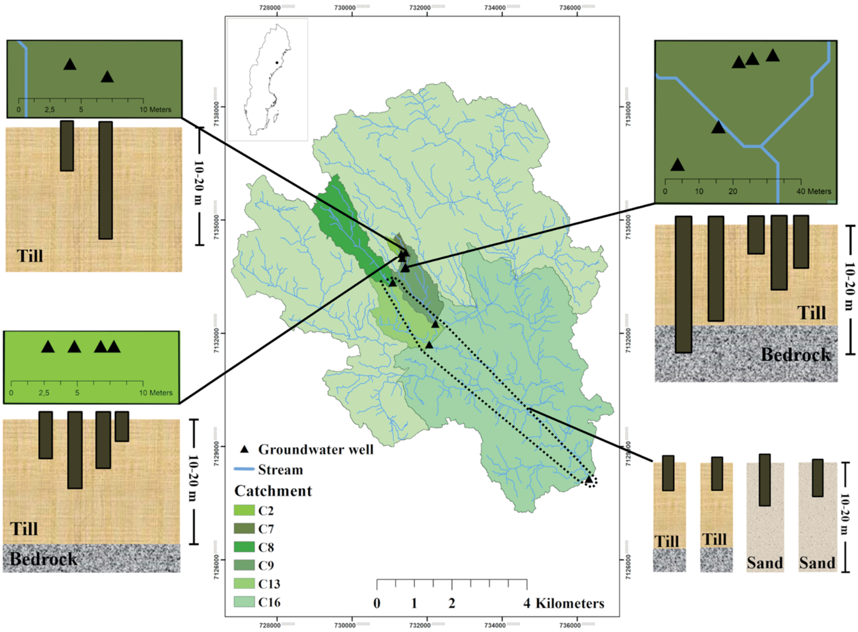

*Figure 1* Map of the Krycklan catchment showing the sampled groundwater wells and the subcatchments included in the study.

willow (*Salix* spp.), are found in the riparian forest along larger streams (Andersson & Nilsson, 2002). Peatlands are dominated by *Sphagnum* species and can be categorized as acid, oligotrophic, and minerogenic mires.

The gneissic bedrocks consist of Svecofennian rocks with 94% metasediments/metagraywacke, 4% acid and intermediate metavolcanic and 3% basic metavolcanic rock (Ågren et al., 2007). Altitude ranges from 127 m above sea level (a.s.l.) at the outlet to 372 m a.s.l. at the highest elevation of the catchment. The region was glaciated and is undergoing isostatic rebound following the last deglaciation. The highest postglacial coastline traverses the catchment at approximately 255-260 m a.s.l dividing the catchment into two distinctly different parts. The upper part of the watershed is covered by quaternary deposits that are dominated by glacial till varying in thickness from zero to up to tens of meters. The lower part of the catchment is dominated by postglacial sediments largely composed of silt and fine sand that were deposited in the distal of a postglacial delta. The total sediment depth in the area varies between 5 and 40 m (Laudon et al., 2013). The sediment deposits form a thick layer through which the larger traversing streams have deeply incised channels, in some cases forming ravines and bluffs of up to 30 m height (Buffam et al., 2008). In the till soils, well-developed iron podzols dominate the forest floor soils, but near the stream channels, the organic content increases, forming a riparian peat zone along the streams. The mineralogy of the soils in the catchment is spatially relatively homogeneous and consists of 31-43% quartz, 20-25% plagioclase, 16-33% K-feldspar, 7-21% amphiboles, 2-16% muscovite and 1-4% chlorite (1–4%) (Ledesma et al., 2013). Soil water pH usually range from 5.8 at 0.50 m depth to 7.0 at 4.3 m depth (Klaminder et al. 2011). Groundwater levels in the Krycklan catchment vary from 3.1 (±0.4 SE) m below the surface at base flow to 0.43 (±0.1 SE) m below the surface during high flow (based on long-term monitoring data).

### *Groundwater sampling*

Sampling was performed in the main Krycklan catchment (C16) and five sub-catchments (C2, C7, C8, C9 and C13), in which a number of groundwater wells were installed in 2012 (Table 1, Figure 1). The wells were installed to cover the entire catchment to allow for investigations of regional groundwater flow as well as to study local water pathways. Groundwater was collected from 16 wells, at depths ranging from 3.4 to 19.5 m. The diameter of each groundwater well is 8.7 cm. Sampling was performed at three different occasions (summer, autumn and spring) to allow for comparisons across seasons. Summer sampling took place on the 12th to 16th of June 2017, autumn sampling on the 11th to 15th of September 2017 and spring sampling on the 7th to 11th of May 2018. Spring sampling was performed directly after peak spring flood. The water level in each well was measured before sampling to allow calculation of the total volume of water in the well. Prior to sampling at least three times the volume of the well of water was removed using a submersible centrifugal pump (Supernova70®, Proactive Environmental Products) such that only the new infiltrating water was used for analysis. Groundwater was collected from approximately 10 cm above the bottom of the well.

For the partial pressure of $CO_2$ ($p$$CO_2$), triplicate samples of 30 mL were taken with a 60 mL syringe equipped with a three-way stopcock directly from the pump-connected tube to minimize degassing of $CO_2$. Directly after sampling 30 mL of ambient air was added to the syringe creating a headspace. For DIC and $CH_4$ determination, a 5 ml sample of bubble-free water was taken directly from the pump-connected tube using a sterile syringe, which was flushed with groundwater before sampling. The sample was injected into a 22.5 ml glass vial sealed with a bromobutyl septa. The vial was prefilled with 0.1 ml of 85%

*Table 1* Catchment characteristics of the main catchment (C16) and the five sub-catchments sampled in this study.

| **Catchment** | **Name** | **Area ($km^2$)** | **Forest (%)** | **Wetland (%)** | **Till (%)** | **Thin soils (%)** | **Peat (%)** | **Post-glacial sediments* (%)** |
|---|---|---|---|---|---|---|---|---|
| C2 | Västrabäcken | 0.12 | 100 | 0 | 84 | 16 | 0 | 0 |
| C7 | Kallkällsbäcken | 0.47 | 81 | 19 | 65 | 15 | 18 | 0 |
| C8 | Fulbäcken | 2.30 | 81 | 17 | 63 | 19 | 12 | 2 |
| C9 | Nyängesbäcken | 2.88 | 80 | 15 | 69 | 7 | 14 | 2 |
| C13 | Långbäcken | 7.00 | 86 | 12 | 61 | 9 | 10 | 16 |
| C16 | Krycklan | 68.91 | 88 | 9 | 51 | 7 | 9 | 30 |

* dominated by silt

phosphoric acid ($H_3PO_4$) and nitrogen gas ($N_2$) at atmospheric pressure. Samples for DOC and total nitrogen (N) were collected without headspace in 250 ml acid washed high-density polyethylene bottles. Samples for pH and conductivity analysis were collected without headspace in 50 ml acid washed high-density polyethylene bottles sealed with a gas tight septa. All samples, except for $p$CO$_2$, which were analyzed directly in the field, were stored in the dark at 4°C for a maximum of seven days until analysis.

### *Chemical analyses*

Analysis of $p$CO$_2$ was conducted in the field using the headspace equilibrium method as modified and described by Kokic et al. (2015). Equilibrated gas samples were analyzed on a portable infrared gas analyzer (IRGA, EGM-4) within 5 min from sampling. The $p$CO$_2$ was calculated using the appropriate Henry's constant Weiss (1974) and after correcting for temperature, atmospheric pressure and the amount of $CO_2$ in the added ambient air.

For DIC and $CH_4$ determination, another headspace method was used (Åberg & Wallin, 2014; Wallin et al., 2010). Headspace $CO_2$ and $CH_4$ concentrations were analyzed by GC-FID (Clarus 580, Perkin Elmer Autosystem Gas chromatograph) and connected to an autosampler (TurboMatrix 110, PerkinElmer). Concentrations of DIC and $CH_4$ were then calculated from the GC determined headspace of $p$CO$_2$ and partial pressure of $CH_4$ ($p$CH$_4$) using temperature-dependent equations for the carbonate equilibrium (Gelbrecht et al., 1998) and Henry's Law (Weiss, 1974; Wiesenburg & Guinasso, 1979), following methods outlined in Wallin et al. (2010, 2014). The detection limit of the instrument was 50 ppm for $CO_2$ and 1 ppm for $CH_4$ in the headspace gas corresponding to a field DIC and $p$CH$_4$ of 0.11 mg L$^{-1}$ and 75 µatm, respectively. Due to the detection limit, $CH_4$ was not detected in all wells.

For $\delta^{13}$C-DIC analysis, a 2 ml sample was injected into a helium flushed 12 ml bromobutyl septa capped Exetainer glass vial which had been prefilled with 200 µl $H_3PO_4$ and 20 µl zinc chloride ($ZnCl_2$) (Campeau et al., 2017). Samples of 100 µL headspace gas were analyzed for $\delta^{13}$C-DIC using an isotope ratio mass spectrometer (DeltaV Plus,Thermo Fisher Scientific, Bremen, Germany) Gasbench II (Thermo Fisher Scientific, Bremen, Germany) measuring the $CO_2$ in the headspace. Each sample was analyzed seven times and the first two injections for each sample were discarded to avoid memory effects and the other five were averaged to give the final result. The $\delta^{13}$C values are expressed in terms of deviation from the standard Vienna Pee Dee Belemnite in per mille. The $\delta^{13}$C-DIC was calibrated against laboratory standards ($NaHCO_3$ and $K_2CO_3$); these standards had been calibrated against certified standards (IAEA-600, USGS 40, IAEA-CH-6, NBS 19). The accuracy of the readings was ±0.15‰.

The DOC was measured as total organic C (TOC) on a Shimadzu TOC-VCPH using catalytic combustion, after acidification with 2M HCl to remove inorganic C. The DOC concentrations can be assumed equal to the TOC concentrations as the particulate fraction of organic C in freshwaters in Krycklan generally is less than 1% (Laudon et al., 2011). Total N was measured by wet chemical oxidation on a Shimadzu TNM1. The pH and conductivity were analyzed using a closed pH cell to avoid any outgassing of $CO_2$ and measured with a Mettler Toledo Digi117-water combined pH meter equipped with a glass electrode with sensor chip stirring gently at ambient temperature (22°C), using a two-point calibration at pH 4.01 and 7.0.

### *Statistical analyses*

We used one-way analyses of variances (ANOVAs) to test for differences of chemical variables between seasons. To test for relationships between $p\mathrm{CO_2}$ and DOC, DIC, $\delta^{13}$C-DIC, $p\mathrm{CH_4}$ and pH we performed linear regressions on the mean of the values measured at the different seasons. Data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Bartlett's test. Only DOC showed a non-normal distribution and these data were log transformed to achieve normal distribution. Statistical analyses were performed in the software package JMP version 13.0.0 (SAS Institute Inc. 2016). Significance was set at an alpha level of 0.05 for all tests.

## Results

The groundwater in the Krycklan catchment was generally highly oversaturated in $p\mathrm{CO_2}$ relative to the atmosphere, with values ranging from 716 up to 12584 µatm (Table 2). Highest $p\mathrm{CO_2}$ was observed at C13, where the well penetrates down to 5.6 m. Lowest $p\mathrm{CO_2}$ was found in the deepest well (18.0 m) at C2. Highest DOC was also found at C13, whereas lowest DOC was observed in the 5.0 m deep well at C9. We also found highest DIC at C13, while lowest DIC was found in the 6.8 m deep well at C2. Highest $\delta^{13}$C-DIC, of -18.1 ‰, was found in the deepest well sampled (19.5 m) located at C7. Lowest $\delta^{13}$C-DIC, of -25.2 ‰, was observed in the 4.9 m deep well at C7. As with the other C species, $p\mathrm{CH_4}$ was highest at C13. In three of the wells at C2 and in three of the wells at C7, $p\mathrm{CH_4}$ were below the detection limit (i.e. below 75 µatm in headspace).

