Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

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Abstract

Many agricultural streams in Sweden exhibit high concentrations of nitrogen, phosphorus, suspended sediments and dissolved organic matter (DOM). Together these substances cause eutrophication in streams, rivers and eventually the Baltic Sea. The interactions between different fractions of DOM and nutrients are not very well understood. The aims of this study were to investigate spatial and temporal patterns in DOM in Swedish agricultural streams and to understand how DOM and nutrients interact. Ten catchments in south – central Sweden that are dominated by arable land were compared. Most of the catchments have intensive crop production with high nutrient turnover and in some the livestock density is also high. All these factors can affect the qualitative and quantitative properties of DOM. Water samples from the catchments and fields were analyzed for week 2, 4, 6 and 8 2017, covering a time period of one and a half month. DOM was analyzed optically using excitation-emission fluorescence spectroscopy (EEM) and absorbance spectroscopy (UV/Vis). A number of spectroscopic indices were used to describe the DOM properties. Turbidity, total organic carbon, dissolved organic carbon, pH and orthophosphate concentration were also analyzed. In addition, information about nitrogen and phosphorus concentrations was available. Only weak temporal trends could be found in the dataset but clear spatial differences in DOM properties were observed between the catchments. Catchment F26 differed from most other catchments by having heavier, more labile and to a greater extent plant derived DOM. Catchments O18 and E21 had opposite characteristics to F26 in nearly all studied indices. The DOM was fresher and the molecular weight lower. Correlations between phosphorus and several particulate properties could be found, which describe the absorbing nature of dissolved phosphorus. Nitrogen correlated with the molecular weight of DOM and with the ratio of humic-like to fuvic-like fluorophores. Neither nitrogen nor phosphorus correlated with the protein-like fluorophore. The results demonstrate the importance of reducing leaching of particles and large dissolved organic molecules to streams, since these fractions are related to nitrogen and phosphorus concentrations. This can be done by installing ponds and wetlands and by preventing erosion along stream banks. However, catchment specific actions need to be taken, since the study clearly demonstrated differences between catchments when it comes to DOM and nutrient properties.

Keywords: dissolved organic matter, excitation-emission fluorescence spectroscopy, absorbance spectroscopy, eutrophication, biogeochemistry, absorbance index, fluorescence index

Sammanfattning

Många svenska vattendrag på jordbruksdominerade avrinningsområden bär höga koncentrationer av kväve, fosfor, suspenderade sediment och löst organiskt material (DOM). Tillsammans bidrar dessa substanser till övergödning av vattendragen. De biogeokemiska mekanismer som ligger bakom substansernas samverkan och sättet på vilket samverkar är inte välkända. Syftet med den här studien var att beskriva temporära och spatiala mönster i DOM egenskaper och koncentrationer, samt att undersöka hur DOM och näringsämnen samverkar. Tio jordbruksdominerade avrinningsområden i södra och centrala Sverige ingick i studien. På de flesta områdena bidrar en intensiv grödproduktion till hög näringsomsättning, vilket kan påverka egenskaperna hos DOM i omkringliggande vattendrag. Flödesproportionella vattenprov togs varannan vecka tillsammans med stickprov. I studien analyserades prover från vecka 2, 4, 6 och 8 år 2017, vilka täcker en tidsperiod av en och en halv månad. DOM analyserades optiskt med excitations-emissions fluorescensspektroskopi (EEM) och absorbansspektroskopi (UV/Vis). Olika spektroskopiska index användes för att beskriva DOM. Turbiditet, totalt organiskt kol, löst organiskt kol, pH och ortofosfat mättes även. Därtill användes tillgänglig information om olika kväve- och fosforkoncentrationer i vattendragen. Statistiska samband mellan de olika indexen och kväve, fosfor och kol analyserades, liksom temporära och spatiala mönster. Enbart svaga temporära trender kunde hittas. Däremot skiljde sig vattendragen tydligt från varandra. I avrinningsområde F26 var DOM av större molekylär storlek, mer labilt och till högre grad av växtursprung jämfört med övriga avrinningsområden. Område O18 och E21 hade motsatta egenskaper till F26 i så gott som alla undersökta index. Det organiska materialet var färskare och molekylerna av mindre storlek än i F26. Samband mellan fosfor och olika partikulära egenskaper kunde hittas, vilket beskriver absorberingen av fosfor till partiklar. Kväve korrelerade med molekylvikten av DOM och även med C:A, vilket beskriver förhållandet mellan humussyror och fulvosyror. Resultaten av studien demonstrerar vikten av att reducera avrinning av suspenderade partiklar och stora lösta organiska molekyler från jordbruksmark. Det kan göras genom att installera sedimentationsdammar eller våtmarker på odlingsområden, samt genom att minimera erosionsrisken längs vattendrag. Platsspecifika åtgärder bör dock planeras eftersom studien tydligt visade att avrinningsområden skiljer sig från varandra då det gäller DOM- och näringsegenskaper.

Nyckelord: löst organiskt material, excitation-emission fluorescensspektroskopi, absorbansspektroskopi, övergödning, biogeokemi, absorbansindex, fluorescensindex

Populärvetenskaplig sammanfattning

Övergödning av vattendrag och kustområden är ett allvarligt problem som berör stora delar av Sverige. Algblomningar, syrefria bottnar och försämrade ljusförhållanden är några av de återkommande problem som kan förknippas med övergödning. Naturvårdsverket har satt upp miljökvalitetsmål för att bekämpa övergödning och förbättra kvaliteten i svenska vattendrag. Målet är att, med hjälp av åtgärder på lokal nivå, uppnå god ekologisk status i svenska vattendrag fram till år 2020. De viktigaste bakomliggande orsakerna till övergödning av vattendrag är förhöjda kväve- och fosforkoncentrationer. I detta spelar avrinning från jordbruksmark en avgörande roll. Löst organiskt material bidrar även till övergödningen genom att det frigör näringsämnen vid nedbrytning, samt genom att det samverkar med kväve och fosfor i vattnet. Intensiteten och typen av samverkningarna beror på det organiska materialets kvalitativa och kvantitativa egenskaper. Ökad förståelse för de biologiska, fysikaliska och kemiska processer som styr övergödningen krävs för att kunna planera och verkställa effektiva åtgärder. I den här studien var målet att undersöka det lösta organiska materialets egenskaper i svenska vattendrag på jordbruksdominerade avrinningsområden, samt att undersöka samverkningarna mellan löst organiskt material, kväve och fosfor. Tio svenska vattendrag ingick i studien. Olika index användes för att beskriva det organiska materialets egenskaper i vattendragen. Indexen jämfördes med kväve- och fosforkoncentrationerna i vattnet. Temporära och spatiala mönster undersöktes även för att bättre förstå det organiska materialets roll i övergödningsdynamiken. Resultaten av studien visade att de undersökta vattendragen skiljde sig nämnvärt från varandra vad beträffar det organiska materialets egenskaper. Det innebär att platsspecifika åtgärder mot avrinning av näringsämnen och organiskt material alltid bör planeras. Studien visade även att fosfor främst samverkar med partikulärt organiskt material, medan kväve samverkar med löst organiskt material. Kväve korrelerade med molekylvikten av det lösta organiska materialet, vilket innebär att större organiska molekyler förekommer tillsammans med högre kvävekoncentrationer. Det faktum att fosfor binder till partiklar är ingen nyhet och det beaktas redan i planering av åtgärder mot fosforläckage, bland annat i sedimentationsdammar. Däremot kan resultatet av kvävestudien användas för planering av effektivare åtgärder mot kväveläckage. Genom att minska avrinningen av löst organiskt material, speciellt tyngre molekyler, kan även kväveläckaget minskas. Rekommenderade åtgärder innefattar även här sedimentationsdammar och konstruerade våtmarker. Genom att installera välplanerade dammar och våtmarker på jordbruksmark kan därför en dubbel vinstsituation uppstå där avrinning av fosfor, kväve minskar samtidigt som avrinning av organiskt material kan förhindras.

Table of contents

List of Tables

List of Figures

Abbreviations

a	absorbance coefficient
A:T	ratio between humic- and tryptophan-like fluorescence
AU	animal units
BIX	the freshness index
BOD	biological oxygen demand
C	carbon
C:A	ratio between humic- and fulvic-like fluorescence
CDOM	chromophoric dissolved organic matter
C:M	degree of blue-shift in the fluorescence
C:N	carbon to nitrogen ratio
C:T	ratio between humic- and tryphophan-like fluorescence
DOC	dissolved organic carbon
DOM	dissolved organic matter
DON	dissolved organic nitrogen
EEM	excitation-emission matrix
E2:E3	ratio between absorption at 250 nm and 365 nm, indicator of molecular size and weight
FA	fulvic acid
FDOM	fluorescent dissolved organic matter
FIX	the fluorescence index
HA	humic acid
HIX	the humification index
IR	infrared, refers to spectroscopy using IR light
N	nitrogen
NOM	natural organic matter
OM	organic matter
P	phosphorus
POM	particulate organic matter
SUVA	specific UV absorbance, indicator of aromaticity
T:C	ratio between tryptophan- and fulvic-like fluorescence, used to separate manure/human derived FDOM from plant derived FDOM
TOC	total organic carbon
UV/Vis	ultraviolet-visible, refers to absorbance spectroscopy

1 Introduction

Eutrophication of streams, lakes and marine ecosystems is an ongoing problem globally and in the Baltic Sea region (HELCOM, 2007). Extensive algal blooms, oxygen depletion and reduced biodiversity are examples of negative effects of eutrophication on aquatic ecosystems. International agreements, such as the EU Water Framework Directive (2000/60/EC), Nitrates Directive (1991/676/EEC) and the HELCOM Baltic Sea Action Plan (HELCOM, 2007), were signed in order to combat eutrophication and problems associated with it. The Swedish Environmental Protection Agency (SEPA) has set up 16 national environmental quality objectives with the aim of meeting the international directives and improving the quality of the natural environment. Two of the goals touch eutrophication and surface water quality. These are Zero Eutrophication, which states that:

"Nutrient levels in soil and water must not be such that they adversely affect human health, the conditions for biological diversity or the possible varied use of land and water"

Swedish Environmental Protection Agency, 1999

and Flourishing Lakes and Streams, which states that:

"Lakes and watercourses must be ecologically sustainable and their variety of habitats must be preserved. Natural productive capacity, biological diversity, cultural heritage assets and the ecological and water-conserving function of the landscape must be preserved, at the same time as recreational assets are safeguarded."

Swedish Environmental Protection Agency, 1999

Despite international and national agreements and actions adopted on local catchment level, eutrophication has still not been combated in freshwater systems in the Baltic Sea region. The Swedish national environmental quality objectives will not likely be achieved by 2020, which was the original goal set by the Swedish environmental protection agency (SEPA, 2017). Successful treatment of eutrophic freshwater systems comes down to mitigation measures on local level where nitrogen, phosphorus, suspended solids and organic matter leaching is taken into account (Elmgren & Larsson, 2001). Interactions between these substances are important in eutrophication dynamics, but not very well understood.

The aims of this study were to measure and evaluate organic matter properties in Swedish agricultural streams, including temporal and spatial variation. Secondly, the DOM properties were compared to phosphorus and nitrogen concentrations in order to better understand the dynamics of eutrophication.

2 Literature review

2.1 Organic matter in agricultural streams

2.1.1 Fractions of dissolved organic matter

Natural organic matter (NOM) is ubiquitous in the aquatic environment. It is present in dissolved, colloidal and particulate fraction in natural waters (Hudson *et al*. 2007). Of these, the dissolved fraction is the most abundant and studied and the one that will be the main focus of this report. Particulate organic matter (POM) and total organic carbon (TOC) will also be discussed.

Only around 25% of the dissolved organic matter (DOM) has been characterized (Baker & Spencer, 2004). Because of its heterogeneity, complexity and often low concentration in natural waters, characterization can be challenging (Postnikova, 2015). The character of DOM varies from simple non-humic organic substances like the protein tryptophan to complex humic compounds (Hudson *et al*., 2007), as shown in *Figure 1*. The majority of DOM comprises humic substances. Essington (2015) recently described the composition of DOM, by stating that up to 90% of the DOM comprises humic substances. Humic substances are degradation products that are large and coloured. The macromolecules can have an aliphatic or aromatic core structure with many functional groups. The most common functional groups are carboxylic groups (-COOH) and hydroxyl groups (-OH), but amines ($-NH_2$) and sulfhydyl groups (-SH) are also common. Due to dissociation of carboxylic groups the net charge of humic substances is negative. Partial positive charges can also be found in the structure. The exact structure of humic substances is often not known, because of the large size and complex structure. Instead humic substances are divided into three groups based on solubility: humic acids, fulvic acids and humins. Fulvic acids are the most soluble fraction of humus. They are always dissolved in water regardless of the pH (Piccolo, 2002). One suggested reason to the high solubility is that fulvic acids have more carboxylic groups than the other humus fractions, which would make their solubility less dependent of the acidity of the water (Essington, 2015). Fulvic acids are also smaller and more polar than humic acids (Rosario-Ortiz & Korak, 2017). Humic acids are insoluble in water at pH below 2 but soluble at higher pH. Finally, humins are not soluble under any pH conditions, and thus not part of the DOM of the water (Piccolo, 2002).