Although $p\mathrm{CO_2}$ showed commonly lower values with less than 6500 µatm in wells located at greater depths (> 7 m) (Table 3), we found a high variation in $p\mathrm{CO_2}$ in wells at 3-7 m depths, where values ranged between 1819 and 12584 µatm (Figure 2a). Nevertheless, $p\mathrm{CO_2}$ showed a significant decrease with depth of the well (Table 3). Conversely, $\delta^{13}$C-DIC increased significantly with increasing depth of the wells (Table 3), although they were still rather variable at 3-7 m depths (Figure 2d). Also pH increased significantly with depth of the wells (Table 3), while we did not observe any significant depth relationship for DOC, DIC and $p\mathrm{CH_4}$ (Figure 2).

The chemical composition of the groundwater was stable across all seasons, with no significant differences between seasons for any of the measured variables (Figure 3, Table 4).

Although $p\mathrm{CO_2}$ is a component of DIC, there was only a weak relationship between $p\mathrm{CO_2}$ and DIC (Table 3, Figure 4). A strong negative relationship was however observed between $\delta^{13}$C-DIC and $p\mathrm{CO_2}$ and, although not as strong, there was also a significant negative relationship between $p\mathrm{CH_4}$ and $p\mathrm{CO_2}$ (Figure 4). As expected we observed increasing $p\mathrm{CO_2}$ with decreasing pH, whereas we found no relationship between DOC and $p\mathrm{CO_2}$ (Figure 4).

## Discussion

### *Groundwater pCO$_2$, DOC and pCH$_4$*

The groundwater in the Krycklan catchment was oversaturated with $\mathrm{CO_2}$ relative to the atmosphere across all investigated wells. The degree of oversaturation was however highly variable across well depth and across the catchment. We did observe a weak, but significant, decreasing trend of $p\mathrm{CO_2}$ with depth. There was a large variation in $p\mathrm{CO_2}$, ranging between 1819 and 12584 µatm, in the 11 wells that were between 3 and 7 m deep, which are distributed all across the catchment. High spatial variation in groundwater $p\mathrm{CO_2}$ across a catchment has previously

*Table 2* Range of measured groundwater chemistry variables in the 16 groundwater wells sampled across three seasons (summer, autumn, spring).

| **Catchment** | **Well** | **Elevation (m.a.s.l.)** | **Soil type** | **Depth (m)** | **$p$CO$_2$ (µatm)** | **DOC (mg L$^{-1}$)** | **DIC (mg L$^{-1}$)** | **$\delta^{13}$C-DIC (‰)** | **$p$CH$_4$ (µatm)** | **Total N (mg L$^{-1}$)** | **pH** | **Conductivity (µS cm$^{-1}$)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **C2** | 201 | 259 | Till | 4.2 | 8001 – 11526 | 1.1 - 1.9 | 8.7 - 11.6 | -22.4 - -22.3 | 80.2 - 93.3 | 0.09 - 0.13 | 5.9 - 6.3 | 39.4 - 53.4 |
| **C2** | 301 | 252 | Till | 5.0 | 5698 – 6005 | 1.0 - 1.4 | 5.8 - 15.9 | -22.1 - -21.1 | - | 0.06 - 0.07 | 5.8 - 5.9 | 32.4 - 38.6 |
| **C2** | 302 | 252 | Till | 3.4 | 4000 – 6578 | 1.1 - 1.3 | 4.2 - 5.2 | -21.7 - -21.4 | 79.1 - 80.0 | 0.05 - 0.06 | 5.8 - 6.1 | 20.5 - 25.2 |
| **C2** | 303 | 252 | Till | 6.0 | 1717 – 1846 | 0.9 - 2.0 | 5.4 - 6.7 | -20.7 - -20.4 | - | 0.10 - 0.11 | 6.1 - 6.7 | 77.1 - 79.1 |
| **C2** | 304 | 252 | Till | 11.7 | 2638 – 3199 | 2.0 - 2.3 | 4.4 - 6.8 | -20.9 - -19.9 | - | 0.14 - 0.18 | 6.4 - 6.8 | 37.2 - 91.2 |
| **C7** | 211 | 252 | Till | 6.8 | 2360 – 2570 | 0.7 - 1.4 | 3.7 - 4.1 | -18.9 - -18.3 | - | 0.06 - 0.08 | 6.3 - 6.5 | 29.9 - 32.3 |
| **C7** | 212 | 252 | Till | 19.5 | 1741* | 3.9* | 6.8* | -18.1* | - | 0.12* | 6.7* | 139.6* |
| **C7** | 401 | 240 | Till | 4.8 | 7643 – 8497 | 3.8 - 8.0 | 9.3 - 12.0 | -25.0 - -22.9 | - | 0.17 - 0.21 | 5.9 - 7.2 | 57.3 - 63.4 |
| **C7** | 403 | 240 | Till | 4.9 | 9605 – 11395 | 4.6 - 6.9 | 13.7 - 14.7 | -25.6 - -25.0 | 112 - 223 | 0.18 - 0.21 | 6.0 - 6.4 | 56.3 - 65.7 |
| **C7** | 404 | 240 | Till | 10.8 | 1676 – 1896 | 1.4 - 2.6 | 8.9 - 9.7 | -19.8 - -19.4 | 260 - 346 | 0.11 - 0.13 | 6.4 - 6.8 | 95.0 - 97.2 |
| **C7** | 411 | 239 | Till | 15.0 | 4548 – 4781 | 4.8 - 5.8 | 9.0 - 12.2 | -23.5 - -22.7 | 219 – 310 | 0.17 - 0.21 | 6.5 - 7.0 | 88.3 - 94.1 |
| **C7** | 412 | 239 | Bedrock | 18.0 | 716 – 1196 | 2.0 - 4.1 | 11.7 - 12.5 | -19.5 - -18.7 | 245 – 328 | 0.12 - 0.14 | 6.8 - 7.3 | 126.1 - 128.1 |
| **C8** | 511 | 235 | Till | 5.0 | 8363 – 11709 | 1.3 - 4.6 | 11.7 - 21.5 | -21.4 - -21.3 | 465* | 0.13 - 0.25 | 6.0 - 6.7 | 70.0 - 129.1 |
| **C9** | 501 | 182 | Till | 5.0 | 1309 – 1398 | 0.9* | 18.4 - 11.7 | -20.0 - -19.8 | 78.7 - 82.9 | 0.14* | 6.8 - 7.3 | 99.7* |
| **C13** | 601 | 173 | Sand | 7.0 | 9739 – 12584 | 1.6 - 9.3 | 18.0 - 24.0 | -24.2 - -21.6 | 5955 - 28377 | 0.39 - 0.48 | 5.5 - 6.2 | 64.2 - 87.3 |
| **C16** | 701 | 128 | Sand | 5.6 | 8498 – 9578 | 2.2 - 2.5 | 14.6 - 15.5 | -22.4 - -23.2 | 145 - 197 | 0.07 - 0.08 | 6.1 - 6.5 | 103.1 - 108.1 |

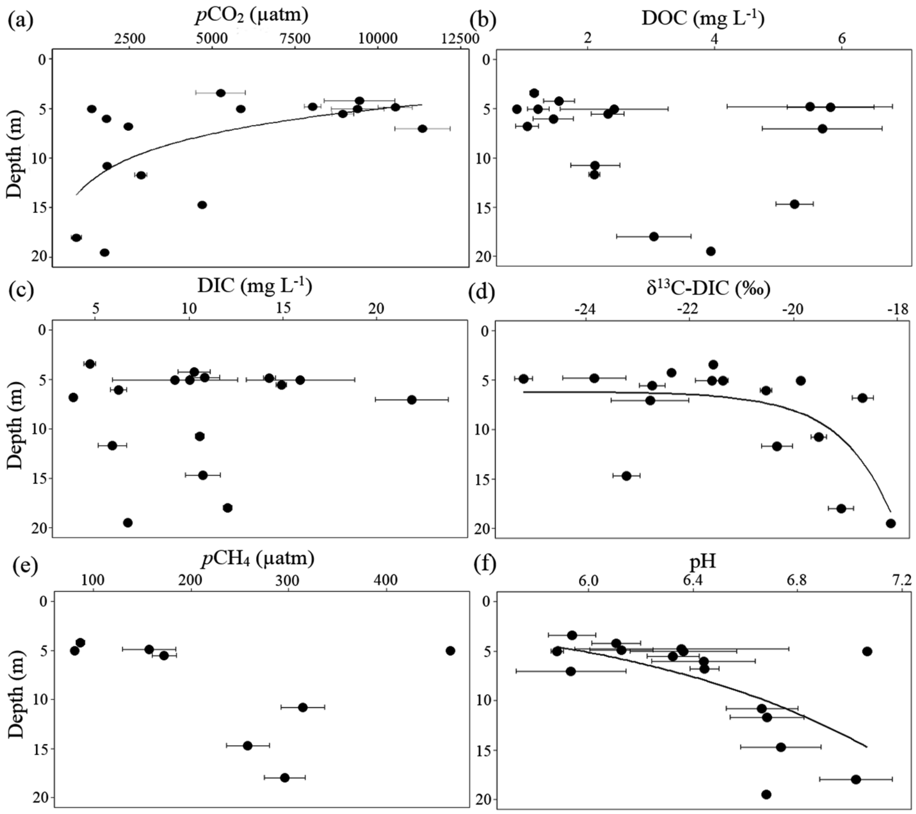

*Figure 2* $pCO_2$ (a), DOC (b), DIC (c), δ$^{13}$C-DIC (d), $pCH_4$ (e) and pH (f) in groundwater wells at different depths. For all variables, data are presented as mean values ± standard error (*n*=3, SE). A linear regression line is shown for variables with a significant relationship to well depth. For statistical details see Table 3.

been demonstrated (Worrall & Lancaster 2005). Soil respiration as well as $CO_2$ diffusion and dissolution are important processes that regulate groundwater $pCO_2$ (Kessler & Harvey, 2001). Our observed variation in $pCO_2$ across the catchment demonstrates that these processes are spatially heterogeneous. Soil respiration could be spatially variable even at local scales, which would affect groundwater $pCO_2$ and could potentially explain the observed patterns in $pCO_2$ (Vincent et al., 2006). The observed variations in groundwater $pCO_2$ may also be due to differences in soil porosity, the degree of soil saturation and permeability (i.e., the hydraulic conductivity) as this would affect both $CO_2$ diffusion and dissolution (Hu et al., 2018; Moldrup et al., 2000). Differences in local and regional topography (i.e., hydraulic gradient) could also be important as both hydraulic conductivity and hydraulic gradient affects the sub-surface hydrological pathways, and thus regulate the movement of groundwater