The non-humic substances are produced in order to sustain life processes and they include substances such as carbohydrates, nitrogen compounds, lipids and lignin (Essington, 2015). Free amino acids are present only in low concentrations in natural waters (Reynolds, 2003), while phenols are an important part of DOM (Sleighter

et al., 2010; Coble *et al.*, 2014). Phenols are produced by higher plants and algae. They are found in most aromatic acids (Coble, 2014). Polyphenolic compounds such as tannins are common in terrestrial biomass and they can be used as indicators of terrestrially derived DOM (Sleighter *et al.*, 2010). They should be used as indicators with caution, though, since they can also be produced by brown algae (Chkhikvishvili & Ramazanov, 2000). Other common compounds in the non-humic DOM fraction are indoles and phenylpropanes. Indoles are important in biosynthesis of tryptophan and phenylpropanes are associated with lignin, which is one of the major components in plant tissues. Lignin is the second most abundant terrestrial biopolymer after cellulose and therefore an important constituent of DOM (Boerjan *et al.*, 2003).

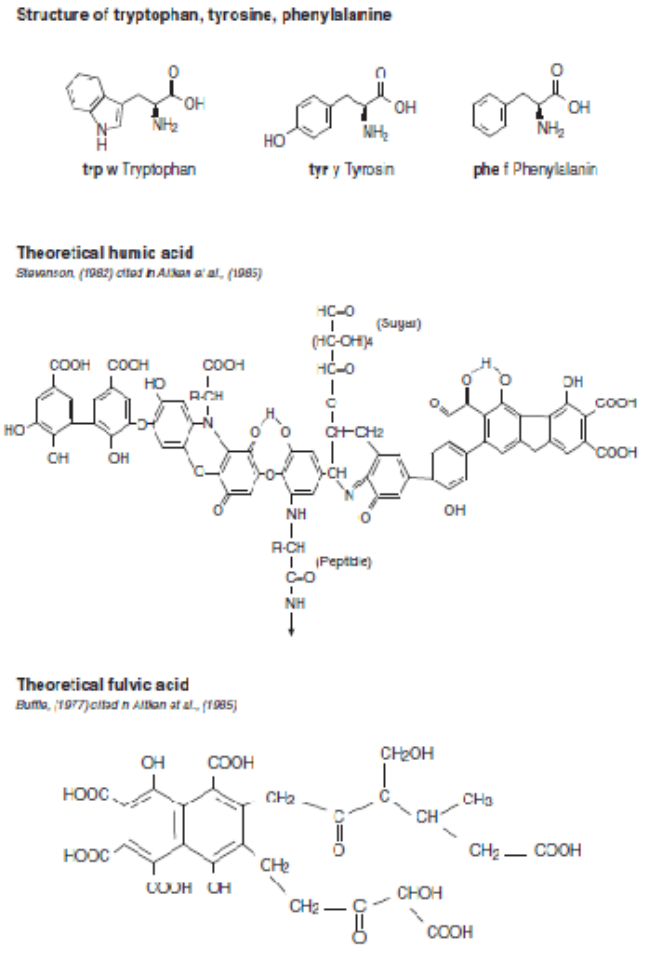


Figure 1. Molecular structures of the aminoacids tryptophan, tyrosin and phenylalanin along with hypothetical structures of humic and fulvic acids (Figure: Hudson *et al.*, 2007).

2.1.2 Spatial variation in DOM concentration and composition

Spatial variation in DOM concentration and composition can be explained by land use, land use changes, topography, hydrology and other catchment properties (Baker & Spencer, 2004; Wilson & Xenopoulos, 2009; Kothawala *et al*., 2014). These factors affect the formation, transport and transformations of DOM in streams (Hudson *et al*., 2007). Downstream water quality depends directly on the quality of headwaters (Withers *et al.*, 2009). In headwaters the DOM concentrations are typically higher than in downstream sites because DOM can be mineralized to CO_2 through decomposition or sedimented along the stream (Aufdenkampe *et al.*, 2011).

DOM can originate from various sources. Allochtonous DOM is produced outside of the water course and transported to it along the hydrological pathways or produced in upstream sites. Autochtonous DOM is formed *in situ* through metabolism of aquatic organisms or by carbon fixation (Wilson & Xenopoulos, 2009; Stedmon & Cory, 2014; Ledesma *et al.*, 2015). The composition and complexity of DOM can be used to define the origin of the material (Wilson & Xenopoulos, 2009). *In situ* production of DOM can take place over all levels of the food chain (Stedmon & Cory, 2014), but some levels might be of greater importance than others in given ecosystems. For example DOM released by phytoplankton is more common in the open ocean than in coastal areas and freshwater systems (Fleming-Lehtinen *et al.*, 2015). *In situ* production of DOM depends on external factors in the aquatic system. It can be inhibited through a negative feedback mechanism where the depth of the photic zone is decreased as a result of increased concentration of coloured DOM (CDOM) in the water, which absorbs light in the photosynthetic range and inhibits production of new organic matter (Ferrari *et al.*, 1996). Flocculation of DOM can also change the concentration and production of DOM in riverine systems. Generally DOM in streams show a conservative behaviour, but some removal may take place through flocculation (Amon & Benner, 1996). Flocculation can be an important removal mechanism of metals bound to DOM, but it may also lead to deficiency of essential trace metals in the surface water (Wells, 2002).

The three main land use types that can affect the DOM content instreams are agriculture, forestry and urban areas. Most of the global terrestrial carbon is stored in boreal regions (Ledesma *et al.*, 2015). Forests and peatlands are therefore potential important contributors of DOM in Swedish agricultural streams, when present in the catchment (Ledesma *et al*., 2015). The quality of DOM originating from forests and peatlands differ from that of cultivated areas, by having a high carbon to nitrogen ratio (C:N), low bioavailability and high aromaticity (Asmala *et al*., 2013). These properties can be explained by the dominance of humic, highly aromatic compounds found in forest soils, which are often polyphenolic and of plant origin (Kothawala *et al.*, 2015). Overall the structures of DOM derived from peatlands and

wetlands are more complex than DOM derived from agricultural land (Wilson & Xenopoulos, 2009).

Agriculture has proved to contribute with more DOC and DON to headwaters than boreal forests, despite the high carbon pool in forested areas (Wilson & Xenopoulos, 2009; Heinz *et al.*, 2015). DOM originating from agriculture is often more reactive than DOM from natural systems, which can be seen in a lower molecular weight, low C:N ratio and a higher bioavailability (Wilson & Xenopoulos, 2009; Asmala *et al*., 2013; Heinz *et al.*, 2015). Agriculturally derived DOM is to a great extent of microbial origin, which explains the high reactivity and biodegradability (Wilson & Xenopoulos, 2009). Agricultural management practices such as continuous fertilization, ploughing and drainage optimize the conditions for mineralization, which also contributes to the increased reactivity of DOM, higher turnover of organic matter and increased leaching through changes in the hydrology. The productivity in aquatic ecosystems may therefore increase if the input of agriculturally derived DOM increases (Heinz *et al.*, 2015). The composition of agriculturally derived DOM is more processed by microbes than DOM from forests, which is reflected in a highly aromatic structure (Heinz *et al*., 2015). Increased cropland coverage and reduced wetland coverage will lead to degradation and decreased structural complexity of DOM, which leads to lower molecular weight, reduced aromaticity and increased lability of the DOM compounds. This makes the transition from wetlands to croplands one of the most important land use changes that will affect the DOM characteristics (Kalbitz *et al.*, 1999; Zsolnay *et al.*, 1999).

In agriculture the use of slurry and manure as fertilizers may be a source of DOM. Some researchers have shown that DOM from slurry applied to fields may end up in and can be detected from the recipient soon after application (Naden *et al.*, 2010), while others have shown no such leaching of DOM even after storm events (Old *et al.*, 2012). Old *et al*. (2012) explained the low transport of DOM from slurry by rapid adsorption to soil particles and immobilization through microbial breakdown. Soil type, organic matter content and the composition of the microbial community are therefore factors that may be of importance in DOM runoff from fields. Intensive farming will also increase the concentrations of dissolved organic and inorganic nitrogen in the catchment, leading to a higher nutrient load and potential eutrophication (Kyllmar *et al.*, 2006; Graeber *et al.*, 2015). In Sweden the livestock density in a catchment, often expressed in animal units (AU), is determining the maximum use of manure and the potential leaching of DOM. The maximum allowable animal density has been set to 1.6 AU per hectare (Ulén *et al.*, 2012).

DOM and nutrient leaching from fields is often referred to as diffused compared to point sources, such as waste water treatment plants and urban areas. Farmyards can be thought of as intermediate sources between diffuse and point sources. Drainage from farmyards has higher concentrations of both tryptophan-like compounds and fulvic- and humic-like compounds compared to sites without farmyards. High

flow rates during and after storm events lead to a more dilute leachate from farmyards, compared to grasslands where a higher discharge leads to higher amounts of tryptophan-like and humic/fulvic-like compounds (Old *et al*., 2012). Finally, important urban sources of DOM include sewage effluent inputs from waste water treatment plants and discharge from septic tanks (Withers *et al*., 2009). The number of household in a catchment and the type of sewage treatment applied can affect the DOM composition in the surrounding streams. Human wastes can be difficult to separate from animal wastes and animal feed in water samples, since they have similar DOM properties (Old *et al*., 2012). Both sources can explain elevated concentrations of protein-like compounds (Baker & Spencer, 2004).

2.1.3 Temporal variation in DOM concentration and composition

The main temporal variation in surface water quality in Sweden is seasonal with key events such as snowmelt, primary production and storm events. These events alter the hydrology (see *Figure 5*) and availability of newly produced DOM in the aquatic system. Spring floods bring suspended and dissolved organic matter from the catchments to the streams and ultimately increase the concentrations of DOM (Stedmon & Cory, 2014). The quality of DOM may also change during snowmelt through an increase in the percentage of humic substances (Gabor *et al*., 2014). Spring and summer are ecologically sensitive periods when the biological activity is the most intense. Biological events such as algal blooms increase the autochtonous DOM input, while consumption and degradation may decrease the DOM concentration and alter the properties of DOM (Garbor *et al*. 2014). Degradation and its impacts on DOM properties and nutrient release will be discussed in further detail in the next chapter. Another example of a biological event that is important for the DOM quality during spring and summer is the spawning period of fish, which may temporally increase the abundance of proteins in streams (Hood *et al.*, 2007). The highest DOM concentrations in Sweden are often recorded during the summer in southern Sweden where the microbial activity is high and the water throughput reduced (Stedmon & Markager, 2005). Autumn storms are finally key events that may bring plant residues and DOM to streams. Storm events may alter the DOM in the catchment by increasing flow, runoff and biodegradability, which leads to lower proportion of protein-like compounds and higher proportion of humic-like compounds. The trend may be revered in wetlands (Fellman *et al.*, 2009).

The bioavailability of DOM and dissolved organic nitrogen (DON) can also change seasonally, with the highest availability during spring flood when labile nitrogen compounds, such as dissolved amino acids, are abundant. In some cases the seasonal bioavailability of DOM can be diminished because of seasonal adaptations by the bacterial community (Asmala *et al*., 2013). In addition, seasonal variation in

DOM can be reduced in catchments dominated by drained agricultural land because precipitation is drained rapidly (Stedmon & Cory, 2014).

Long-term changes in DOC and DOM quality have been reported globally (Evans *et al.*, 2005). A uniform increase in surface water DOC concentrations has been observed in northern and central Europe as well as in eastern North America (Monteith *et al.*, 2007). Many explanations to the increase have been suggested in the literature. The main hypothesis links long-term DOC increase to reduced sulphur deposition. According to this hypothesis the increased DOC concentration is a result of recovery from acidification and return to pre-industrial DOM levels (Monteith *et al.*, 2007). Some researchers have focused on climate change and states that increased temperatures has led to a greater microbial breakdown of peat and thereby a higher export of DOM from land to water over the last 10-20 years in agricultural areas of Great Britain (Baker & Spencer, 2004). Others have shown that land use changes towards more agricultural land and less peatlands can explain increasing DOM concentrations (Kalbitz *et al.*, 1999; Zsolnay *et al.*, 1999). In line with global increases in DOM and DOC concentrations, the DOM inputs to the Baltic Sea have also increased since the 1990s (Hoikkala *et al.*, 2015) as well as the DOM concentrations in boreal surface waters in northern Sweden (Ledesma *et al*., 2015). The turnover time of DOM in lake systems ranges between hundreds to a few thousands of years. Because of the long turnover time of DOM and the increased DOM inputs, the effects on the ecosystems will be long-lasting (Ledesma *et al*., 2015).

2.1.4 Organic matter as a nutrient source

One important feature of DOM is the interaction with and alteration of other substances such as nutrients and metals. DOM provides binding sites which means it can store, transport and immobilise nutrients. Especially tryptophan-like fluorescence has been shown to correlate with phosphate, nitrate and ammonia and may be a source and storage of these nutrients (Baker & Inverarity, 2004). Similarly, DOM interacts with POM through physicochemical processes, such as absorption. According to Postnikova (2015) up to 90% of the total DOM can be absorbed to particle surfaces. In this way the DOM concentration can be determined by the presence of POM and mineral particles.

Nutrients can also be released from NOM (Bushaw *et al.*, 1996) through structural changes caused by degradation (Hudson *et al*., 2007). Humic substances hold 0.5 – 2% nitrogen by weight, which can be released as bioavailable nitrogen through degradation (Bushaw *et al.*, 1996). As a consequence organic carbon can explain up to 95% of the variation in organic nitrogen in pristine areas. In areas influenced by human activity, such as agriculture, the relation between carbon and nitrogen is not as clear (Asmala *et al*., 2013). Different types of degradation can take place in the water course. These can be divided into physical (photo-degradation by UV-light),

chemical (reactions with free radicals) and biological degradation (Amon & Benner, 1996). Biodegradation and photo-degradation are typically targeting DOM compounds of different molecular size and complexity. Microbes generally degrade labile molecules with low molecular size while larger molecules can be degraded by the impact of UV light (Hansen *et al.*, 2016). However, photo- and microbial degradation are often coupled in a way that DOM initially is transferred into compounds with lower molecular mass by solar radiation, which in turn are degraded by microbes. Not only the molecular structure but also the origin of DOM can influence the degradability. Generally soil derived DOM is recalcitrant and will not degrade further in the aquatic environment, at least on a short-term perspective (Hedges *et al.*, 1994). Finally, an intense degradation of reactive DOM fractions may cause oxygen depletion, which in turn will impact the aquatic organisms and the quality of the water course.

DOM also alters the pH through protonation and de-protonation and affects metal speciation, which can impact the biological and physico-chemical properties of the water (Hudson *et al.*, 2007). Fulvic acids have shown to be the only fraction that contributes to proton and metal binding in water, whereas the humic acid fraction can contribute in soils. The acid-base and metal binding properties of humic and fulvic acids are characterized by the site heterogeneity and poly-electrolytic behaviour of the compounds (Essington, 2015).

2.2 Organic matter measurements using fluorescence and absorbance spectroscopy

Some organic molecules have characteristic adsorption patterns that can be detected using different types of spectroscopic methods. Compounds can be characterized as chromophores (CDOM) and fluorophores (FDOM) based on their optical properties. Chromophores are substances that absorb light. These can be detected using absorbance spectroscopy, such as infrared (IR) or ultraviolet-visible spectroscopy (UV/Vis). Fluorophores are compounds that absorb light and re-emit light after excitation. They can be detected using excitation-emission fluorescence spectroscopy (EEM) (Hudson *et al*., 2007). Fluorescence and absorbance spectroscopy are related methods that both are based on excitation of electrons. More information about the sources and fluxes of DOM can be obtained if the two methods are applied together, compared to the single use of any of them (Baker & Spencer, 2004). However, only a small fraction of the DOM can be characterized as CDOM and of the photons absorbed by the CDOM less than 3% is emitted as fluorescence (Del Vecchio & Blough, 2004). How representative the FDOM is for the total DOM should therefore

always be considered when using fluorescence spectroscopy (Rosario-Ortiz & Korak, 2017). In the next chapter absorbance spectroscopy will be covered and in the following three fluorescence spectroscopy will be discussed.

2.2.1 Absorbance spectroscopy

Molecules can exist in discrete energy states that are determined by the electronic, vibrational and rotational energies of the molecules. When a transparent material is irradiated with electromagnetic radiation some of the radiation can be absorbed if the material contains one or several chromophores. The radiation that is left after absorption is passed through a prism, which will scatter the light and show it as a spectrum with gaps at the absorbed wavelengths. This absorption spectrum is characteristic for the molecule. When the molecule absorbs radiation, its energy state can increase to an excited state as depicted in *Figure 2*. This type of absorption spectra is called electronic and it occurs in the ultraviolet and visible region from below 400 nm to 800 nm (Suzuki, 1967).

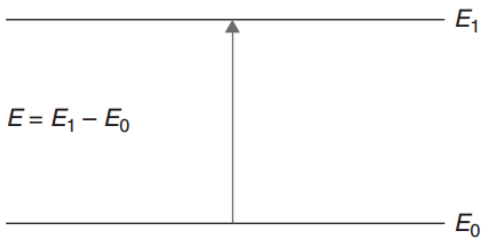


Figure 1. Absorption of energy E leads to excitation of an electron from the ground electronic state E_0 to the excited state E_1. (Image: Hudson et al. 2007)

Two other types of molecular absorption spectra are distinguished, namely vibrational and rotational spectra. Rotational spectra are caused by energy losses to rotations of molecules or parts of molecules and they occur in the far infrared region at wavelengths of 0.1-10 cm of order. Vibrational absorption is often associated with rotational absorption, which shows the fine structure of rotations. Vibrational spectra are mainly found in the near and middle infrared region (Suzuki, 1967). The increase in energy after absorption is determined by the energy of the absorbed photon, given by:

$$E = hv = \frac{hc}{\lambda} \quad \text{Eq. 1}$$

E = energy
h = Planck's constant
v = frequency of the radiation
λ = frequency and wavelength of the radiation
c = velocity of light

Electronic excitation occurs only if the energy corresponds to the difference in energy between the basic electronic state E_0 and the electronically excited state of the absorber, E_1. The amount of radiation that is absorbed is proportional to the number of molecules. It is expressed by Beer and Lambert's law and states that the optical density should remain uniform as long as the product of the concentration and path length is constant (Coble *et al*., 2014). Beer and Lambert's law:

$$I_t = I_0 exp^{-\epsilon cl} \quad \text{Eq. 2}$$

I_t = transmitted light intensity
I_0 = incident light intensity
Є = molar absorptivity
c = concentration of absorbing species
l = path length of the sample

The outcome of absorption spectroscopy is a two-dimensional spectrum showing absorbance in the unit less range between 0 and 2, at different wavelengths set by the spectrophotometer. Absorbance at given wavelengths can be converted to absorption coefficients by taking into account the pathway of the light (Helms *et al.*, 2008). Traditionally indices based on absorption ratios at different wavelengths have been used to present and interpret absorption spectroscopy data. A major advantage with such ratios is that they are independent of the CDOM concentration (Helms *et al*., 2008). Using indices information about the relative size of DOM (Peuravuori & Pihlaja, 1997), aromaticity (Piccolo, 2002), degree of humification (Chen *et al*., 2003) and other DOM properties can be obtained. Examples of indices that will be used later in this study are the *E2:E3* ratio, the specific UV absorbance (SUVA) and the spectral slope. These are summarized in *Table 1*. The *E2:E3* ratio, where the absorption at 250 and 365 nm are compared, can be applied as an indicator of the molecular size and weight (Peuravuori & Pihlaja, 1997). SUVA is calculated by dividing the absorption coefficient, often at 254 nm or 280 nm, by the DOC concentration. SUVA is strongly correlating with the aromatic fraction and can

therefore be used for estimating and comparing the concentrations of dissolved aromatic carbon in samples with different DOC concentrations (Weishaar *et al.*, 2003). The spectral slope is derived from log-transformed absorbance spectra and it gives information both about the molecular weight and aromaticity of the DOM. The spectra slope can therefore be compared to the SUVA and *E2:E3* ratio (Helms *et al*., 2008). The size of the slope, where a higher slope means a lower molecular weight (Hansen *et al.*, 2016), depends on the wavelength interval at which it is calculated (Twardowski *et al.*, 2004). The main advantage with narrow intervals is that possible variations in slope caused by dilution can be overcome compared to when broader intervals are used (Brown, 1977). Too narrow intervals may give different and non-representative slopes though, which means that the position and range of the interval has to be chosen carefully (Twardowski *et al.*, 2004).

Table 1. Absorbance indices used in the study. The calculations are presented in the method part.

Index	***Full name***	***Interpretation***	***Calculation/Reference***
E2:E3	E2:E3	Molecular size/weight	Eq. 7 (Peuravuori & Pihlaja, 1997)
$SUVA_{254}$	Specific UV absorbance at 254 nm	Aromaticity	Eq. 6 (Weishaar *et al*., 2003)
Slope	Spectral slope	Molecular weight Aromaticity	Eq. 8 (Twardowski *et al*., 2004)
Abs220	Abs220	Nitrate signal	

2.2.2 Fluorescence spectroscopy

Fluorescence is a type of spectroscopy, where light is emitted from a substance when loosely held electrons return to their ground state after excitation (Coble *et al.*, 2014). The characteristic adsorption and light-emitting properties of molecules can be explained by interactions between electrons and orbitals within atoms, in other words different types of chemical bonds in the molecule (Coble *et. al*., 2014). Excitation, vibrational relaxation and emission of light are the main processes explaining the optical properties of FDOM. Similarly as in absorbance spectroscopy, excitation of loosely held electrons initiates fluorescence spectroscopy (*Figure 2*). The electrons are excited with visible or UV light. When the electrons return to their ground state some of the energy will be released as light, which can be detected with a fluorescence spectrometer. Some of the energy will also be lost as non-detectable

heat (Lakowicz, 2006). The molecular structure controls the pathway in which the excited molecule returns to the ground state. Molecules that can rotate and vibrate freely are prone to thermal deactivation while less free molecules, such as aromatic molecules, will fluoresce (Coble *et al*., 2014). As mentioned earlier, less than 3% of the absorbed photons are emitted as fluorescence. This limits the possibility to describe the majority of the present DOM present in the sample (Del Vecchio & Blough, 2004).

In molecules where excitation takes place some degree of vibration will always be observed. The vibrations are caused by the changed electrostatic forces between the nucleus and the electrons. Because of the vibrations, not all of the energy required for electron transitions within the molecule will be used for the actual transition of electrons. Some energy is also lost as heat when the excited electron collides within the molecule. Because of this the emission energy will always be lower than the excitation energy. This leads to a so called Stokes shift, which is seen in a shorter wavelength in the excitation spectra compared to the emission spectra (Coble *et al*., 2014). Both excitation and emission wavelengths are molecular specific and thereby important when characterizing molecules (Hudson *et al*., 2007).

2.2.3 Interpreting fluorescence data

Many different types of fluorescence spectroscopy methods have been developed during the last 50 years (Hudson *et al*., 2007). Two of the main advantages with the more recent types of fluorescence spectroscopy is that they are rapid (1-5 min per sample) and that they operate on short wavelengths down to 240 nm. This means they can provide more detailed information about analysed compounds and their reactions in water samples (Hudson *et al*., 2007; Knapik *et al*., 2014). Fluorescence spectroscopy has proved to be useful in analyses of labile organic matter, which is not always the case with traditional methods (Knapik *et al*., 2014). Another advantage with fluorescence spectroscopy is the low detection limit. In modern fluorescence spectroscopy FDOM can be detected at ppb or ppm level depending on fluorophore (Hudson *et al*., 2007). Fluorescence spectroscopy is mainly a qualitative measure of DOM, but the fluorescence intensity is also correlated to the DOC concentration, making the measure also quantitative. However, the fluorescence intensity may vary if the concentration of the solute varies over time. Causes to this kind of variation could be molecular association at high concentrations, for example ionization of the solute when it comes to acids, bases and salts (Coble *et al*., 2014).

The outcome of fluorescence spectroscopy can be excitations at single wavelengths, synchronous spectra from a range of different wavelengths or an excitation-emission matrix (EEM). In modern fluorescence spectroscopy EEM is the most

common way of presenting and collecting data (*Figure 3*). EEM is a three-dimensional optical map that contains information about the excitation wavelength, emission wavelength and fluorescence intensity (Coble *et al*., 2014). It typically covers wavelengths from 200 nm to 500 nm, which corresponds to the range from UV to visible blue-green light (Baker & Spencer, 2004).

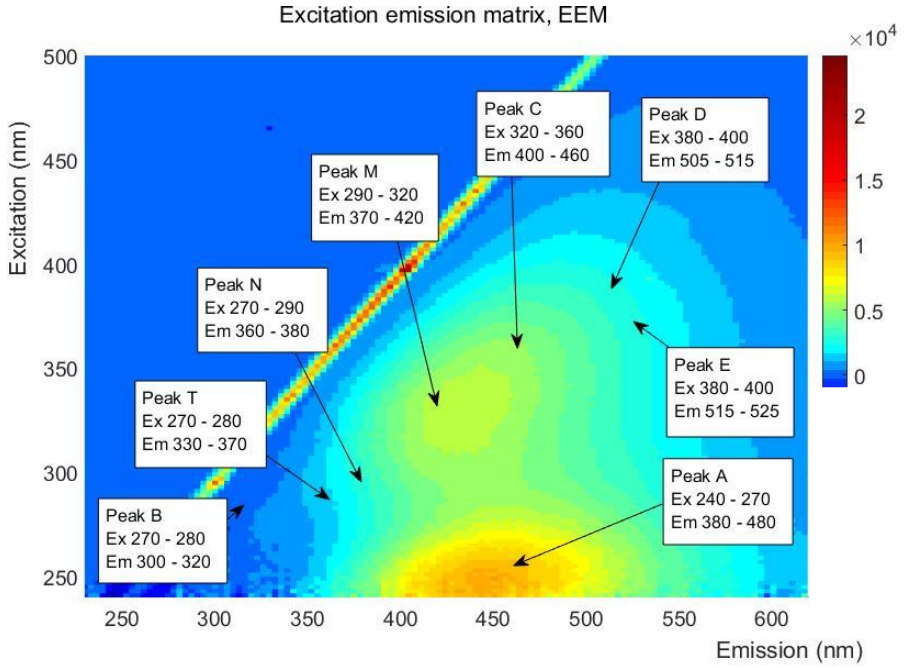


Figure 3. Excitation emission matrix (EEM) showing the positions of common fluorescence peaks. The example is from one of the studied catchments (Figure: Betty Ehnvall).

It can be challenging and in some cases not accurate to compare EEMs from different samples. Another common way of processing and presenting fluorescence data is therefore by calculating indices (Hansen *et al.*, 2016). The most common indices are the fluorescence index (FIX), humification index (HIX) and freshness index (BIX) (Coble *et al.*, 2014). These are shown in *Table 2* together with other fluorescence indices explained later. In studies of seasonal variation in DOM the fluorescence index has often been used because it changes with seasonal DOM inputs and also with changes in the flow rate and residence time of the water (Coble *et al*., 2014). In addition, terrestrial and microbial derived DOM can be identified using the fluorescence index. A higher degree of microbially derived DOM results in a higher index (McKnight *et al*., 2001; Wilson & Xenopoulos, 2009). The humi-

fication index can be used to distinguish between DOM fractions of different degrees of humification and their quantities and sorption capacities (Zsolnay *et al.*, 1999; Ohno, 2002; Hansen *et al.*, 2016). Finally, the freshness index is used to indicate newly produced DOM (Parlanti *et al.*, 2000). All these indices provide additional information about the properties and sources of DOM when used together with the absorbance indices (Coble *et al*., 2014).