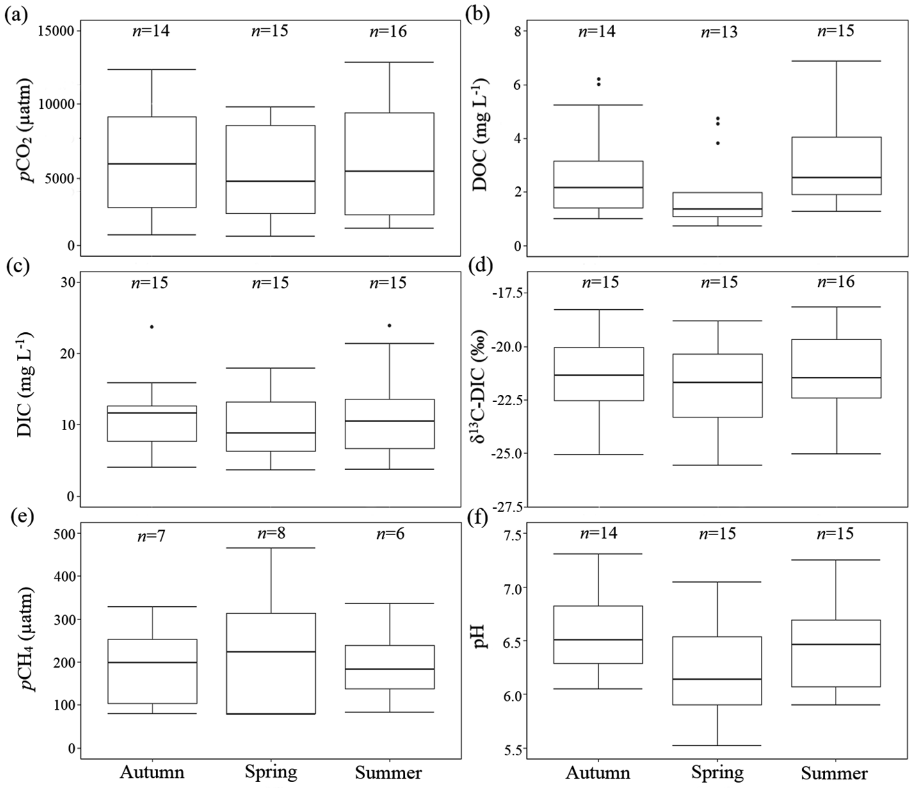

*Figure 3* Normal quantile boxplots for (a) $pCO_2$, (b) DOC, (c) DIC, (d) $\delta^{13}$C-DIC, (e) $pCH_4$ and (f) pH during autumn, spring and summer. For statistical details, see Table 4.

*Table 3* Summary of results from linear relationships presented in Figure 2 and Figure 4, showing the adjusted $R^2$ values, number of observations (*n*) and *p*-values for the regression models.

| | **Equation** | **$R^2_{adj}$** | ***n*** | ***p*** |
|---|---|---|---|---|
| *Linear regression with depth (Fig 2)* | | | | |
| $pCO_2$ | $\log(pCO_2) = 9.11 - 0.10*\text{depth}$ | 0.27 | 16 | 0.022 |
| $\delta^{13}$C-DIC | $\exp(\delta^{13}\text{C-DIC}) = -1.62e^{-9} + 4.72e^{-10}*\text{depth}$ | 0.39 | 16 | 0.006 |
| pH | $\exp(\text{pH}) = 412 + 29*\text{depth}$ | 0.32 | 16 | 0.013 |
| *Linear regression with $pCO_2$ (Fig 4)* | | | | |
| DIC | $pCO_2 = -349 + 546*\text{DIC}$ | 0.42 | 16 | 0.004 |
| $\delta^{13}$C-DIC | $pCO_2 = -26983 – 1520* \delta^{13}\text{C-DIC}$ | 0.65 | 16 | 0.0002 |
| $pCH_4$ | $pCO_2 = 10750 – 23*pCH_4$ | 0.42 | 9 | 0.035 |
| pH | $pCO_2 = 51847 – 7232*\text{pH}$ | 0.49 | 16 | 0.002 |

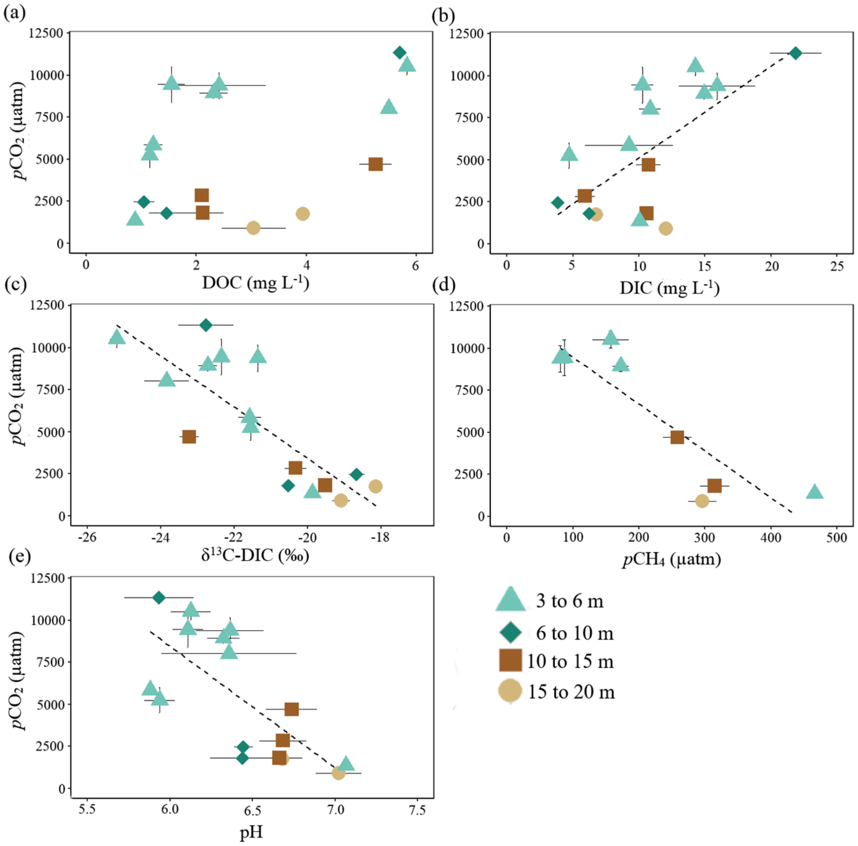

*Figure 4* Relationships between (a) DOC, (b) DIC, (c) $\delta^{13}$C-DIC, (d) $p$CH$_4$ and (e) pH and $p$CO$_2$ and in the groundwater of the Krycklan catchment. The symbols are color coded according to the depth of the sampled well. For all variables, data are presented as mean values ± standard error ($n$=3, SE).

*Table 4* F-statistics and $p$-values from one-way analyses of variances (ANOVA) for differences in the chemical variables $p$CO$_2$, DOC, DIC, $\delta^{13}$C-DIC, $p$CH$_4$ and pH between three seasons (summer, autumn and spring).

| | F | $p$ |
|---|---|---|
| $p$CO$_2$ (µatm) | 0.575 | 0.567 |
| log DOC (mg L$^{-1}$) | 3.099 | 0.056 |
| DIC (mg L$^{-1}$) | 0.251 | 0.779 |
| $\delta^{13}$C-DIC (‰) | 0.623 | 0.541 |
| $p$CH$_4$ | 0.460 | 0.638 |
| pH | 2.604 | 0.086 |

(LaBaugh, 2009; Wu & Selvadurai, 2016). Variations in hydraulic properties (i.e., hydraulic conductivity and gradient) have been shown to be of importance for surface and groundwater interactions at both sub-catchment and full catchment-scale in the Krycklan catchment (Sterte et al., 2018).

The observed spatial variation in $p$CO$_2$ may also be due to local differences in silicate weathering and/or accumulation of base cations as these would affect the pH and consequently $pCO_2$ (Buffam et al., 2008; Stets et al., 2017; Warfvinge et al., 1993). In the upper more organic parts of the soil, higher soil and root respiration can be expected. This would lead to increased $pCO_2$ and subsequently lower soil water pH near the surface. Further down the soil profile, lower organic content and increased mineral weathering will lead to an enhanced pH through the release of base cations and increased ANC (Klaminder et al. 2011). Additionally, weathering of silicate minerals consumes $CO_2$ and produces $HCO_3^-$, which subsequently increases pH (Brady, 1991). It has previously been shown that concentrations of base cations and pH in groundwater between 0.5 to 4.3 m depth in the Krycklan catchment increased along a 70-m long hillslope (Klaminder et al. 2011). Shallow groundwater (at 0.5 m depth) had a pH of 5.8, whereas deeper groundwater, at 2.9 to 4.3 m depth, had a pH of 6.9 to 7.2, respectively. This could be attributed to increased influence of silicate weathering because of long residence time of water (Klaminder at al., 2011). Weathering processes are slow, however groundwater age generally increases with depth (Kim et al., 2008), hence it is possible that there has been enough time for significant silicate weathering to occur resulting in enhanced pH in the groundwater of our deep wells. The change in pH could also explain the observed negative relationship between $pCO_2$ and depth. There was a strong negative relationship between pH and $pCO_2$ (Figure 4) suggesting that the pH driven carbonate buffering system plays an important role for carbon dynamics in groundwater at intermediate depths. Concurrently, the change in pH with depth could also be due to decreased soil respiration with depth as this may have caused $pCO_2$ to decrease, subsequently increasing the pH. It is difficult to elucidate whether pH is driving $pCO_2$ or vice versa in the study catchment, but it is likely that both increased silicate weathering and decreased soil respiration with depth are important factors. As there are no carbonate minerals in the Krycklan catchment carbonate weathering could not explain the observed trends in $pCO_2$ or pH.

In shallow soil water, DOC concentrations have generally been found to decrease with increasing soil depth (Grabs et al., 2012; Kaiser & Kalbitz, 2012; Lyon et al., 2011). In constrast we did not observe any relationship between DOC and sampling depth across the 3-20 m range included in our study. Instead DOC concentrations were varaible ranging between 1 and 6 mg $L^{-1}$. This lack of DOC depth dependency could be due to the overall low DOC concentrations, which in turn are controlled by biodegradation rates, adsorption and co-precipitation (Graham et al., 2015). A lack of DOC mobilization could perhaps also explain the lack of relationship between DOC and sampling depth. In the upper soil profile, DOC may be immobilized by iron oxide (Kindler et al., 2011). Reducing conditions favouring the dissolution of iron oxides, and thus leaching of DOC, are created when the groundwater reach superficial organic horizons (Stemmler et al., 2004). When the groundwater are below superficial organic horizons, DOC mobilization is limited resulting in no relationship between DOC and depth.

As with DOC, there was no relationship between $p$CH$_4$ and depth of the sampling well. There were a number of wells where $p$CH$_4$ was below detection limit of the analytical method and all these wells are located in the upper part of the catchment (i.e., C2 and C7). However, in the three deeper wells at C7, which are between 10 and 18 m deep, $p$CH$_4$ was between 258 and 314 µatm. One important factor for CH$_4$ production is whether the environment is anoxic or not. We found a negative relationship between $p$CO$_2$ and $p$CH$_4$ which suggests that the oxygen (O$_2$) supply exerts a control on the metabolic process responsible for the decompostion of organic carbon. Hence, the negative relationship between $p$CO$_2$ and $p$CH$_4$ could also be due to higher aerobic soil respiration at more shallow depth and increased methanogeneis towards deeper soil layers. There are a few environmental factors, other than organic C substrates and O$_2$ concentrations, that also influence CH$_4$ metabolism, such as soil temperature and redox potential (Song et al., 2006; Wang et al., 2017). Such environmental factors could vary between and within catchments but were not measured in this study, but should be considered in future work.