Table 2. Fluorescence indices used in the study.

Index	***Full name***	***Interpretation***
FIX[a]	Fluorescence index	Separate terrestrial and microbial FDOM Seasonal changes in flow, residence time and DOM input
HIX[b]	Humification index	Degree of humification, quantity of fraction Sorption capacity
BIX[c]	Freshness index	Newly produced or old FDOM
A:T ratio	Humic- to tryptophan-like fluorescence	Amount of humic-like vs fresh-like fluorescence Recalcitrant vs labile fluorescence
C:A ratio	Humic- to fulvic-like fluorescence	Amount of humic-like vs fulvic-like fluorescence
C:M ratio	Humic- to marine-like fluorescence	Degree of blue-shift in the fluorescence
C:T ratio	Humic- to tryptophan-like fluorescence	Amount of humic-like vs fresh-like fluorescence Recalcitrant vs labile fluorescence
T:C ratio	Tryptophan- to humic-like fluorescence	Manure/human waste origin vs plant origin

[a] (McKnight *et al*., 2001); [b] (Ohno, 2002); [c] (Coble *et al*., 2014)

Identification of molecules can be performed in different ways using EEMs. The most common practice is to identify compounds based on the position of the maximum wavelength of excitation and emission (Coble *et al*., 2014). Using this method eight groups of fluorophores can be identified (*Table 3*). Three amino acids are used as indicators of proteins and peptides in water; tryptophan, tyrosine and phenylalanine (*Figure 1*). These molecules have shared electrons, either in a benzene ring or in some other aromatic ring structure. The shared electrons are loosely held in the structure and the compounds therefore fluoresce. Compounds with a fluorescence in the same area as these amino acids are called protein-like. These correspond to

peak B (tyrosine-like fluorescence) and peak T (tryptophan-like fluorescence; Hudson *et al*., 2007). Tyrosine could also be considered as a phenolic compound because it is the phenol group of the molecule that is responsible for the fluorescence (Coble *et al*., 2014).

Table 3. Peak labelling and fluorescent groups (From Coble et al,. 2014).

Peak	*Excitation maximum (nm)*	*Emission maximum (nm)*	*Description of fluorophores*
B	270-280	300-320	Tyrosine-like, protein-like
T	270-280	330-370	Tryptophan-like, protein-like
A	240-270	380-480	Humic-like
M	290-320	370-420	Marine humic-like
C	320-360	400-460	Humic-like
D	380-400	505-515	Soil fulvic acid
E	380-400	515-525	Soil fulvic acid
N	270-290	360-380	Plankton derived

The two other main groups, based on the location of the fluorescence, are humic-like and fulvic-like fluorophores (Hudson *et al*., 2007). Compounds that are humic-like correspond to peak M (marine humic), peak C and peak A (humic-like material). Fulvic-like compounds correspond to peak D and E (soil fulvic acid) (Hudson *et al*., 2007; Coble *et al.*, 2014). Of these peaks C is the most commonly found in both fresh and marine water, but it is always observed together with peak A in a ratio specific of the location (Coble *et al*., 2014). Humic acids have low fluorescence efficiencies and are often not very abundant in aquatic systems. In addition the fluorescence areas of humic and fulvic acids overlap somewhat. Interpretation of these fractions should therefore be done carefully (Rosario-Ortiz & Korak, 2016). The relative concentrations of fluorophores within the humic-like and fulvic-like regions can be indicated using the C:A ratio. Despite the fact that both peak C and A represent humic-like substances (*Table 2*) the application of the C:A ratio is relevant, since the two peak areas have shown to vary independently (Hansen *et al.*, 2016).

Different sources of DOM can be interpreted from FDOM data. In freshwater ecosystems protein-like fluorescence often indicates bacterial production and respiration, the overall respiration of the community and the bioavailability of the DOM (Coble *et al*., 2014). A more powerful way of presenting different DOM sources is by using peak intensity ratios of protein-like fluorescence to fulvic/humic-like fluorescence, which can be expressed as the T:C ratio (Old *et al*., 2012). In general, DOM originating from animal manure and human waste has a higher T:C ratio than

DOM derived from plants (Old *et al*., 2012). The T:C ratio has been shown to correlate with the BOD/DOC ratio, which is one of the ways in which fluorescence measurements relate to traditional water quality measurements (Gabor *et al*., 2014). The presence of protein-like fluorophores is not only indicating biological activity, but also often indicating an autochtonous DOM origin (Stedmon & Cory, 2014). When it comes to humic substances, those derived from plant litter and soil are generally more chromophoric than substances from microbial biomass (Gabor *et al*., 2014). Humic substances are predominantly allochtonous formed from degraded plant and soil organic matter, but they can also be autochtonous (Stedmon & Cory, 2014). The intensity at peak C and T can also be used to distinguish between recalcitrant (humic-like) and labile (newly produced) fluorophores and can thus be used together with the humification index. In this case the ratio is more commonly expressed as the C:T-ratio (Baker *et al*., 2008). Similarly the A:T ratio can be used to describe the ratio between recalcitrant and labile fluorophores (Hansen *et al*., 2016). In the case of fulvic acids the position of the emission peak can be used to distinguish between microbially and terrestrially derived fluorophores. Microbially derived fulvic acids occur at shorter wavelengths and have a more distinct emission peak than terrestrially derived acids (McKnight *et al*., 2001).

The position and maximum intensity of peak C can be used for dating DOM. Older or more terrestrial material is shifted towards longer (red-shift) wavelengths (Ohno, 2002; Coble *et al*., 2014). A red shift may be explained by increased hydrophobicity in the structure, in other words a more humic-like character. Photochemical degradation shifts peak C towards shorter (blue-shift) wavelengths, which may be explained by a decrease in aromaticity (Wu *et al*., 2003). Photochemical degradation may also decrease the fluorescence intensity (Coble *et al*., 2014). Peak M consists of newly produced fluorescent humic-like material. The name "marine humic-like" is somewhat misleading since recent research has showed that the peak also can be found in non-marine environments. Peak M is therefore rather a sign of recent biological activity (Coble *et al*., 2014). Using the C:M ratio the degree of blue-shifting can be discovered (Hansen *et al*., 2016). All fluorescence indices used in the study are summarised in *Table 3*.

The traditional peak areas can be used to identify classes of organic matter, but they can reflect several groups of fluorophores (Gabor *et al*., 2014). Interpretation of peak intensities and peak ratios can therefore be complicated and not straightforward. Alternative methods to distinguish fluorophores have also been suggested. These are based on the chemical character of the compounds, such as conjugation, hydrophobicity and acid/base character (Coble *et al*., 2014).

2.2.4 Quenching and enhancing of fluorescence intensity

Fluorescence spectroscopy is generally a more sensitive method than absorbance spectroscopy when it comes to identifying the chemical properties and sources of DOM, since not only the fluorescence intensity is measured but also the wavelength and possible shifts in it (Garbor *et al.*, 2014). Fluorescence peaks are well correlated with biological oxygen demand (BOD), total organic carbon (TOC) and dissolved organic carbon (DOC) and could replace these traditional measures of organic matter in water and also bring more detailed information about the organic matter (Knapik *et al.*, 2014). Fluorescence spectroscopy has been claimed to be a more flexible and accurate indicator than BOD (Hudson *et al.*, 2008). However, several physico-chemical properties of the water can affect the wavelength and intensity at which organic compounds fluoresce. This may limit the accuracy of fluorescence methods. The most important ones are changes in pH and temperature, chelation and other interactions between DOM and substances present in the water (Hudson *et al*., 2007). Quenching is a term used to describe reduction or elimination of fluorescence intensity. Quenching can be caused by interactions between the quenching species and the fluorescent molecule either in its ground state or excited state. The interactions can be of chemical or physical nature, such as chemical reactions or collisions between the quencher and fluorescent. The most common quencher is oxygen, which reacts with most fluorophores (Coble *et al*., 2014). Quenching can also result from non-molecular mechanisms. An example of such a mechanism is the so called inner-filter effect that can be of primary or secondary type (Ohno, 2002). In the case of primary inner-filter effect the quenching molecules absorb excitation light, which means less light is available for excitation of FDOM. In the case of secondary inner-filter effect the quencher absorbs the light emitted by the fluorophore, which means all emitted light will not be detected. The inner-filter effect is an important issue to consider when natural waters are assessed (Ohno, 2002; Coble *et al*., 2014).

Changes in pH can lead to exposing or hiding fluorescent parts of the molecule (Hudson *et al.*, 2007). In addition protonation and dissociation of functional groups bound to aromatic fluorophores can lead to a shift in the fluorescence emission of a compound, because the non-radiative processes that compete with the fluorescence may increase or decrease. Electron withdrawing groups, such as carboxylic groups, will shift towards longer wavelengths when protonated and towards shorter when de-protonated. For electron-donating groups like hydroxyl and amine the trend is the opposite; protonation will shift to shorter wavelengths while de-protonation will shift towards longer wavelengths. Because of the pH dependency of different fluorophores, comparison of spectra from waters with different pH may cause problems (Coble *et al*., 2014).

In a similar manner as protonation, metal binding influences the electronic state of the fluorescent. Depending on the nature of the metal and the functional groups in the DOM, the fluorescence may be quenched or enhanced. In most cases quenching will take place, but some CDOM compounds can become fluorescent when they react with metals and are thereby enhancing the fluorescence. Iron is a metal of relevance when it comes to quenching of natural waters because the concentrations are often high enough to alter the optical properties of DOM. Iron (Fe^{3+}) can decrease the fluorescence intensity by absorbing wavelengths that are important for excitation and emission, in other words act as an inner-filter. Despite the important effect of iron in natural waters, it is often not considered in studies of optical properties of DOM (Coble *et al*., 2014).

Thermal quenching, which is highly relevant in the sense of sample handling and storage, can be expressed in different ways. Both high and low temperatures can change the fluorescence of a sample. As temperature raises the collision frequency increases, which leads to a decrease in the fluorescence since energy is lost in the collisions. At higher temperatures it is also more likely that the excitation energy will be lost via radiation-less pathways, which leads to lower fluorescence intensity (Coble *et al*., 2014). Thermal changes are reversible, since no changes in the molecular structure take place. Thermal quenching has shown to be linear against temperature change both for humic-like and tryptophan-like substances (Carstea *et al.*, 2014).

Considering the physico-chemical properties of water discussed above, some degree of quenching can always be assumed to take place and the intensity of the EEM results always somewhat lower than the actual ones (Coble *et al.*, 2014). In the present study all mentioned quenching types might affect the results. Quenching caused by suspended particles, in other words the inner-filter effect, might be of greatest importance since the turbidity in some of the studied steams is high. Possible quenching effects were considered during the analysis and will be discussed further.

3 Materials and methods

3.1 Catchment properties

Ten catchments in southern and central Sweden were included in the study (*Figure 4*). Some of the catchments are part of the Swedish National Agricultural Monitoring Programme, while others are part of regional programmes (Kyllmar *et al.*, 2006). The ten sites were selected based on their high proportion of agricultural land, as shown in *Table 4*. Soil and crop type, as well as climate vary between the catchments. All catchments are dominated by arable land and most of them have intensive crop production with high inputs of fertilizers and high nutrient addition. Thus, the risk of nutrient and especially nitrogen leaching is high (Kyllmar *et al.*, 2014). In *Figure 5* flow rates between years 2010 and 2016 are presented for each catchment.

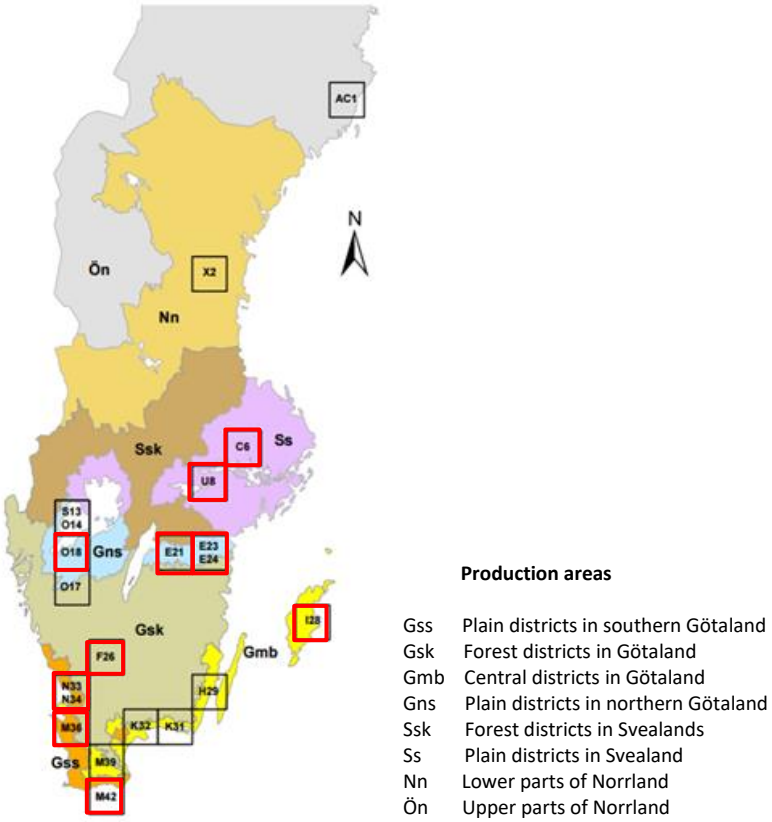


Figure 4. Catchments included in the Swedish national *Agricultural Monitoring Programme* are marked with squares and the catchments included in the study are marked in red. Swedish production areas are shown in different colours in the background map. (Figure adopted from Stjernman Forsberg *et al.*, 2015).

Table 4. Catchment properties (Adopted from Kyllmar et al., 2014)

Catchment code	***Soil texture[a]***	***Area (ha)***	***Arable land (%)***	***Temperature (ºC)***	***Precipitation (mm)***
I28	Sandy loam	4.8	84	6.9	587
N34	Sandy loam and silt loam	13.9	85	7.2	886
F26	Loamy sand	1.8	70	6.2	1066
M36	Clay, sandy loam	7.8	86	7.6	719
C6	Clay loam	33.1	59	5.5	623
E21	Sandy loam	16.3	89	6.0	506
O18	Clay	7.7	92	6.1	655
M42	Sandy loam, loam	8.2	93	7.7	709
U8	Clay	5.7	56	5.9	539
E23	Clay	7.4	54	6.3	594

Catchment code	***Production***	***Pasture (%)***	***Drained area (%)***	***Livestock density (AU ha^{-1})***	***Scattered households (persons km^{-2})***
I28	Cereals, grass, potato	2	99	0.3	11
N34	Cereals, grass, potato	2	93	0.3	19
F26	Grass	3	-	1.3	33
M36	Cereals, grass, potato	1	88	0.3	37
C6	Cereals	2	95	<0.1	10
E21	Cereals	1	95	0.2	9
O18	Cereals	0	100	<0.1	8
M42	Cereals	0	100	0.1	10
U8	Cereals, grass	2	-	0.2	-
E23	Cereals, grass	8	-	0.6	-

a. Dominant soil texture according to the United States Department of Agriculture (USDA) soil taxonomy.

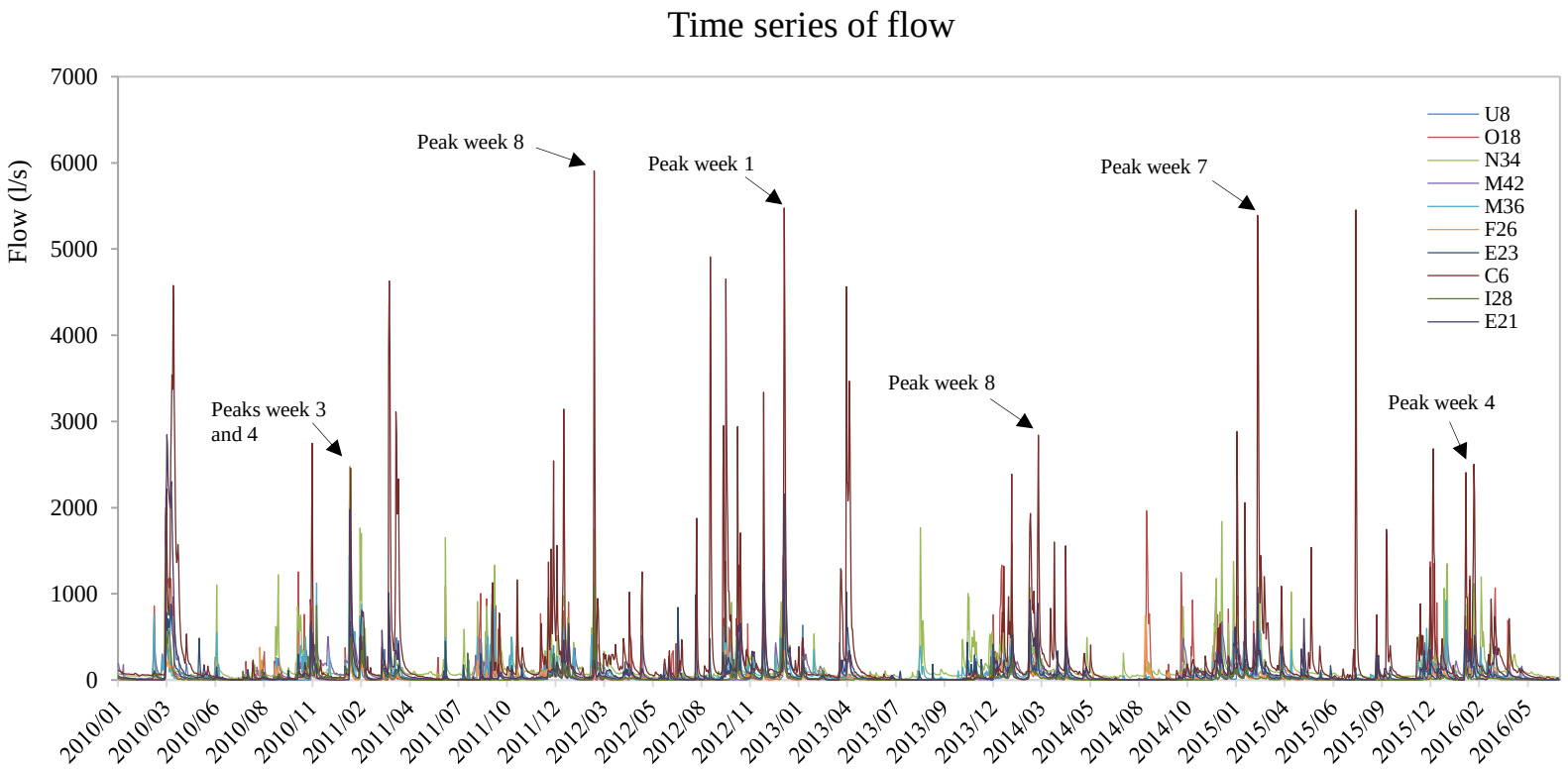


Figure 5. Average daily flow rates between years 2010 and 2016 are shown for each catchment. Peak flows for catchment C6 that occur between January and February 2011 – 2016, which corresponds to the weeks used in the present study for year 2017, are marked with arrows. The data was extracted from jordbruksvatten.slu.se (12.04.2017).

3.2 Laboratory measurements

From all catchment outlets flow proportional and grab samples were delivered bi-weekly. Samples from weeks 2, 4, 6, and 8 year 2017 were analysed, which covers a period of one and a half month during the winter-early spring months. In order to make the statistical comparison robust, fourteen observational fields around Sweden were included in the analyses. Samples from the fields were taken and treated as the catchment samples. All samples were transported to the lab and stored refrigerated (8 ^{o}C) in dark until analysis. The samples were split into three, where one was filtered using a 0.45 µm filter (F45), one using a 0.20 µm filter (F20) and one left unfiltered (UF). The following properties were analysed: DOM quantity and quality (fluorescence peak intensities and spectroscopic indices listed in *Tables 1 and 2*), TOC and DOC, turbidity, orthophosphate and pH. In addition information about the nitrogen (total nitrogen, dissolved nitrogen and dissolved nitrate), phosphorus (total phosphorus, dissolved phosphorus and dissolved phosphate), carbon (TOC) and suspended solids was available from independent water chemistry monitoring measurements, which also provided long term averages for the sites (jordbruksvatten.slu.se). Long-term averages were calculated between years 1990 and 2016.

Fluorescence in UF, F45 and F20 was analysed using Aqualog (Horriba, US) spectrophotometer. Excitation and absorbance wavelengths at 240-600 nm and emission wavelengths at 211-620 nm were used with 1 s integration time and 2 nm scan width. A high precision quartz cuvette with a light path of 10x10 mm was used. The Raman intensity of distilled water was measured from a sealed cuvette. The same cuvette was used as a reference (blank). Absorbance was analysed using AvaSoft (Avaspec-3648) spectrophotometer. Absorbance wavelengths at 180-800 nm and the same cuvette as in the fluorescence spectroscopy were used. TOC and DOC were measured with a Shimadzu TOC-Vcph analyzer. UF was used to measure TOC and F45 to measure DOC, assuming that all carbon present in the filtered sample is in dissolved form. Turbidity was measured optically in nephelometric turbidity units (NTU) using Hach Lange 2100 AN spectrophotometer. Ortophosphate was measured colourimetrically using a Hach Lange Dr2800 UV/Vis spectrophotometer at 880 nm.

3.3 Data processing and statistical analysis

Data processing and statistical analyses were performed in MATLAB (R2016a), JMP Pro 12 and Microsoft Excel 2010. EEMs were pre-processed by removing the Raman scatter, and carrying out the inner-filter correction. Maximum intensity within each peak area (C, A, B, T, M, D, E and N), and the corresponding excitation and emission wavelengths were identified. Because of the complex dataset where extraction of meaningful information can be challenging, several absorbance and fluorescence indices were analysed. The following peak ratios were calculated based on the maximum intensity within each defined peak area: C:A, C:M, A:T, T:C and C:T. Six descriptive indices were calculated from the EEMs: fluorescence index (FIX, Eq. 3), freshness index (BIX, Eq. 4), humification index (HIX, Eq. 5), $SUVA_{254}$ (Eq. 6), *E2:E3* (Eq. 7) and spectral slope (Eq. 8). Several ways of calculating the fluorescence, freshness and humification index have been suggested in the literature (Parlanti *et al.*, 2000; McKnight *et al.*, 2001; Ohno, 2002; Chen *et al.*, 2003; Old *et al.*, 2012; Coble *et al*., 2014). In this study calculations described by McKnight *et al*. (2001) for fluorescence index, Coble *et al*. (2014) for freshness index and Ohno (2002) for humification index were used.

$$FIX_{ex370} = \frac{(I\ em\ 450\ )}{(I\ em\ 500)} \qquad \text{Eq. 3}$$

$$BIX_{ex310} = \frac{(I\ em\ 380)}{(max\ I\ (em\ 420 \rightarrow em\ 436))} \qquad \text{Eq.4}$$

$$HIX_{ex225} = \frac{(\sum I\ em\ 436\ \rightarrow I\ em\ 480\ )}{(\sum I\ em\ 436 \rightarrow em\ 480) + (\sum I\ em\ 300 \rightarrow em\ 346\ )} \qquad \text{Eq.5}$$

SUVA was calculated as described by Weishaar *et al*. (2003) for absorbance at 254 nm wavelength. The absorption coefficient was given directly by the instrument. $SUVA_{254}$ was calculated as:

$$SUVA_{254} = \frac{A}{DOC} * 100 \qquad \text{Eq. 6}$$

A = absorbance
DOC = concentration of DOC (mg/l)

The *E2:E3* ratio (Eq. 7) indicates molecular weight and size of the DOM (Peuravuori & Pihlaja, 1997) and was calculated as:

$$E2{:}\,E3 = \frac{Abs\ 250\ nm}{Abs\ 365\ nm} \qquad \text{Eq. 7}$$

Spectral slope was calculated from log-transformed absorption data. Many ways of calculating the spectral slope has been suggested in the literature, summarised by Helms *et al*. (2008). Here the slope was derived from the calculation presented by Twardowski *et al*. (2004) (Eq. 8). Wavelength intervals of 275-295 nm and 350-400 nm were used because these intervals are applicable to various water types. In the outcome of the calculation the slopes are shown as positive numbers. A steeper slope thus indicates a more rapid decrease in absorption with increasing wavelength.

$$a_\lambda = a_{\lambda ref} e^{-S(\lambda - \lambda ref)} \qquad \text{Eq. 8}$$

a_λ = absorption coefficient at wavelength λ
$a_{\lambda ref}$ = absorption coefficient at reference wavelength
S = spectral slope (nm^{-1})

Finally, the absorbance at 220 nm, which correlates with nitrate, was extracted from all CDOM matrices. All indices that are used and their interpretation are summarised in *Tables 1* and *2*.

The normality of the data was explored prior to statistical analyses using descriptive statistics (scatter plot, histogram, probability plot, calculation of skewness and kurtosis). Indices and intensity peaks were compared between filtrations (variation between molecules of different size), between catchments (spatial variation) and within and fractions over time (temporal variation). Because of skewed data distribution in all indices the non-parametric Kruskal-Wallis test was used for the comparisons. Indices and peak intensities were also compared to each other and to nutrient levels. The non-parametric Spearman's rank correlation was used to investigate correlations.

4 Results

4.1 Spatial and temporal changes in DOM properties

Figures 6 – 12 show the absorbance and fluorescence indices with greatest spatial variation. Additional figures showing peak intensities and ratios with less difference between catchments are presented in Appendix 2. Because of deviation from normal distribution, spatial variation across catchments was analyzed using the non-parametrical Kruskal-Wallis test. The results are given in *Figures 6 – 12* and in Appendix 2. Since some of the indices indicate similar properties, such as the spectral slope and *E2:E2*, correlations between indices were analyzed using the non-parametric Spearman's rank correlation test. Spearman's correlation coefficients (ρ) and the significance of the correlations (p) are presented in *Table 8*. In *Tables 6* and *7* indices with differences in means between weeks (temporal variation) and fractions (variation between molecules of different size), as a result of Kruskal-Wallis test with a significance level of $p < 0.05$, are presented. All indices, TOC, DOC and turbidity are given in *Table 5*, where an (*) marks the indices that differ either between weeks or fractions and thus are separated in *Table 6* or *7*. In Appendix 1 detailed DOM data is summarized for each catchment.