In the well at C13, which is 5.6 m deep, we observed the highest $p$CO$_2$, DOC, $p$CH$_4$ and DIC. A mire is situated just upslope of C13 and it is possible that water from the mire infiltrates the soil profile and reaches the groundwater well. Hence, it is possible that the mire is a major source of the high C concentrations also observed in the groundwater at C13. Lowest $p$CO$_2$ was observed in the deepest well at C7, which is 18 m deep. This was not surprising as this well is the only one that was drilled into bedrock, hence influence of soil respiration would be limited. Although the well reaches down into bedrock, $p$CO$_2$ was still oversaturated relative to the atmosphere. This is likely due to downward diffusion of soil CO$_2$ produced in the above soil horizon. Another possible explanation for the observed $p$CO$_2$ concentration in the bedrock could be infiltration of groundwater enriched in dissolved CO$_2$ into bedrock cracks, pores and hollows (Rodhe & Bockgård, 2006).

### *Groundwater DIC and $\delta^{13}$C-DIC*

Similar to $p$CO$_2$, DIC concentrations were found to be variable around 3 to 7 m depth ranging between 4.7 and 21.9 mg $L^{-1}$ with highest concentraion in the well at C13. However DIC did not show a dependency with well depth. On the contrary, we found a positive relationship between the $\delta^{13}$C-DIC and sampling depth, with less negative $\delta^{13}$C-DIC at greater depths. The vegetation in the Krycklan catchment consists only of $C_3$ plants, which have a $\delta^{13}$C between -22 and -34‰ (Vogel, 1993). We observed slightly less negative $\delta^{13}$C-DIC values in the groundwater, between -18.1 and -25.2 (mean -21.3 ±2.0‰). Closer to the surface we observed more negative $\delta^{13}$C-DIC, which are in the range of $\delta^{13}$C for $C_3$ plants, however further down the soil profiles the $\delta^{13}$C-DIC increased. Our observed positive relationship $\delta^{13}$C-DIC and depth contradict previous findings by Amundson et al., (1998) who found a negative relationship between $\delta^{13}$C-$CO_2$ and depth. They found that the isotopic value of soil $CO_2$ was closer to atmospheric values near the soil surface, whereas at greater depth the isotopic signature was closer to that of the biological source. This pattern was suggested to be regulated by diffusion-related fractionation as $CO_2$ diffusion could increase the $\delta^{13}$C of up to 5‰ (Amundson et al., 1998). The study by Amundson et al. (1998) was, however, confined to the upper 4 m of the soil layer whereas our study focused on groundwater below 4 m. At shallow depth the diffusion of soil $CO_2$ to groundwater is facilitated by the proximity of the upper soil layers and thus have a higher $\delta^{13}$C-DIC due to diffusion-related fractionation. At greater depth (10-20 m), soil respiration is likely low and $CO_2$ may diffuse downwards resulting in a fractionation in the $\delta^{13}$C-DIC towards more positive values.

As there are no carbonate containing minerals in the soils of the Krycklan catchment (Klaminder et al., 2011), less negative $\delta^{13}$C-DIC values cannot be explained by carbonate weathering. Since silicate weathering does not yield any additional carbon, it only fixes soil $CO_2$, the DIC must have a pure biogenic origin. The $\delta^{13}$C-DIC of silicate weathering and soil respiration are similar, hence silicate weathering is supposed to have only a minor direct effect on the groundwater $\delta^{13}$C-DIC (Telmer & Veizer, 1999). Another potential explanation for the observed postitive relationship between $\delta^{13}$C-DIC and depth could be a change in the quality of the soil C with depth. Earlier studies have shown that there is an increase in $\delta^{13}$C of soil organic matter with depth, and it was argued that this was due to the accumulated influence of isotopic discrimination during microbial decomposition (Buchmann et al., 1997; Nadelhoffer & Fry, 1988; Formanek & Ambus 2004). As a result, the heavier isotope in the oldest and most degraded material is left behind in the deeper soil layers (Formanek & Ambus 2004). This vertical enrichment in $\delta^{13}$C was also reflected in the soil-$CO_2$ evolved from respiration (Formanek & Ambus 2004). It is possible that the enriched $\delta^{13}$C-DIC at greater depth in our study catchment is a result of soil respiration utilizing older C with higher $\delta^{13}$C. Likewise, the variation in $\delta^{13}$C-DIC in groundwater at 3 to 7 m depth across the catchment could also be due to local differences in carbon quality resulting in isotopic discrimination during soil respiration.

Another process which could affect the enhanced $\delta^{13}$C-DIC is methanogenesis, which could result in a fractionation of up to 10‰ on the $\delta^{13}$C-DIC (Wimmer et al., 2013). Anaerobic conditions are likely to be common at the depths investigated in this study, and $CH_4$ was higher in the deeper wells. Although methanogenesis related fractionation of $\delta^{13}$C-DIC could contribute to the observed positive relationship with depth, the $CH_4$ production is likely too low in the groundwater at Krycklan, due to low organic C content to  have any major influence on the $\delta^{13}$C-DIC.

### *Groundwater – surface water connectivity*

As small headwater streams transition into larger streams and further into rivers, the hydrological flowpaths that deliver water and solutes may shift from being dominanted by shallow groundwater in the headwaters to increased influence from deep groundwater further downstream (Carroll et al., 2018; Creed et al., 2015). The combination of shallow and deep groundwater contribution to downstream chemistry vary not only with catchment size but also with season and runoff conditions (Sophocleous, 2002; Winter, 1999). For instance, during baseflow the contribution of deep groundwater to stream surface water could be up to 80% for boreal catchments larger than 10 km$^2$ (Peralta-Tapia et al., 2015). Due to the large spatial variation in C concentrations across the Krycklan catchment it is however difficult to assess what proportion of surface water C originates from deep groundwater, particularly as adjacent subcatchments may function very differently despite seemingly similar characteristics (Karlsen et al., 2016; Sterte et al., 2018). Our study shows that the groundwater chemistry of a small catchment can vary substantially both within and between subcatchments. To fully understand the contribution of deep groundwater to surface water C concentrations we need a better understanding of the catchment hydrology as well as combined measurements of surface water and groundwater chemistry.

## Conclusions

In conclusion, our study shows that there is large spatial variation in groundwater chemistry across a boreal catchment and that local differences in both soil respiration and weathering processes could play a major role in controlling C dynamics of groundwater at intermediate depths. Deep groundwater will eventually connect with surface water, although it could take centuries (Alley, 2009). Considering that about one third of the global total volume of freshwater is groundwater (Shiklomanov, 2000), deep groundwater might play a more important role in the global C cycle than previously understood. We suggest that, due to the great variation in C concentrations over depth and across the catchment, it is essential to have multiple sampling locations when aiming to upscale C concentrations in intermediate groundwater to regional or global scale. Our study provides a step towards a better understanding of the C content and dynamics of intermediate and deep groundwater, which is of great importance as these waters could be an indirect source of $CO_2$ to the atmosphere.

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# Paper IV

# Highly variable explanations of long-term $pCO_2$ increases in boreal lakes and streams

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**Abstract** Understanding the mechanisms driving inland water carbon dioxide ($CO_2$) concentrations is important to foresee inland water $CO_2$ responses to environmental change, yet there are still knowledge gaps regarding which processes are the key $CO_2$ drivers. Here we investigated possible drivers for an observed 21-year partial pressure of $CO_2$ ($pCO_2$) increase in 13 Swedish lakes and streams. Overall, we could not identify a single dominating mechanism responsible for the observed $pCO_2$ increase. In the eight lakes we found that $pCO_2$ had increased either by a possible DOC driven stimulation of microbial mineralization or by a water color driven primary production suppression. In streams, the dominating mechanism for a $pCO_2$ increase was either a change in the carbonate system distribution or a possible nutrient driven decrease in primary production. We conclude that drivers for a $pCO_2$ increase are generally site-specific, which limit the ability to predict future $CO_2$ emissions from inland waters.

## Introduction

Inland waters are active components of the global carbon cycle, by processing, transporting and storing vast amounts of carbon (Cole *et al.* 1988, Cole *et al.* 2007, Lapierre and del Giorgio 2012). Because of the large amounts of carbon being processed in situ, as well as in the surrounding catchment and imported as dissolved inorganic carbon (DIC) to inland waters, the majority of the world's inland waters are supersaturated with carbon dioxide ($CO_2$), therefore being net sources of $CO_2$ to the atmosphere(Cole *et al.* 1994, Raymond *et al.* 2013). Although some of the mechanisms driving the partial pressure of $CO_2$ ($pCO_2$) in inland waters have been intensely studied, there is still some controversy regarding which processes are the key $CO_2$ drivers, in particular when it comes to determine key drivers on a spatial versus the temporal scale. Understanding the mechanisms driving inland water $pCO_2$ across spatial and temporal scales is important to allow for generalizations and predictions of inland water $pCO_2$ responses to environmental change

(Seekell and Gudasz 2016, Nydahl *et al.* 2017). Overall, there are four key processes regulating $p\mathrm{CO_2}$ in inland waters: $\mathrm{CO_2}$ production through microbial and photochemical mineralization of dissolved organic carbon (DOC) (e.g. Tranvik 1992, Hope *et al.* 1996), $\mathrm{CO_2}$ consumption via primary production (e.g. Balmer and Downing 2011), distribution changes within the carbonate system as a result of pH changes (e.g. Lazzarino *et al.* 2009) and $\mathrm{CO_2}$ input from the surrounding catchment driven by catchment hydrology (e.g. Jones and Mulholland 1998, Striegl and Michmerhuizen 1998, Palmer *et al.* 2001).

Among all processes, direct microbial mineralization of DOC has been suggested to be an important driver of $p\mathrm{CO_2}$ in lakes (Hope *et al.* 1996, del Giorgio *et al.* 1997), and it has been shown that there is a positive spatial relationship between DOC and $p\mathrm{CO_2}$ (Sobek *et al.* 2003, Lapierre and del Giorgio 2012). Concentrations of surface water DOC have been increasing in the boreal region over the last decades (Monteith *et al.* 2007, Filella and Rodriguez-Murillo 2014), which could potentially lead to enhanced $p\mathrm{CO_2}$ due to increased substrate availability for microbial mineralization. Microbial mineralization is also dependent on the water temperature resulting in increasing $\mathrm{CO_2}$ production at higher temperatures (Gudasz *et al.* 2010).