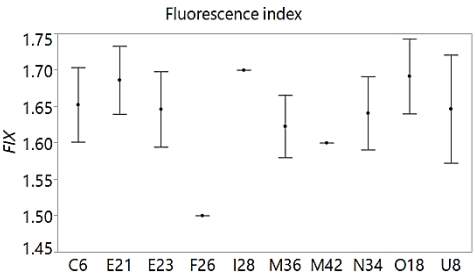


Figure 6. Spatial variation in FIX, with standard deviation given by the bars. F26 differs from all other catchments ($p<0.001$), while only pairs of some of the other catchments differ from each other, such as I28-M34

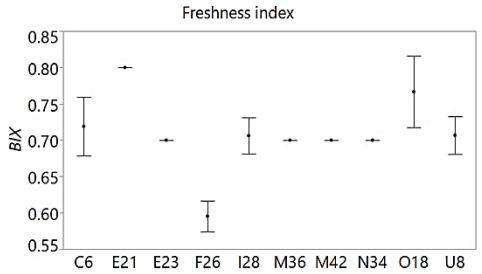


Figure 7. Spatial variation in BIX, with standard deviation given by the bars. F26, O18 and E21 differs from all other catchments ($p<0.001$), while pairs of other catchments differ from each other, such as E21-C6

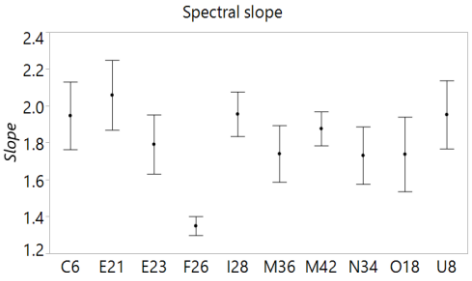


Figure 8. Spatial variation in spectral slope (Eq. 8), with standard deviation given by the bars. F26 differs from all other catchments ($p<0.001$), while pairs of other catchments differ from each other

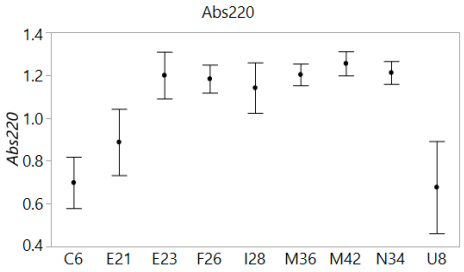


Figure 9. Abs220 with standard deviation given by the bars. F26 and M42 differ from all other catchments ($p<0.05$). Pair-wise differences also between many of the other catchments

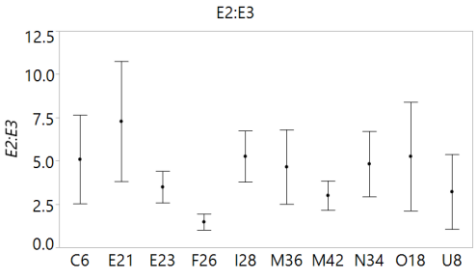


Figure 10. E2:E3 with standard deviation given by the bars. F26 differ from all other catchments ($p<0.05$). Pair-wise differences between some of the other catchments such as U8 – E21

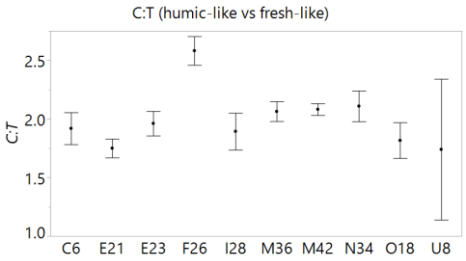


Figure 11. C:T ratio with standard deviation given by the bars. F26 differ from all other catchments ($p<0.01$) and most other catchments differ from each other ($p<0.05$)

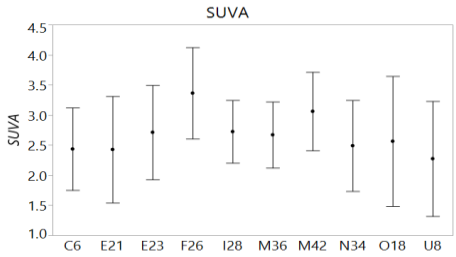


Figure 12. $SUVA_{254}$ with standard deviation given by the bars. No significant difference between the catchments

Table 5. Means and standard deviations for peaks and indices. Properties marked with (*) differ either between fractions or weeks and properties marked with (**) differ both between weeks and fractions.

Index	*Mean*	*SD*	*Index*	*Mean*	*SD*
Peak C	1.24	0.68	T:C	0.52	0.20
Peak A	3.52	2.95	FIX	1.63*	0.09*
Peak T	0.64	0.61	HIX	0.91	0.05
Peak B	0.30*	1.06*	BIX	0.71	0.06
Peak M	1.23	0.68	*E2:E3*	4.47**	2.78**
Peak D	0.57	0.34	Slope	1.77*	0.25*
Peak E	0.50	0.30	$SUVA_{254}$	2.78*	1.31*
Peak N	0.82	0.53	Abs220	1.06*	0.24*
A:T	5.69	1.13	TOC	9.86	7.03
C:A	0.36	0.06	DOC	8.41	3.93
C:M	1.00	0.03	Turbidity	13.98*	42.76*
C:T	2.00	0.31			

Table 6. Significant ($p<0.05$) temporal variation where at least one week differs from at least one other week was found for E2:E3, slope, FIX, peak B, $SUVA_{254}$ and Abs220. In no of the indices a continuous increase or decrease could be seen.

	Week 2		*Week 4*		*Week 6*		*Week 8*	
Index	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
E2:E3	5.48	3.07	3.77	1.60	2.87	1.60	5.71	3.17
Slope	1.83	0.26	1.67	0.18	1.70	0.20	1.84	0.27
FIX	1.65	0.08	1.64	0.07	1.64	0.08	1.61	0.07
Peak B	0.38	1.05	0.17	0.07	0.41	1.75	0.23	0.22
$SUVA_{254}$	2.91	0.81	3.56	0.32	2.62	2.08	2.30	0.70
Abs220	1.10	0.22	1.05	0.21	1.10	0.21	1.06	0.28

Table 7. Significant ($p>0.05$) variation between size fractions could only be found in Abs220, E2:E3 and turbidity, where both E2:E3 and turbidity are functions of the molecular size.

	UF		*F45*		*F20*	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
E2:E3	4.1	3.0	4.9	2.6	4.3	2.7
Turbidity	32.03	61.70	0.89	1.06		
Abs220	1.11	0.27	1.08	0.25	1.06	0.24

Table 8. Correlations between FDOM indices. Numbers above the diagonal line give the Spearman's correlation coefficients (ρ) and numbers below give the significance level (p).

	FIX	BIX	HIX	T:C	C:T	C:A	A:T	*E2:E3*	SUVA	Slope
FIX	-	0.72	-0.08	0.49	-0.49	-0.19	-0.15	0.43	-0.25	0.20
BIX	<0.01	-	-0.30	0.62	-0.62	-0.57	0.03	0.55	-0.37	0.30
HIX	0.56	0.03	-	-0.46	0.46	0.26	0.37	-0.26	0.19	-0.12
T:C	<0.01	<0.01	<0.01	-	-1.00	-0.57	-0.37	0.52	-0.43	0.38
C:T	<0.01	<0.01	<0.01	<0.01	-	0.57	0.37	-0.52	0.43	-0.38
C:A	0.18	<0.01	0.07	<0.01	<0.01	-	-0.43	-0.24	0.32	-0.26
A:T	0.31	0.85	0.01	0.01	0.01	<0.01	-	-0.30	0.14	-0.18
E2E3	<0.01	<0.01	0.07	<0.01	<0.01	0.09	0.05	-	-0.15	0.37
SUVA	0.09	0.01	0.19	<0.01	<0.01	0.03	0.35	0.31	-	-0.31
Slope	0.16	0.03	0.41	0.01	0.01	0.07	0.21	0.01	0.03	-

4.2 Relations between DOM and nutrients

Nutrient data was analyzed together with the DOM data to find correlations between organic matter properties and nutrients. All possible correlations were analysed in order to avoid exclusion of unexpected correlations. Temporal variation in nitrogen and phosphorus concentrations, TOC and suspended solids (see Appendix 3), which possibly could affect the correlations, were analyzed using Kruskal-Wallis test for significance. No significant ($p < 0.05$) differences between the weeks could be found. Correlations between nutrient concentrations and DOM properties were analyzed using Spearman's rank correlation test. Significant correlations for carbon, nitrogen and phosphorus are shown in *Figures 13*, *14* and *15*. Carbon correlated with the spectral slope, $SUVA_{254}$, C:T and Abs220. Nitrogen correlated with the spectral slope and C:A and phosphorus correlated with the turbidity and suspended solids. Spearman's correlation coefficients (ρ) and the significance level (p) are given in the figures. Abbreviations used in the figures correspond to DP = dissolved phosphorus, PO4 = dissolved phosphate, TP = total phosphorus, OP = orthophosphate, TN = total nitrogen, NO3 = NO_3 + NO_2, SS = suspended solids and TUR = turbidity.

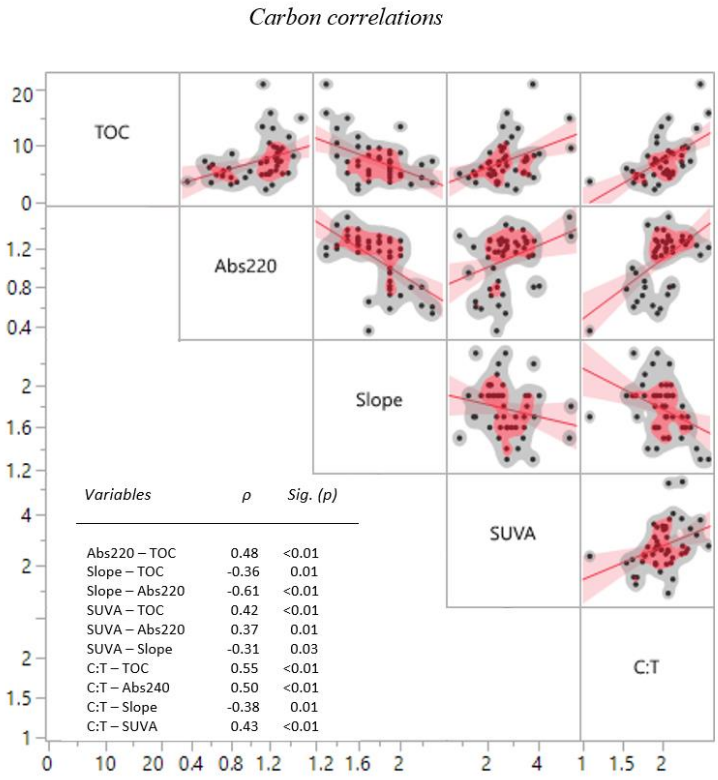


Figure 13. Significant or close to significant ($p < 0.05$) carbon correlations described by Spearman's correlation coefficient (ρ). The non-parametric density is given by the coloured area. The units for each variable are: TOC (mg/l). Abs220, spectral slope, $SUVA_{254}$ and C:T are unitless indices.

Nitrogen correlations

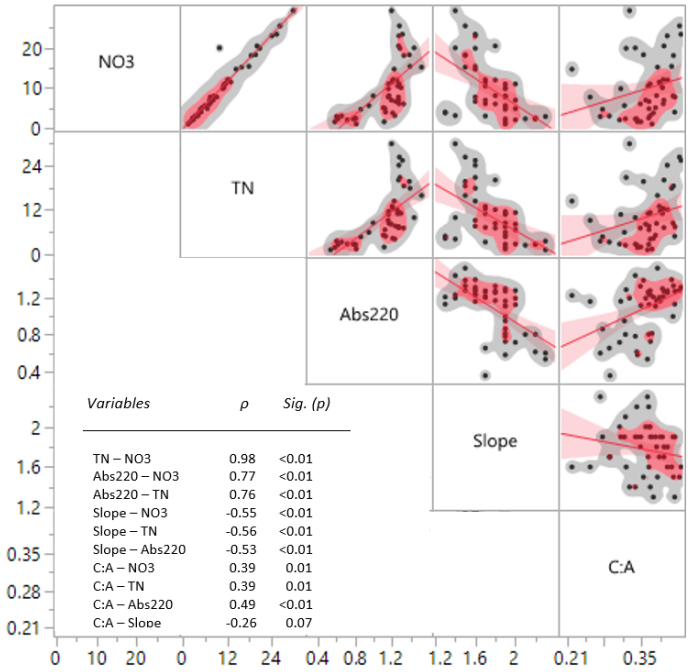


Figure 14. Significant and close to significant ($p < 0.05$) nitrogen correlations described by Spearman's correlation coefficient (ρ). The non-parametric density is given by the coloured area. Units on the axes are according to the variables: NO3 (mg/l) and TN (mg/l). Abs220, spectral slope, and C:A are unitless indices.

Phosphorus correlations

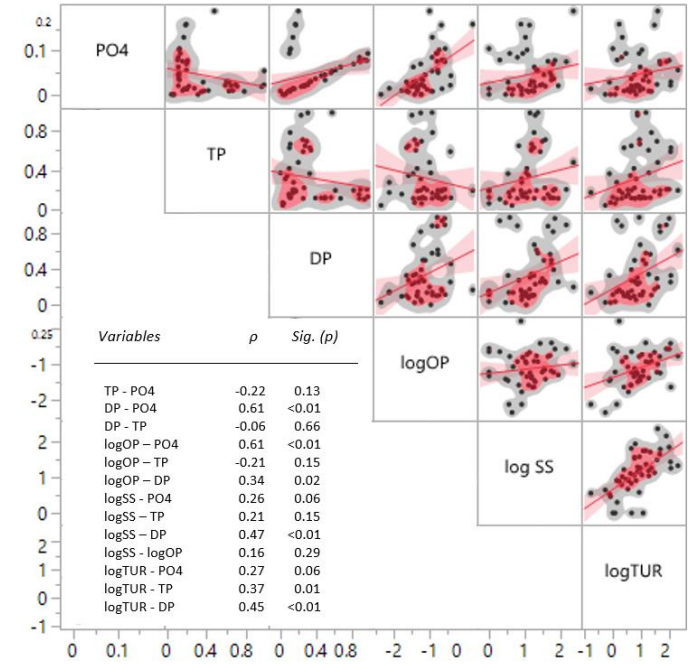


Figure 15. Significant and close to significant ($p < 0.05$) phosphorus correlations described by Spearman's correlation coefficient (ρ). The non-parametric density is given by the coloured area. Units on the axes are according to the variables: PO4 (mg/l), TP (mg/l), DP (mg/l), logOP (mg/l), SS (mg/l) and TUR (NTU.) Suspended solids, orthophosphate and turbidity were log-transformed due to skewed distribution.

5 Discussion

The quantity and quality of DOM in ten Swedish agricultural streams and their relations to nutrient concentrations have been studied. Because of the complexity and heterogeneity of DOM, various spectroscopic indices, of which some indicate similar properties, were analysed and compared. Spatial and temporal patterns in FDOM were investigated as well as the difference between unfiltered and filtered samples. In the following chapters these results will be discussed. Possible issues regarding the quality and comparability of the studied samples from the catchments and fields will also be covered. Finally implications for controlling eutrophication will be discussed.

5.1 Organic matter properties in Swedish agricultural streams

5.1.1 Spatial variation in organic matter properties

Spatial variation was analysed by comparing FDOM properties in the catchments. The most apparent variation was the one between catchment F26 and all other studied catchments. Catchment F26 differed in most of the studied indices (fluorescence index, freshness index, spectral slope, Abs220, *E2:E3*, C:T, C:M, A:T; *Figures 6 – 12* and Appendix 1 – 2) and peak intensities (Peak C, Peak A, Peak E, Peak T, Peak M and Peak N; Appendix 1 and 2). Especially catchment E21 and O18 showed somewhat opposite properties to F26. Spectroscopic indices that were high in F26 were low in E21 and O18, and *vice versa*. Because of this the discussion about spatial variation will from now on focus on the differences between F26 on the one hand, and E21 and O18 on the other.

Weather conditions during the sampling period, land use and soil properties will be considered in order to understand the reasons behind the spatial differences and similarities in FDOM. To start off with the weather conditions during the sampling period and on a longer term also micro-climatic differences between the catchments can result in differences in discharge rates. In this case the weather conditions propose a null hypothesis where catchment specific properties, such as land use, are of secondary importance in DOM leaching. The annual precipitation is high in catchment F26 (1066 mm y^{-1}), while the annual precipitation in E21 and O18 is only half of that in F26 (500 – 650 mm y^{-1}). The average annual temperature is similar in all three catchments (*Table 4*). Despite the lower precipitation in O18 and E21, the annual flow rate is similar as in F26 (*Figure 5*). This indicates that the differences in the FDOM pool are not due to differences in weather or leaching, but in catchment properties. A closer look into the precipitation and temperature during the sampling

period reveals more accurate dependencies between weather conditions and FDOM input. During the sampling period the precipitation and temperature were low in all three catchments (SMHI, 16.05.2017), which indicates that spatial differences in FDOM properties during this time of the year are likely not caused by differences in weather conditions, but in catchment characteristics.

Site specific characteristics will now be discussed as they could explain some of the spatial variation in FDOM properties. F26 is a small catchment located in the forest districts in Götaland, while E21 and O18 are larger catchments in the plain districts in Northern Götaland. The soil texture differs between all catchments. Loamy sand, sandy loam and clay are the dominating soil types in catchment F26, E21 and O18 respectively. Finally the livestock density and number of scattered households varies between the catchments. The livestock density in F26 is highest among the studied catchments, close to the maximum allowable density in Sweden of 1.6 AU ha^{-1}. The number of scattered households is also high (33 persons km^{-2}). The livestock density (<0.1 – 0.2 AU ha-1) and number of households is similar in E21 and O18 (8 – 9 persons km^{-2}), and low compared to F26 (*Table 4*). All these land use and soil factors could influence the quality and quantity of FDOM in the stream water. If the high livestock density and human population was the reason to the different FDOM pools in F26, E21 and O18, it should be reflected in the T:C ratio (Hansen *et al*., 2016). A high T:C ratio, close to 1.0, in the stream water would indicate that the majority of the FDOM originates from manure or human waste (Baker, 2001). In the present study it would mean that the T:C ratio should be higher in F26 than in E21 and O18. This was not the case. Because of a high peak C intensity in catchment F26, the T:C ratio was instead lower than in most other catchments, indicating a potentially low impact from manure and/or sewage. The relatively low protein-like fluorescence indicates low bacterial production and an overall low respiration rate of the organismal community (Coble *et al*., 2014). In addition it indicates that most of the FDOM is of allochtonous origin (Stedmon & Cory, 2014).

The freshness of the organic matter describes productivity and can result in variations in FDOM properties. The freshness was described by several indices, such as the freshness index and the ratio of humic-like to fresh-like fluorophores (C:T and A:T). The ratio of humic-like to fresh-like fluorophores (C:T ratio) was high in catchment F26 and the freshness index low (*Figure 7*). These results indicate that little newly produced material was present (Parlanti *et al*., 2000). Interestingly, the humification index was not higher in F26 than in the other catchments (Appendix 2), which would have been expected based on the high C:T ratio and low freshness index. A low degree of humification means that labile fluorescents with a low sorption capacity are present in the catchment. The overall nutrient dynamics in the stream could be affected by this fact, since the coarse soil texture does not provide the same amount of binding sites as finer particles, and the humus fraction is not

covering up for the low sorption by the mineral particles. The freshness index and spectral slope were high in E21 and O18 (*Figures 6 – 8*), indicating a recent input of fresh microbial material derived FDOM (McKnight *et al*., 2001) with low molecular weight.

Part of the spatial variation between the catchments could be explained by the origin of the FDOM. The origin can be described using the fluorescence index, where FDOM of microbial origin has a fluorescence index of around 1.8 and terrestrially derived FDOM has a fluorescence index of around 1.2 (McKnight *et al*., 2001). In catchment F26 the fluorescence index was lower compared to the other catchments (*Figure 6*). The fluorescence index was 1.5, which indicates an equal amount of terrestrial and microbial derived FDOM. This finding is in line with the low T:C ratio in the catchment, which also suggests a high influence from plant-derived FDOM. The fluorophores in F26 can be summarised as large (low *E2:E3* and slope), plant derived (low T:C) molecules that are labile (low humification index) but not newly produced (low fluorescence and freshness index). In catchment O18 and E21 the FDOM was more newly produced (high fluorescence and freshness index, and low C:T ratio) and the molecules of lower molecular weight (high spectral slope) than in F26.

It is important to keep in mind that the study period fell in a hydrologically intense period where some of the catchments might be in a spring flood phase. Many FDOM characteristics are seasonal, such as bioavailability. The bioavailability has been shown to culminate during the spring flood, resulting in a high abundancy of labile compounds (Stepanauskas *et al.*, 2000). This means that the spatial differences recorded in the present study may be different during other periods of the year. Additional considerations regarding temporal variation in FDOM properties will be discussed in the next chapter.

5.1.2 Temporal variation in organic matter properties

The studied temporal variation covered a period of seven weeks in January – February 2017. Since most of the catchments are located in southern Sweden (*Figure 4*), the study period fell in the hydrologically interesting period late winter – early spring with meltwater present, high flow and leaching potential (*Figure 5*). At these sites the winters are mild and rainy (Kyllmar *et al*., 2014). The streams do not necessarily freeze during the winter, which means they will be hydrologically active during the majority of the year (SMHI, 02.02.2017). The soil responds quickly to rainfall during this time of the year because it is more saturated than during the vegetation period. Despite these conditions, only one temporal trend (fluorescence index) and little variation in the spectroscopic indices, peak intensities and quantitative particulate properties could be found.

The fluorescence index was constantly decreasing from week 2 until week 8, by 2% in total. Even though the decrease was only slight, it should be noted since the overall variation in the fluorescence index was low. The change in the fluorescence index can be interpreted in terms of FDOM origin (McKnight *et al*., 2001) or in a shift in the flow rate, residence time and fresh input of FDOM (Coble *et al*., 2014). According to McKnight *et al*. (2001) a difference in the fluorescence index of at least 0.1 units may indicate a shift in the source of fulvic acids. Low fluorescence indices, like the ones in the present study, are typical during snowmelt (Hood *et al.*, 2003). Since the study period covered a time period that in previous years has shown great variation in flow rate (*Figure 5*), the decrease in the fluorescence index could rather be used as an indication of a changed flow rate than a change in fulvic acid source. Earlier studies have reported that the relation between discharge and the fluorescence index depends on the land use (Old *et al*., 2012). In this way temporal and spatial variation in FDOM properties are linked. Fluorescence indices on grasslands are directly related to flow, while indices from farmyards are inversely related so that a higher flow rate dilutes the water rather than increases the leaching, which in the end brings more FDOM to the streams (Old *et al*., 2012). The land use varied in the studied streams but on most catchments both grassland and farmyards are present (*Table 4*).

The spectral slope and *E2:E3* also varied between single pairs of the studied weeks. Both indices were higher in week 2 and 8 than in week 4 and 6. Additionally the intensity of tyrosine-like fluorescents, the Abs220 and $SUVA_{254}$ varied between weeks, but in individual patterns (*Table 4*). Earlier studies have shown a continuous decrease in $SUVA_{254}$ during the period spring – autumn (Asmala *et al*., 2013). No decrease in $SUVA_{254}$ could be found in the present study, likely because of the limited number of analysed weeks. Overall the $SUVA_{254}$ was high in the studied catchments, closer to earlier reported peat soil leachates than plant and algae leachates, which would have been the expected outcome based on the land use and soil type in the catchments (Hansen *et al.*, 2016). The relative amount of humic substances, and through them the aromaticity of the organic matter, generally increase along the winter months and during the spring flood when the biological activity is low (Gabor *et al*., 2014). This is likely partly the explanation to the high aromaticity in the studied samples expressed by the high $SUVA_{254}$.

5.1.3 Correlations between indices

The spectroscopic indices used in this study were correlated to see how well they describe FDOM in Swedish agricultural streams. As could be seen in *Table 8* many of the indices describing the same FDOM property are correlated, such as *E2:E3* – spectral slope describing the molecular size/weight (Peuravuori & Pihlaja, 1997; Helms *et al*., 2008), $SUVA_{254}$ – spectral slope describing aromaticity (Weishaar *et*

al. 2003; Helms *et al*., 2008) and A:T – C:T giving the ratio between humic-like and fresh-like fluorescents (Hansen *et al*., 2016). These correlating indices can advantageously be used together to describe certain properties of FDOM in the streams. From *Table 8* strong correlations can also be seen between the fluorescence and freshness index, between the freshness index and the C:T ratio, as well as between the freshness index and the T:C ratio. The correlation between the fluorescence index and freshness index can be interpreted in terms of DOM balance. The more newly produced DOM (i.e. higher freshness index), the higher the net DOM input is to the system (i.e. higher fluorescence index) despite the fact that some degradation might also take place. The relation between the two indices can also be interpreted in terms of DOM origin: during this time of the year plants induce more freshly produced DOM than bacteria to the streams.

Furthermore, the humification index correlates inversely with the freshness index, which is expected since a higher degree of humification (i.e. higher humification index) can be seen as a function of time and decreasing freshness of the organic material (i.e. lower freshness index). This is in line with previous studies showing an inverse correlation between the humification index and biodegradation (Kalbitz *et al.*, 2003). A higher degree of humification also means more recalcitrant molecules are present that are not readily degradable (Zsolnay *et al*., 1999; Ohno *et al*., 2002; Hansen *et al*. 2016) and thus less prone to form new DOM of lower molecular size.

Biodegradation has been correlated inversely to $SUVA_{254}$ in previous studies (Kalbitz *et al.*, 2003). In general, humic substances derived from plant litter have a higher aromaticity (higher $SUVA_{254}$) than substances derived from microbial biomass (Gabor *et al*., 2014). This would suggest a correlation between the fluorescence index and $SUVA_{254}$. No such correlation could be found in the present study, but instead an inverse correlation that was close to significant. This finding suggests that the aromaticity of FDOM increases with an increasing degree of microbial derived DOM. Finally, humification has earlier been reported to correlate with aromaticity (Kalbitz *et al.*, 1999). No correlation could be found in the present study between the humification index and $SUVA_{254}$. Indirectly peak ratio A:T and C:T describes the humification since they both give the ratio between humic substances and newly produced organic matter. Significant correlations could be found both between A:T and $SUVA_{254}$, and C:T and $SUVA_{254}$.

5.2 Linkages between DOM properties and nutrient status

Linkages between FDOM properties and the nutrient status in Swedish agricultural streams were analysed through correlations between nitrogen, phosphorus and carbon concentrations and the different FDOM characteristics. The linkages will be

discussed in this chapter. Factors that may influence the simultaneous leaching of nutrients and organic matter will also be discussed since they control the quantity and quality of FDOM and nutrients, and their possibilities to interact.

Analysis of correlations between nutrients and DOM properties revealed that the only significant correlations between phosphorus (total phosphorus, dissolved phosphate and orthophosphate) and organic matter properties were related to suspended solids and turbidity (*Figure 15*). Suspended solids and turbidity are both describing the presence of suspended particles in the water samples. The correlation between phosphorus and suspended solids was interpreted in terms of adsorption; the more suspended particles in the solution, the higher sorption capacity and the more phosphorus will be associated with the particles. In line with this, one would expect a correlation between phosphorus and the humification index, which describes the sorption capacity FDOM (Zsolnay *et al.*, 1999; Ohno, 2002; Hansen *et al.*, 2016). No such correlation could be found. Another expected outcome based on previous studies, which was neither confirmed by the analysed data, was the correlation between peak T and phosphorus (Baker, A. & Inverarity, R., 2004).