Microbial mineralization is not only affected by DOC concentration and water temperature, the quality of the DOC can also be a key regulator and thereby influencing $\mathrm{CO_2}$ in the water column (Bodmer et al. 2016). The quality of DOC depends on its composition (Jaffe *et al.* 2008), which often is divided into humic-like or protein-like components (Kothawala *et al.* 2014). Bioavailability of DOC has been related to a decreasing proportion of humic-like DOC and an increasing proportion of protein-like DOC (Hosen *et al.* 2014). Hence, an increased proportion of protein-like DOC could lead to enhanced microbial mineralization with subsequent increased $\mathrm{CO_2}$ production (Hosen *et al.* 2014). Humic-like DOC is predominantly derived from terrestrial vascular plants and soil organic matter (i.e., allochthonous) (Miller and McKnight 2010) and is typically considered as aromatic with more color and high molecular weight (Kothawala *et al.* 2014). Owing to these characteristics, humic-like DOC is commonly viewed as recalcitrant (Miller and McKnight 2010). In contrast, protein-like DOC is generally derived from algae and in-situ heterotrophic processes (i.e., autochthonous), has low molecular weight and is often labile (McKnight *et al.* 2001). Autochthonous DOC generally has a higher nitrogen (N) content (C:N between 4 and 10) then does allochthonous DOC (C:N of 20 and higher) (Meyers and Ishiwatari 1993). The ratio between DOC and dissolved organic N has been found to be positively related to humic-like substances (Kothawala *et al.* 2014). Consequently, the C:N ratio could be used as a proxy indicator of DOC quality (Bernal *et al.* 2005). Likewise, as humic-like substances generally have more aromatic structures and are thus more colored than protein-like substances, absorbance at 420 nm ($abs_{420}$) divided by DOC concentration ($abs_{420}$/DOC) could also be used as a proxy indicator for DOC quality. More colored DOC, i.e., higher $abs_{420}$/DOC, suggests that there is a higher proportion of humic-like substances. Thus, decreased C:N ratios and/or $abs_{420}$/DOC would indicate an increase in reactive protein-like substances which might lead to enhanced $p\mathrm{CO}_2$.

Primary production can be an important regulator of $p\mathrm{CO}_2$ in inland waters by reducing $CO_2$ through photosynthesis. Primary production is often nutrient-limited, with phosphorus (P) often being the limitation for inland water (Schindler 1977, Elser *et al.* 1990). Consequently, P concentrations are commonly used as a proxy indicator for primary production in inland waters. Another factor regulating primary production, and hence $p\mathrm{CO}_2$ in inland waters is water color (Jones 1992). Increased water color can constrain primary production as a large fraction of photosynthetically active radiation (PAR) is absorbed by the DOC and is thus not available for photosynthesis (Jones 1992). Increasing water color has been observed in surface waters in the boreal region and this has been attributed to increasing DOC (Monteith *et al.* 2007, Haaland *et al.* 2010). This increased water color could lead to decreased $CO_2$ uptake through primary production subsequently enhancing $p\mathrm{CO}_2$.

Another important mechanism regulating $p\mathrm{CO}_2$ in inland waters is the carbonate system (Stets *et al.* 2017), which is partially driven by changes in pH. Although many inland waters in the boreal region are recovering from acidification due to acid deposition reduction, pH has continued to decrease in some Swedish lakes and streams over the past two decades (Nydahl *et al.* 2017, Huser *et al.* 2018). This decrease in pH could be due to the increased DOC concentrations with subsequent increase in organic acidity, or as a result of increased use of fertilizers in agriculture as inorganic nitrogenous fertilizers promotes soil acidity (Collins and Jenkins 1996, Nohrstedt 2001). A decrease in pH would lead to increased $p\mathrm{CO}_2$ as the distribution within the carbonate system shifts and the proportion between free $CO_2$, bicarbonate ($HCO_3^-$) and carbonate ($CO_3^{2-}$) changes towards more free $CO_2$. A recent study including more than 100 streams across the conterminous USA showed that carbonate buffering was the primary control on $p\mathrm{CO}_2$ in surface waters (Stets *et al.* 2017). Likewise, pH was found to be the best predictor of lake surface water $p\mathrm{CO}_2$ for more than 900 Florida lakes (Lazzarino *et al.* 2009).

It is not only internal processes that are important drivers for $p\mathrm{CO}_2$ in inland waters, hydrological inputs (ground and surface water) that mobilize organic and inorganic C from the catchment soils are also known to be important (Hotchkiss *et al.* 2015, Ledesma *et al.* 2015, Weyhenmeyer *et al.* 2015). These hydrological inputs are often driven by precipitation. Although the precipitation has generally increased across Sweden over the past couple of decades, in some areas precipitation has decreased (Chen *et al.* 2015). Where precipitation has decreased, surface water discharge may also has decreased leading to an increased water retention time (WRT). The lake and stream internal $\mathrm{CO}_2$ production is partly controlled by landscape WRT with more efficient production in waters with a long WRT (Hanson *et al.* 2011). Decreased precipitation would also lead to a higher relative contribution by groundwater to surface water with effects on the surface water chemistry (Carroll *et al.* 2018). Shallow groundwater is generally supersaturated with $\mathrm{CO}_2$ and has been shown to be a large contributor to surface water $p\mathrm{CO}_2$, particularly in headwater streams (Jones and Mulholland 1998, Hotchkiss *et al.* 2015). In larger downstream rivers, both shallow and deep groundwater have been found to regulate water chemistry (Hagedorn *et al.* 2000), while concurrently in-stream metabolism increases in its relative importance as a $p\mathrm{CO}_2$ driver further downstream (Hotchkiss *et al.* 2015). Despite being a minor volumetric source of water to lakes, groundwater is also a major driver of lake water chemistry due to the high concentration of dissolved species in groundwater (Schmidt *et al.* 2009, Shaw *et al.* 2013).

In a recent study it has been shown that out of a total data set of 71 lakes and 30 streams, eight lakes and five streams demonstrated an increase in $p\mathrm{CO}_2$ over the past two decades (Nydahl *et al.* 2017). Here we investigated the reasons behind the observed synchronous $p\mathrm{CO}_2$ increase in the nine boreal surface waters with focus on four key driving processes: DOC mineralization, primary production, the carbonate system and catchment hydrology. We tested the hypotheses that $p\mathrm{CO}_2$ has increased due to (1) stimulation of microbial mineralization by increased DOC concentrations and/or increased temperature; (2) stimulation of microbial mineralization as a result of DOC quality changes towards more reactive DOC subsequently enhancing carbon mineralization rates and $\mathrm{CO}_2$ production; (3) decreased primary production with a consequent decrease in $\mathrm{CO}_2$ bio-uptake; (4) shift in the carbonate system towards a higher proportion of free $\mathrm{CO}_2$ due to a pH decrease and; (5) a greater proportion of groundwater to surface water input and longer WRT due to decreased precipitation.

## Methods

Out of a database of 101 Swedish lakes and streams with available time series since 1997 we selected the waters which previously had shown increasing surface water $p\mathrm{CO}_2$ (Nydahl *et al.* 2017). The waters with increasing $p\mathrm{CO}_2$ comprised eight lakes and five streams and were distributed across the boreal and hemiboreal region of Sweden but showed a dominance towards southern Sweden (Figure 1). The lakes were generally small, having a median surface area of 0.37 $\mathrm{km}^2$, and shallow, with a median mean depth of 4.4 m. The catchment areas of the lakes were mainly forested (average 81.2%) with some agricultural land (average 5.2%). The catchment areas of the streams also had large percentage of forest (average 54.5%), however in the catchment of Vindbron the greatest proportion of land use was semi urban (41.1%) and in Akkarjåkka the largest land use type was tundra (54.5%). Many surface waters in Sweden have been limed since the late 1970s to counter surface water acidification caused by acid deposition (Henrikson *et al.* 1995); however, none of the waters included in this study have been limed.

From the Swedish national freshwater monitoring program, described by Fölster *et al.* (2014) we acquired water chemistry data from the lakes and streams for the period 1997 to 2017, freely available at http://www.slu.se/ vattenmiljo. In this study we considered data on total organic carbon (TOC), pH, alkalinity, total phosphorous (total P), total nitrogen (total N), inorganic nitrogen (IN), absorbance at 420 nm measured in a 5 cm cuvette ($\mathrm{abs}_{420}$) and water temperature. Total organic carbon concentrations were used as a proxy indicator for DOC concentrations as the particulate fraction of organic carbon in boreal and hemiboreal inland waters generally is less than 1% (Laudon *et al.* 2011). Additionally, total-P was used as a proxy indicator for primary production (Wetzel 1992). We calculated dissolved organic nitrogen (DON) as total N minus IN. We used DOC:DON (C:N) ratios for DOC quality since these ratios are positively related to humic-like substances (Kothawala *et al.* 2014). We also used $\mathrm{abs}_{420}$/DOC as a proxy indicator for DOC quality as a higher $\mathrm{abs}_{420}$/DOC is equivalent to more color, thus indicating a higher proportion of humic-like substances. All lakes and streams included in this study had been sampled at least four times per year during the study period 1997 to 2017.

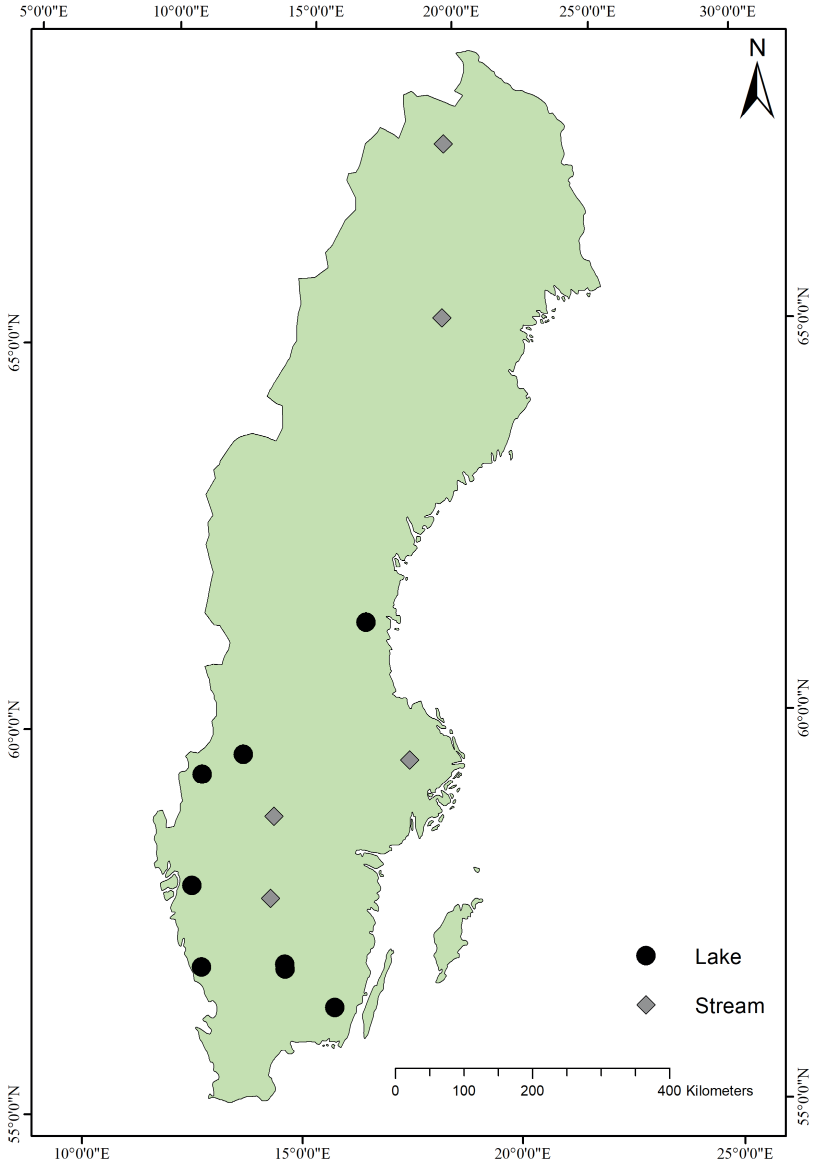

**Figure 1** Location of the Swedish study lakes (circles) and streams (diamonds).

All samples were collected at a water depth of 0.5 m, except in more shallow streams where samples were taken closer to the surface. All samples were analyzed by the SWEDAC (Swedish Board for Accreditation and Conformity) accredited laboratory at the Swedish University of Agricultural Sciences following standard limnological procedures. A detailed method description including analytical precision and range can be found at http://www.slu.se/en/departments/aquatic-sciencesassessment/laboratories/geochemical-laboratory/water-chemical-analyses/. All water chemistry analyses considered in this study were made on unfiltered water, except absorbance which was based on filtered (0.45 µm) water.

From the available water chemistry data, we calculated concentrations of $CO_2$ using water temperature, alkalinity (using only positive values), and pH (only values >5.4 were used; see below for reasoning) according to (Weyhenmeyer *et al.* 2012). From the calculated $CO_2$ we determined $pCO_2$ (in µatm) using Henry's constant according to Henry's law while adjusting for atmospheric pressure at sample site elevation (Weyhenmeyer *et al.* 2012). According to Abril *et al.* (2015), calculation of $pCO_2$ from alkalinity and pH may result in overestimated values in organic-rich and acidic waters, and $CO_2$ cannot be calculated from pH values of less than 5.2 (Hunt *et al.* 2011, Raymond *et al.* 2013). As pH slightly above 5.2 also has been shown to provide uncertain estimates of $pCO_2$ (Raymond *et al.* 2013, Wallin *et al.* 2014), we discarded all data with pH values of less than 5.4 to minimize the uncertainty. We further applied the triprotic model by Hruska *et al.* (2003) as it allows for an estimation of the dissociation of organic acid anions ($RCOO^-$) from measured pH and TOC. The calculated organic alkalinity was subtracted from the measured total alkalinity, and this new value for alkalinity was used to calculate $pCO_2$ according to Weyhenmeyer *et al.* (2012). Lastly, we used median values rather than means to avoid the impact of outliers to minimize uncertainties in the $pCO_2$ calculations.

In addition to the water chemistry data, we downloaded hydrological data for precipitation, discharge and groundwater storage for each subcatchment of the lakes and streams from the Swedish Meteorological and Hydrological Institute's Vattenweb at http://vattenweb.smhi.se/. For precipitation and discharge, average monthly values for the entire subcatchments were modeled using the hydrological model S-HYPE (Lindström *et al.* 2010). Groundwater in the moraine and glacifluvial sediments were also modeled using S-HYPE. The groundwater levels in the moraine and glacifluvial sediments were then averaged to provide one value for groundwater levels. Groundwater levels were modeled for every day of the year and reported as the percentage of the daily groundwater level during the period 1961-2017. A groundwater level of 0% represents the historically lowest groundwater level on the same day of the year, whereas a groundwater level of 100% is equivalent to the highest groundwater level on the same day of the year.

### *Statistics*

In this study we used two main statistical approaches, one to evaluate variables affecting surface water $pCO_2$ and one to evaluate changes over time. For the first approach we applied partial least squares (PLS) regressions on the whole dataset to quantify the effect of DOC, temperature, $abs_{420}$/DOC, C:N, pH, $abs_{420}$, total-P, precipitation, discharge and groundwater on surface water $pCO_2$. Data were normalized using log-transformation where necessary. Since we applied PLS on the whole dataset we also included time as an x variable. We performed three separate PLS analyses. Firstly, we performed a PLS on all available lake and stream data combined which included water type (i.e., lake or stream) as an x-variable, adding up to a total of 11 x-variables. The other two PLS analyses were performed on the lake and stream data separately, having a total of ten x-variables each (x predictor variables listed in Table 1). PLS is a reliable technique when identifying relevant variables and their magnitude of influence, particularly if the sample size is small (Abdelhady *et al.* 2018). The Variable Importance for Projections (VIPs), which are linear coefficient plots between the predictors and the response variable (i.e., $pCO_2$) across all model components, were used to identify the explanatory variables that contribute most to the models (i.e., the weight of each predictor). Here we consider VIP-values greater than 1.0 as significant explanatory variables (Eriksson *et al.* 1999). PLS analyses were performed using the software package SIMCA version 15.0.2 (Umetrics AB, Umeå Sweden). For the second approach we used the non-parametric Mann Kendall trend tests to determine if there was a significant increase or decrease in DOC, temperature, $abs_{420}$/DOC, C:N, pH, $abs_{420}$, total-P, precipitation, discharge and groundwater during 1997 to 2017. Input data for the test were yearly median values for the period 1997 to 2017. The significance was set at $p < 0.05$ for all Mann Kendall trend tests. We used an Excel-Macro for the Mann Kendall trend test (Microsoft Office 2015).

## Results

All our eight lakes and five streams showed a significant increase in surface water $pCO_2$ during the period 1997 to 2017 (Mann Kendall trend test: $p < 0.05$). All waters were oversaturated with $pCO_2$ relative to the atmosphere, the long-term median ranged between 770 and 1236 µatm and between 967 and 4124 µatm in the lakes and streams, respectively (Table 1). The long-term median DOC concentration ranged between 7.4 and 19.5 mg $L^{-1}$ in the lakes and between 1.9 and 17.4 mg $L^{-1}$ in the streams (Table 1). The lakes were generally acidic, having a pH ranging from 5.7 to 6.9, while most streams had slightly higher pH ranging from 6.5 to 7.2 (Table 1). Total-P concentrations were generally low (<12.5 µg $L^{-1}$) in the waters, except for two streams where total-P concentrations were 42 and 55 µg $L^{-1}$ (Table 1).

### *Predictor variables for $pCO_2$ in lakes and streams*

Using PLS to predict the synchronous increase in surface water $pCO_2$ in the studied lakes and streams we found that total-P, C:N and DOC were important variables for explaining the variability in surface water $pCO_2$ when lakes and streams were combined in the same model. The first PLS component explained 19% of the total variance and showed that total-P and DOC were positively correlated to $pCO_2$, whereas C:N was negatively correlated to $pCO_2$ (Figure 2a). Overall, the PLS model for the lakes and streams combined extracted three significant components which collectively explained 59% of the variation in surface water $pCO_2$ ($R^2$), by using 50% of the variation in the environmental predictors (Table 2). The second and third PLS components explained 17% and 13% of the variance, respectively (Figure 2a). Water type (i.e., lake and stream) were found on opposite sides in the PLS plot, although water type was not a significant explanatory variable for surface water $pCO_2$ (VIP<1, Figure 3a). The importance of total-P, DOC and C:N for explaining the variability in $pCO_2$ was further supported by the VIP scores, which for total-P, DOC and C:N were > 1.0 (Figure 3a). None of the three catchment hydrological variables, precipitation, discharge and groundwater, had a significant impact on surface water $pCO_2$ in lakes and streams combined (Figure 2a).

In our second PLS model we considered lakes only, having a total of ten x-variables (x predictor variables listed in Table 1). The first PLS component explained 31% of the variation and showed that pH and temperature were negatively related to $pCO_2$ whereas $abs_{420}$ and $abs_{420}$/DOC were positively related to lake $pCO_2$ (Figure 2b). The VIP scores further confirmed the importance of the variables pH, temperature, $abs_{420}$ and $abs_{420}$/DOC for explaining the variability in lake $pCO_2$ as these all had VIP scores > 1.0 (Figure 3b). Overall, the PLS model explained 51% of the variation in $pCO_2$ ($R^2$), by using 61% of the variation in the environmental predictors, divided over four significant components (Table 2). The second PLS component explained 16% of the variation whereas the third and fourth components only explained 8 and 7%, respectively.

In our third PLS model we considered stream data only, resulting in a model with ten x-variables (x predictor variables listed in Table 1). The first PLS component explained 24% of the total variance and showed that total-P and DOC were positively related to stream

*Table 1* Median water chemical and catchment hydrological values for eight Swedish lakes and five Swedish streams for the 21-year period 1997 to 2017. The sign within parentheses refers to results from Mann Kendall trend tests where + is a significant increase, - is a significant decrease and nc refer to no change at an alpha level of 0.05.

| *System* Site | $p\mathrm{CO_2}$ (µatm) | DOC (mg $L^{-1}$) | Temperature (°C) | $\mathrm{Abs_{420}}$/ DOC | C:N | pH | $\mathrm{Abs_{420}}$ | Total-P (µg/l) | Precipitation (mm $mth^{-1}$) | Discharge ($m^3$ $s^{-1}$) | Groundwater (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Lake* | | | | | | | | | | | |
| Brunnsjön | 1421 (+) | 19.5 (nc) | 18.1 (nc) | 0.021 (nc) | 27.8 (nc) | 5.71 (nc) | 0.402 (nc) | 12 (nc) | 62.9 (nc) | 0.138 (+) | 33.2 (nc) |
| Fiolen | 770 (+) | 7.6 (+) | 14.2 (nc) | 0.009 (nc) | 15.4 (+) | 6.68 (+) | 0.064 (nc) | 11 (nc) | 71.8 (nc) | 0.061 (nc) | 50.5 (nc) |
| Fräcksjön | 1213 (+) | 10.0(+) | 12.8 (-) | 0.014 (nc) | 23.1 (nc) | 6.43 (nc) | 0.135 (+) | 9 (nc) | 76.9 (-) | 271 (nc) | 49.7 (nc) |
| Skärsjön | 718 (+) | 4.5(nc) | 11.6 (nc) | 0.006 (nc) | 11.5 (+) | 6.86 (nc) | 0.026 (nc) | 9 (nc) | 104.5 (nc) | 0.252 (nc) | 60.8 (nc) |
| Stensjön | 912 (+) | 7.4 (+) | 11.8 (+) | 0.016 (nc) | 29.5 (+) | 6.39 (nc) | 0.116 (nc) | 6 (nc) | 59.8 (nc) | 0.030 (nc) | 46.0 (nc) |
| Stora Skärsjön | 1036 (+) | 5.0 (+) | 15.9 (nc) | 0.014 (+) | 17.3 (+) | 6.77 (nc) | 0.064 (+) | 9 (+) | 74.9 (nc) | 0.176 (nc) | 50.1 (nc) |
| Ulvsjön | 1236 (+) | 8.4 (nc) | 7.2 (nc) | 0.014 (nc) | 22.9 (+) | 6.05 (nc) | 0.114 (+) | 7 (nc) | 68.5 (nc) | 0.390 (nc) | 51.9 (nc) |
| Översjön | 1100 (+) | 6.8 (nc) | 7.1 (nc) | 0.011 (nc) | 24.8 (nc) | 5.82 (+) | 0.079 (+) | 6 (nc) | 63.3 (nc) | 0.195 (nc) | 53.0 (nc) |
| *Stream* | | | | | | | | | | | |
| Akkarjåkka | 866 (+) | 1.9 (-) | 3.5 (nc) | 0.014 (nc) | 11.3 (+) | 7.12 (nc) | 0.027 (nc) | 7 (-) | 63.3 (nc) | 12.5 (nc) | 56.5 (nc) |
| Bergmyrbäcken | 1040 (+) | 6.0 (-) | 4.1 (-) | 0.021 (nc) | 26.7 (+) | 6.79 (+) | 0.132 (-) | 5 (-) | 61.4 (nc) | 0.171 (-) | 56.5(-) |
| Svedån Sved | 967 (+) | 7.6 (nc) | 6.3 (nc) | 0.021 (-) | 23.5 (nc) | 6.93 (nc) | 0.162 (nc) | 9 (-) | 57.6 (nc) | 0.499 (nc) | 51.8 (nc) |
| Vindbron | 4124 (+) | 15.0 (nc) | 7.3 (nc) | 0.011 (nc) | 6.1 (nc) | 7.58 (-) | 0.170 (nc) | 55 (nc) | 47.9 (nc) | 5.350 (nc) | 43.0 (nc) |
| Visman Nybble | 2713 (+) | 17.4 (+) | 11.1 (nc) | 0.016 (nc) | 10.9 (nc) | 6.49 (-) | 0.267 (nc) | 42 (nc) | 57.2 (nc) | 1.305 (nc) | 44.7 (nc) |

surface water $pCO_2$, whereas C:N was negatively related to $pCO_2$ (Figure 2c). Overall, the model resulted in three significant components explaining 74% of the variation in stream $pCO_2$ ($R^2$), using 43% of the variation in the explanatory variables (Table 2). The second PLS component explained 12% of the variance, whereas the third PLS component explained 7% of the total variance (Figure 2c). The dependency of stream $pCO_2$ on total-P, DOC and C:N was further confirmed by the VIP scores which all were > 1.0 (Figure 3c).

### *Changes in lake water chemical variables since the late 1990's*

During our study period, 1997 to 2017, we found that in six of the eight lakes there were significant trends in at least one of the variables we used as proxy indicators for DOC mineralization, i.e., DOC, temperature, $abs_{420}$/DOC and C:N. Half of the eight lakes had increased in DOC, Fiolen, Fräcksjön, Stensjön and Stora Skärsjön (Figure 4, Table 1). In Stensjön we also observed an increase in temperature, whereas in Fräcksjön we found a reduction in temperature over the 21-year study period (Table 1). Regarding our measures for DOC quality, both $abs_{420}$/DOC and C:N had increased in Stora Skärsjön. We also observed enhanced C:N in the lakes Fiolen, Skärsjön, Stensjön and Ulvsjön, however $abs_{420}$/DOC had remained constant in the remaining seven lakes.

For primary production, significant trends were observed for at least one of the two primary production proxy indicators (total-P and $abs_{420}$) in four of the eight lakes (Figure 3, Table 1). The water color had increased in Fräcksjön, Stora Skärsjön, Ulvsjön and Översjön, as indicated by increased $abs_{420}$ during the period 1997 to 2017 (Table 1). Total-P had only increased in one lake, Stora Skärsjön (Figure 4, Table 1).

Another strong driver of $pCO_2$ in inland water is the carbonate system and we used pH to identify distribution changes within the carbonate system. We found that pH had increased in Fiolen and Översjön, while in all other lakes pH had remained the same during the period 1997 to 2017 (Table 1).

During our 21-year study period, we only observed a few significant changes in regards to catchment hydrological drivers of surface water $pCO_2$ (precipitation, discharge and groundwater) in the catchments of our study lakes.

Precipitation had decreased in the catchment of Fräcksjön, while discharge had increased in the catchment of Brunnsjön. Groundwater levels had not changed in any of the lake catchments (Figure 4, Table 1).

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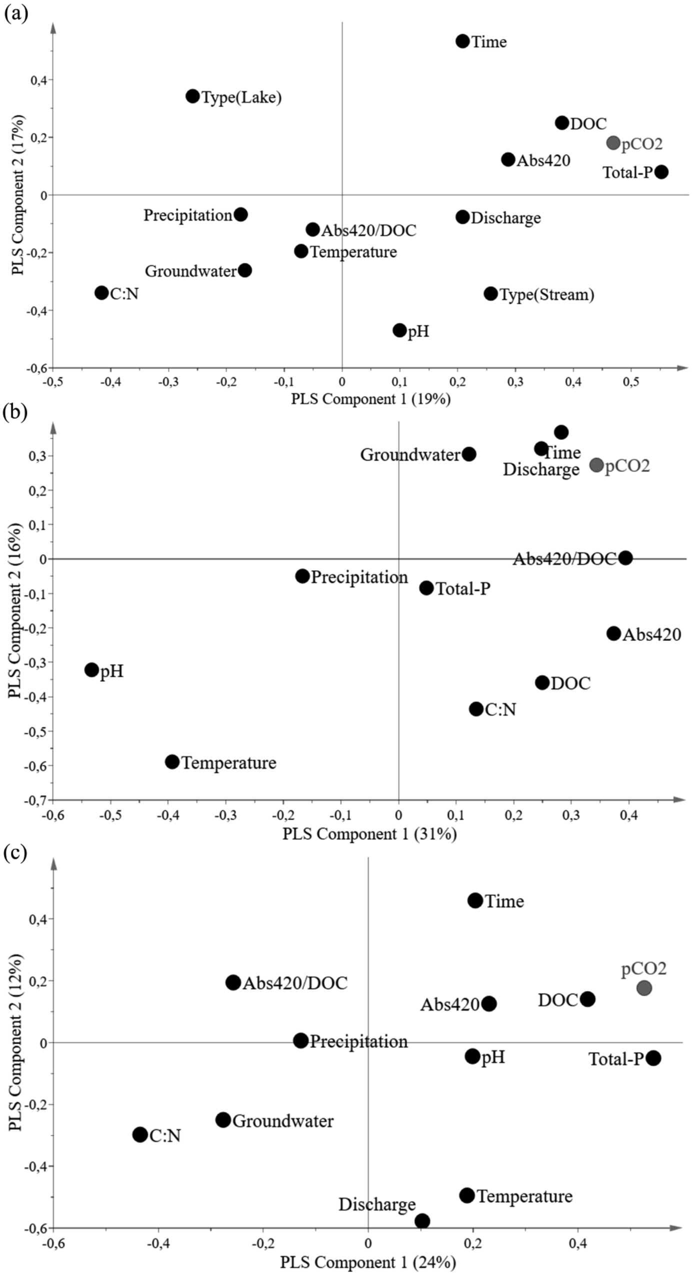

*Figure 2* Loading plots of the partial least squares regression (PLS) analyses for (a) all surface waters, (b) only lakes and (c) only streams as given by the PLS weights (w*$c_1$ and w*$c_2$). The graphs represent the correlation structures between the X variables (11 X variables for all surface waters, 10 X variables for only lakes and 10 X variables for only streams) and $p\mathrm{CO}_2$. The Y-variable ($p\mathrm{CO}_2$) is shown in grey whereas the explanatory variables are shown in black. Only the first and second components were shown in the graphs for reasons of clarity.

*Table 2* Percent variation in the X-matrix (X), cumulative percent variation explained ($R^2$) and predictive ability ($Q^2$) of PLS models on $pCO_2$ in surface waters of lakes and streams vs. 10 environmental predictors (see Table 1).

| PLS component | Percent variation | | |
|---|---|---|---|
| | X | $R^2$ | $Q^2$ |
| *Lakes and streams* | | | |
| 1 | 19 | 51 | 51 |
| 2 | 37 | 57 | 56 |
| 3 | 50 | 59 | 59 |
| *Lakes* | | | |
| 1 | 31 | 35 | 34 |
| 2 | 47 | 45 | 45 |
| 3 | 55 | 50 | 48 |
| 4 | 61 | 51 | 49 |
| *Streams* | | | |
| 1 | 24 | 69 | 69 |
| 2 | 36 | 73 | 72 |
| 3 | 43 | 74 | 73 |

### *Changes in stream water chemical variables since the late 1990's*

During the period 1997 to 2017, four of the five study streams showed a significant change in at least one of the variables influencing DOC mineralization (DOC, temperature, $abs_{420}$/DOC, C:N), however there was no obvious trend that could allow us to elucidate if one of the DOC mineralization driving variables was a particularly strong driver of $pCO_2$ in the streams (Figure 4). In one of the streams, Visman Nybble, DOC had increased during the 21-year period, while in two of the streams, Akkarjåkka and Bergmyrbäcken, DOC concentrations had decreased (Table 1). Temperature had also decreased in Bergmyrbäcken. In regards to the DOC quality measures, $abs_{420}$/DOC had decreased in Svedån Sved, whereas C:N had increased in Akkarjåkka and Bergmyrbäcken (Table 1).

The proxy indicators for primary production, total-P and $abs_{420}$ had changed significantly in three of the five streams over the 21-year period (Table 1). In Bergmyrbäcken, both total-P and $abs_{420}$ had decreased. Total-P had also decreased in Akkarjåkka and Svedån Sved (Table 1). No other changes in our primary production proxy indicators were observed in the streams.

During the 21-year study period we found that in three of the five streams the distribution within the carbonate system had changed as demonstrated by changes in pH (Figure 4). In Bergmyrbäcken, pH had increased, whereas in Vindbron and Visman Nybble there was a decrease in pH during 1997 to 2017 (Table 1).

As for the lakes, little changes were observed for the hydrological variables in the streams. Only in the catchment of Bergmyrbäcken significant changes were observed with decreased discharge and groundwater level (Table 1, Figure 4).

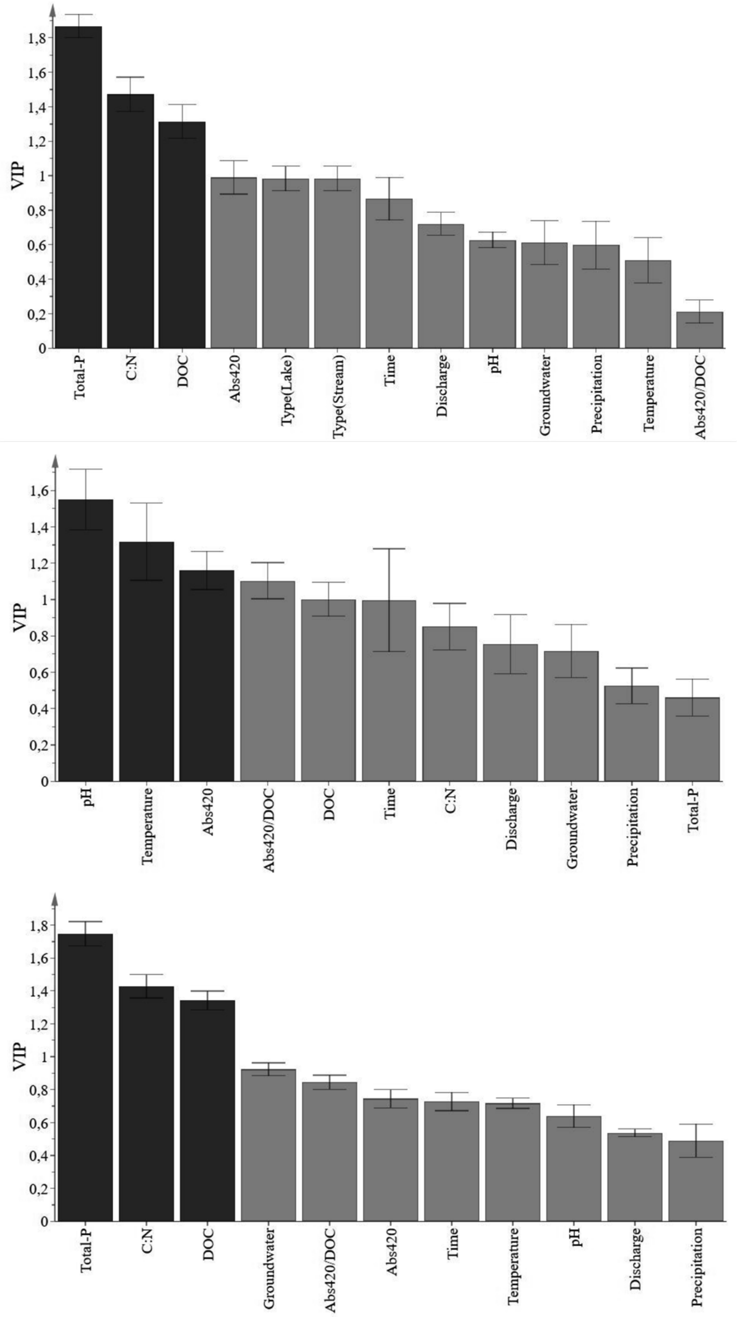

*Figure 3* Importance of the ten environmental input variables for the prediction of $p\mathrm{CO}_2$ in (a) all surface waters ($n$=2439), (b) only lakes ($n$=1000) and (c) only streams ($n$=1439) using PLS regression. The higher the VIP values the more important is the input variable for the model performance. VIP values > 1 are considered as important for the model performance. Dark grey bars represent variables with VIP > 1 whereas light grey bars represent variables with VIP < 1.

# Discussion

The dominating mechanism driving the observed synchronous $p\mathrm{CO}_2$ increase across a variety of Swedish lakes and streams was highly variable and generally site-specific, and clearly differed between lakes and streams (Figure 1a). Considering the temporal scale, which was the main focus of this study, the site-specific patterns became even clearer. Overall, our results indicate that the dominating mechanisms driving $p\mathrm{CO}_2$ in lakes are increased microbial mineralization as a response of increased DOC and/or change in DOC quality as well as suppressed primary production as a response of a water color induced light limitation. In streams, the key $p\mathrm{CO}_2$ driving mechanisms were a distribution change within the carbonate system as a response to decreased pH as well as a decreased primary production due to nutrient limitation.

## *Effects of DOC mineralization on pCO₂*

Half of our eight study lakes showed an increase in DOC simultaneously to the $p\mathrm{CO}_2$ increase during the 21-year study period, suggesting that microbial mineralization has been an important driver for $p\mathrm{CO}_2$ in these lakes (Tranvik 1992, Hope *et al.* 1996). On the contrary, the remaining four lakes were in agreement with a recent study showing that DOC concentrations and $p\mathrm{CO}_2$ are uncoupled over time (Nydahl *et al.* 2017). We also found opposing trends in the streams, where DOC concentrations were found to have increased, not changed, or even decreased. Hence, we cannot fully confirm or disprove our hypothesis that $p\mathrm{CO}_2$ has increased due to stimulation of microbial mineralization by increased DOC concentrations. However, DOC concentrations appeared to be of more importance for $\mathrm{CO}_2$ production in lakes than in streams. This is likely due to the longer WRT in lakes, allowing more time for carbon transformation processes to occur.

Our finding that changes in DOC could be more important in lakes than in streams are further supported by our finding that DOC quality changes were more pronounced in our study lakes. In five of the lakes, C:N had increased, suggesting that the proportion of humic-like substances had increased (Kothawala. *et al.* 2014) The C:N had also increased in two of the five study streams. Humic-like substances have been considered less labile than algal derived DOC with low C:N (Miller and McKnight 2010, Hosen et al. 2014). If this is the case, the enhanced C:N should lead to decreased microbial mineralization and a subsequent pCO2 decrease. There is however, some controversy regarding the relationship between DOC quality and DOC reactivity and high molecular weight DOC has been shown to be more bioreactive than low molecular

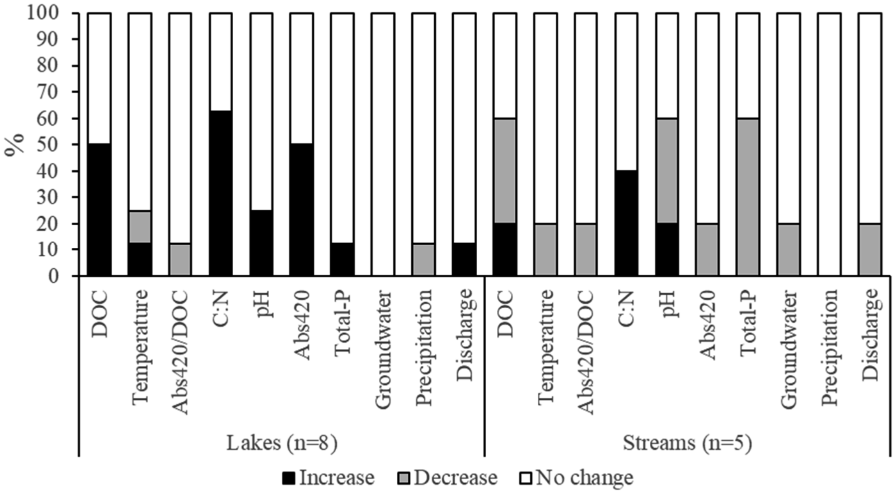

*Figure 4* Percentage of significant increases, decreases or no change in dissolved organic carbon (DOC), temperature, absorbance at 420nm ($abs_{420}$)/DOC, carbon:nitrogen (C:N), pH, $abs_{420}$, total phosphorus (total P) and catchment area precipitation, discharge and groundwater in surface waters of boreal lakes (*n*=8) and streams (*n*=5) during the period 1997 to 2017.

weight compounds (Amon and Benner 1996). A positive relationship between high molecular weight DOC and $CO_2$ has also previously been demonstrated (Bodmer *et al.* 2016). Likewise, D'Amario and Xenopoulos (2015) found that larger and more complex organic carbon compounds were more important for $CO_2$ than smaller molecules. Our results are in agreement with Amon and Benner (1996), D'Amario and Xenopoulos (2015) and Bodmer et al. (2016), however, more studies on the reactivity of DOC of different quality are needed, particularly to better understand its relation to $CO_2$ production in inland waters.

### *Effects of primary production on pCO₂*

None of the 13 waters included in our study had a concurrent decrease in total-P and increase in $abs_{420}$. One of the lakes had increased in total-P, while in the remaining seven lakes, total-P had remained constant. If primary production was the key mechanism explaining the observed $pCO_2$ increase in our study waters, we would expect to see a decrease in total-P as P concentrations could be a limiting factor for primary production (Schindler 1977, Elser *et al.* 1990). Consequently, this increase or lack of change in total-P in the lakes suggests that the observed $pCO_2$ increase in our study lakes was not due to a nutrient controlled decrease in primary production. In eutrophic lakes, primary production could exert strong controls on $pCO_2$, whereas in oligotrophic lakes, microbial mineralization likely has a stronger control on $p\mathrm{CO_2}$ (Cole and Caraco 2001, Pacheco *et al.* 2014). All of our study lakes are oligotrophic, further supporting our finding that the observed $p\mathrm{CO_2}$ increases were not due to decreased primary production resulting from nutrient limitation.

For streams, the stream size could play an important role in determining the importance of primary production controls on $p\mathrm{CO_2}$. Much of the $\mathrm{CO_2}$ in headwater streams is originally produced in the surrounding catchment and transported via groundwater to the streams (Wallin *et al.* 2013), while further downstream in-stream metabolism becomes more important (Hotchkiss *et al.* 2015). All our study streams are higher order streams (catchment areas between 21 and 1254 $\mathrm{km^2}$), and three of the streams showed a reduction in total-P, hence it is possible that a decreased primary production has resulted in the observed $p\mathrm{CO_2}$ increase in these streams.

In four of the lakes, $\mathrm{abs_{420}}$ had increased, hence, despite no change in total-P, the darkening of the water in these four lakes could have reduced the primary production. As $\mathrm{abs_{420}}$ increases there is less light available for primary production, thus $\mathrm{CO_2}$ bio-uptake would decrease and $p\mathrm{CO_2}$ subsequently increase (Jones 1992, Thrane *et al.* 2014, Nydahl *et al.* 2019). $\mathrm{Abs_{420}}$ had not increased in any of the streams, however in Bergmyrbäcken we observed a decrease in $\mathrm{abs_{420}}$. Since Bergmyrbäcken was one of the three streams showing a decrease in total-P we cannot determine whether primary production was a strong driver for $p\mathrm{CO_2}$ or not in this stream.

### *Effects of changes in the carbonate system on pCO₂*

An increase in pH would theoretically lead to a decrease in $p\mathrm{CO_2}$, however, pH had increased in two of the eight study lakes. This pH increase could be explained by the recovery from acidification of many Swedish inland waters due to reduced atmospheric sulfur deposition (Vuorenmaa *et al.* 2006). Despite the increase in $p\mathrm{CO_2}$, pH had not changed in the other six lakes. Consequently, other processes causing the $p\mathrm{CO_2}$ to increase were generally more important than distribution changes within the carbonate system for the long-term $p\mathrm{CO_2}$ trends in our study lakes. We also found an increase in pH of one stream. Conversely, in two of the streams (Vindbron and Visman Nybble) pH had decreased, hence distribution changes within the carbonate system could potentially explain the observed $p\mathrm{CO_2}$ increase in these streams.

### *Effects of changes in hydrology on pCO₂*

Our hypothesis that $p\mathrm{CO_2}$ has increased as a result of an increased groundwater input could not be confirmed in this study since groundwater levels had remained constant during the 21-year study period in all study catchments, except Bergmyrbäcken where groundwater levels had decreased. Thus, groundwater level changes are probably not the main driving force for any observed increase in $p\mathrm{CO}_2$ in our study waters. Likewise, changed precipitation is probably not key driver of the observed $p\mathrm{CO}_2$ increase, except potentially for Fräcksjön where precipitation in the catchment had decreased during the study period. As there was no other $\mathrm{CO}_2$ driving mechanism (i.e., DOC mineralization, primary production or distribution changes within the carbonate system) that had changed in Fräcksjön during the 21-year study period, the change in precipitation was the most likely driver for the observed $p\mathrm{CO}_2$ increase in this specific catchment. Discharge had increased in the catchment of Brunnsjön, hence WRT would have decreased and could thus not explain the observed $p\mathrm{CO}_2$ increase. However, none of the other key $p\mathrm{CO}_2$ driving mechanisms, DOC mineralization, primary production or distribution changes within the carbonate system, had changed in Brunnsjön during the 21-year study period. Therefore, we cannot establish what the key driver behind the observed $p\mathrm{CO}_2$ increase was in Brunnsjön. Perhaps there had been enough smaller, but not significant, changes among the different mechanisms that combined led to a significant $p\mathrm{CO}_2$ increase. Discharge had decreased in the stream Bergmyrbäcken, and although in-situ carbon transformation processes are less important for $\mathrm{CO}_2$ production in streams than in lakes (Jonsson *et al.* 2003, Hotchkiss *et al.* 2015), the decreased discharge may still have led to the observed $p\mathrm{CO}_2$ increase.

## *Conclusions*

Our detailed analyses of $p\mathrm{CO}_2$ variation in eight lakes and five boreal streams during a period of 21 years clearly demonstrates that there is not a single main driver behind the observed synchronous long-term $p\mathrm{CO}_2$ increase in these inland waters. Instead, we found a large variability in main drivers that showed clear site-specific patterns. In lakes, stimulation of microbial mineralization by increased DOC and/or changed DOC quality to suppressed primary production as a response of light limitation by increased water color were found to be the more common drivers. In streams, the more dominating mechanisms were either carbonate system distribution changes due to decreased pH or a possible decreased primary production due to nutrient limitation. Thus, we conclude that the long-term $p\mathrm{CO}_2$ control is site-specific with complex interactions of drivers and that spatially derived models cannot be applied to predict $p\mathrm{CO}_2$ patterns over time. These site-specific long-term

$p\text{CO}_2$ controls need to be accounted for in order to accurately predict future large scale $\text{CO}_2$ emissions from inland waters.

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