Nitrogen in the form of total nitrogen, dissolved nitrate and nitrate in DOM (Abs220) was correlated against the studied FDOM properties. All nitrogen fractions correlated inversely with the spectral slope (*Figure 14*), which means heavier FDOM molecules are found together with higher nitrogen concentrations (Helms *et al*., 2008). Two possible reasons to this correlation are proposed, namely anion adsorption and mineralization of organic nitrogen. The sorption capacity of organic molecules depends on their surface area and charge. Heavier molecules generally have a higher sorption capacity, which means they can adsorb more substances. FDOM can be polar, with positive and negative partial charges. This means that also anions can interact with FDOM. In this way nitrogen and FDOM could interact in a similar manner as phosphate and suspended solids. The affinity of nitrate is very low, however, which means it is not interacting with other substances in the soil environment (Essington, 2015). Therefore the correlation between nitrogen and the spectral slope could rather be interpreted in terms of mineralization. Larger molecules can contain more nitrogen that can be released from the structure through mineralization. The mineralized nitrogen will be found in the solution together with the FDOM, unless taken up by organisms. A correlation between the ratio of humic-like fluorescents to fulvic-like fluorescents (C:A ratio) and nitrogen was also found. Again, the correlation can be interpreted in terms of sorption or mineralization. Because of the larger size (Rosario-Ortiz & Korak, 2017), humic-like fluorescents can provide more binding sites or they can contain more nitrogen that can be found in and released from the molecular structure compared to fulvic-like fluorescents.

Surprisingly there was no correlation between nitrogen and peak T and B, which represent protein-like compounds. Since several classes of FDOM can fluoresce in

the same area, non-protein compounds might have overridden the protein-like fluorescence (Gabor *et al*., 2014). Also, earlier studies have shown that the relative amount of humic-like substances increases during snowmelt, which could explain the weak correlation between peak T, B and nitrogen (Gabor *et al*., 2014). Other expected nitrogen correlations that were not confirmed by this study, were those between nitrogen and the humification index, freshness index and fluorescence index. A high degree of humification generally leads to an increased content of functional groups containing nitrogen (Ohno, 2002), which would suggest a correlation between the humification index and nitrogen. However, no such correlation was found. The degree of humification has also been shown to correlate inversely with the carbon to nitrogen ratio (C:N), meaning that molecules with a higher degree of humification has a higher nitrogen content (Kalbitz *et al.*, 1999). Earlier studies have shown that the freshness index correlate with total dissolved nitrogen through a co-relationship where microbial productivity increases when the nitrogen levels increase, which in turn leads to increased fluorescence in peak M and T regions and a higher freshness index (Gabor *et al*., 2014). No correlation between the freshness index and dissolved nitrogen could be found in the present study.

In the case of carbon significant correlations could be found between TOC and the spectral slope, $SUVA_{254}$ and the ratio of humic-like to fresh-like fluorescents (C:T; *Figure 13*). The inverse correlation between TOC and the spectral slope indicates that heavier molecules contain more carbon, which is expected. The positive correlation between TOC and $SUVA_{254}$ suggests that molecules with higher carbon content are more likely to contain aromatic features such as ring structures and loosely held electrons. It is worth mentioning that in the calculation of $SUVA_{254}$ the DOC is used, which could have an impact on the correlation between $SUVA_{254}$ and TOC. Finally the correlation between TOC and C:T is expressing the relation between humic substances and TOC. Humic substances are large, complex and recalcitrant. They can therefore contain more carbon than newly produced labile organic molecules (Essington, 2015).

Many catchment specific characteristics might have affected the simultaneous leaching of nutrients and DOM. These characteristics might also have affected the properties of the leached DOM and thereby the type of interactions between nutrients and FDOM, as presented above. Nitrogen and phosphorus often behave in different and sometimes opposite ways in the soil (Kyllmar *et al.*, 2006). It might therefore be necessary to separately assess their interactions with FDOM and the factors controlling their leaching. Soil texture and structure will first be discussed as they are important factors controlling nutrient leaching. Kyllmar *et al.* (2006; 2014) demonstrated that in a long-term perspective those of the studied catchments with coarse soil texture (sand or sandy loam: I28, N34, F26, E21 and M42) had higher nitrogen leaching than fine-textured soils (clay and clay loam: E23, U8, C6 and

M36). The opposite was recorded for phosphorus losses with higher leaching from fine soils. Suggested reasons to the higher nitrogen leaching from coarse-textured soils are that soil water containing nitrate can infiltrate easily down the soil profile, denitrification rate is lower compared to fine textured soils which leads to lower gaseous nitrogen losses and higher leaching potential, and plant uptake is lower in coarse-textured soils (Wetterlind, 2006).

Phosphorus losses are dependent on the soil structure, where large macro-pores may lead to preferential flow and elevated losses of dissolved and particulate phosphorus. Also DOM leaching can be affected by preferential flow if the organic matter is adsorbed to particle surfaces (Postnikova, 2015). The pore-size distribution and presence of macro-pores is determining whether preferential flow will take place. Fine-textured soils generally have a more heterogeneous pore-size distribution with macro-pores present, whereas coarse soils have a more homogenous pore size distribution that often leads to matrix flow (Djodjic et al., 1999). Erosion of soil particles with phosphorus and/or DOM absorbed to the surface is the other main pathway of phosphorus/DOM losses (Ulén *et al.*, 2007). Earlier studies have shown that among the studied catchments the highest phosphorus losses are found in catchment O18 and U8 (Kyllmar *et al.*, 2014).

As discussed in earlier chapters, land use can affect the overall leaching and the properties of leached FDOM (Old *et al*., 2012). Similarly, nutrient leaching is affected by land use factors. The percentage of arable land in the studied catchments has been shown to correlate with nitrogen leaching (Kyllmar *et al.*, 2006). In the case of phosphorus the soil texture overrides the impact of the arable land percentage, and is thus a more powerful factor explaining phosphorus leaching (Kyllmar *et al*., 2006). Wilson & Xenopoulos (2009) showed that also the continuity of cropland is related to the dissolved nitrogen concentration. Phosphorus did not respond in the same way.

The presence and type of drainage is another important land use factor on agricultural land that influences nutrient leaching. A high percent of the areas in the studied catchments are tile-drained (*Table 4*). The discharge is greater from drained catchments than undrained, which can lead to a greater nutrient leaching (Ulén *et al*., 2012), especially when it comes to nitrogen which moves with the soil water. Because of the increased discharge from drained sites, the rain intensity will directly affect the leaching of nutrients. In this way climate properties are related to drainage and discharge. Because of these climatic factors the largest nitrogen losses have been found in the sandy loam catchments with high precipitation and mild winter climate (M42, N34 and F26; Kyllmar *et al*., 2014). The catchments with drier climate have lower losses of nitrogen, but in higher concentrations because of the lower dilution (Kyllmar *et al*., 2014). Nutrient leaching also depends on the length of the vegetation period and crop type in the catchment. Longer vegetation periods

enable higher mineralization rates, which can bring more mineralized nutrients into the aquatic system (Kyllmar *et al*., 2014). When it comes to crop type, sites where perennials are cultivated generally have a lower area-specific leaching than sites with annuals (Blombäck *et al*., 2011). In around half of the catchments included in the present study perennials are grown and in the rest cereals are cropped (*Table 4*).

Finally, animal density is a land-use factor that can influence the nutrient leaching, since organic and mineralized nutrients can be desorbed or released from the manure after degradation and transported to the streams (Bushaw *et al.*, 1996). In catchments with high livestock density (F26 and E23; Ulén *et al*., 2012) animal production could be of importance in nutrient and DOM leaching. Kyllmar *et al*. (2006) did not find a correlation between animal density and nitrogen leaching when the catchments were analysed, but if the animal density was compared to the area of ley where the manure is spread, a correlation between animal density and nitrogen leaching could be seen (Kyllmar *et al*., 2006). In the present study the T:C ratio was used to describe the presence and ratio of manure derived FDOM to plant derived FDOM. A high T:C ratio would not only indicate presence of manure derived FDOM, as has been discussed in earlier chapters, but also a higher risk that nutrients transported with the FDOM might have been released in the aquatic environment. This would be in line with the earlier reported correlations between animal density and nitrogen leaching (Kyllmar *et al*., 2006). However, no correlation between animal density and T:C could be found in the present study.

5.3 Quality of the FDOM data

The results of the study were discussed in the previous chapters. Before putting them into a larger context, which will be done in the final chapter, the quality of the results will be discussed. This will be done by considering handling and storage of the water samples, possible quenching effects and ways of overcoming these.

Correct handling and storage of water samples is important in order to avoid degradation of DOM and DON. Ideally the samples would have been filtered and analysed within 24 hours after sample collection to prevent microbial and photochemical degradation and the impact of particles on CDOM (Coble *et al*., 2014). Because of the sample collection setup, with samples being delivered from the catchments and observational fields around Sweden, instant analysis was not possible. Instead the samples were stored in 8 ^{o}C in dark until analyzed, which in the case of week 2, 2017 lasted several weeks. The samples from weeks 4 – 8, 2017 were analyzed within two weeks after sampling. The effect of storage on the particular samples was analysed by comparing fluorescence indices in samples with high and low fluorescence and absorbance in the initial measurements to the indices one month later

(result not shown). The comparison showed changes in many of the studied indices, which exemplifies the importance of instant analyzation of organic matter. The changes were more evident in samples with initially low fluorescence. In these samples the humification index and the ratio between humic-like fluorescents and fresh-like fluorescents (A:T and C:T ratio) increased after storage, whereas the spectral slope and *E2:E3* ratio decreased. All these changes point towards degradation of FDOM during storage (Peuravuori & Pihlaja, 1997; Helms *et al.*, 2008). Even though the samples in the present study were stored during a shorter period of time the same type of changes in FDOM caused by degradation could have altered the accuracy of the results.

Filtration is another point worth rising. A large proportion of the DOM, up to 90%, can be adsorbed to particle surfaces (Postnikova, 2015). Filtration and the size of the filter used will therefore not only influence quantity of POM but also the degree of adsorption and thereby the DOM concentration. In the present study two size fractions of DOM were separated from the total organic matter using filters with 0.45 µm and 0.20 µm mesh size. Fine filters remove selectively most of the suspended particles and DOM absorbed to it, which may have an impact on the spectral absorption of the sample. Adsorption of FDOM can be detected from shifts in fluorescence wavelengths. The absorbed FDOM has a lower energy yield than the dissolved FDOM leading to a shift towards longer (redder) wavelengths in the fluorescence (Postnikova, 2015). In the present study a red-shift was detected in samples from catchment M42 and E21 for peak areas A and C, which could be caused by absorption (Postnikova, 2015). However, FDOM of old or highly terrestrial origin can also cause red shifting of fluorescence (Coble *et al*., 2014).

Another important aspect to consider concerning the quality of FDOM data is the acidity (pH) of the water samples. As discussed in the literature review, fluorophores show individual pH dependences that may cause problems if spectra from water samples with different pH are compared (Hudson *et al*., 2007; Coble *et al*., 2014). To assure that the fluorescence data in the present study was comparable, pH was measured in unfiltered and filtered samples from all sites. As can be seen in Appendix 1 the pH was alike and rather high in all studied catchments. Quenching effects caused by pH can therefore be assumed to be similar across the sites and the samples. In association with the pH measurements the electrical conductivity was measured. Conductivity can be used to find potential quenching effects caused by metals (Baker, 2002). Variations in conductivity (not shown) were greater than the variations in pH and the effect of metals could therefore be important. The effect of metals was not studied in further detail in this study.

Finally turbidity is a useful measure in water analyses both when used as a quantitative measure of colloids in the sample, and when validating results of other measurements. High turbidity may scatter the fluorescence in EEMs and cause adverse effects on index calculations (Karanfil *et al.*, 2005). In addition biological processes

that can alter the FDOM composition may proceed in samples with high turbidity (Coble *et al*., 2014). In the current study the turbidity varied between the studied sites (*Table 5* and Appendix 1) and also between filtered and unfiltered samples, as expected. Some of the catchments, such as U8, E21 and F26, showed high turbidity also in the filtered samples, which brings a higher risk of inner-filter effect. In these sites the colloidal particles were of such nature that they could pass through the 0.45 µm filter, as has also been reported in previous studies (Karanfil *et al.*, 2005). To overcome the effect of suspended particles in the water samples, the fluorescence data was corrected for primary and secondary inner-filter effect prior to the data analyses. Despite the inner-filter correction quenching effects might have affected the FDOM and TOC results.

5.4 Implications for controlling eutrophication

The water quality varied between the studied streams. Some of the sites, such as F26, differed from the other catchments by having a poorer water quality reflected in many of the DOM indices. The BOD was not measured in the present study, but since earlier studies have shown strong correlations between the BOD and optical properties of DOM (Baker & Inverarity, 2004; Hudson *et al.*, 2008; Knapik *et al.*, 2014), the BOD can be assumed to be high in catchments like F26 and the overall oxygen status poor. The oxygen status and light conditions in the streams can be improved by treating DOM leaching.

The results of the study clearly demonstrate the importance of catchment specific mitigation measures for controlling eutrophication. In successful eutrophication control nutrient and DOM characteristics should be taken into account as well as land use and climatic factors. When it comes to DOM mitigation different measures might have to be taken for allochtonous and autochtonous DOM. Nutrient conditions in the stream are central in formation of new autochtonous DOM, while catchment properties might be of greater importance in leaching of already produced allochtonous DOM. It is important, however, to bear in mind that DOM plays a central role in the aquatic food web and that DOM concentrations should not be reduced below natural levels (Asmala *et al*., 2013). Knowledge about the studied watercourses is therefore central in order to combat eutrophication successfully.

A wider understanding of the relation between DOM and nutrients, which has been discussed in previous chapters, is helpful when planning remediation actions against eutrophication and understanding the dynamics of eutrophication. Recent recommended mitigation measures against nutrient losses in the studied catchments include cultivation of catch crops and ley (Fölster *et al*., 2012), improvement of soil structure and permeability by applying subsoil cultivation, structural liming and

changes in crop rotation (Kyllmar *et al*., 2013). Also, reduction of stream bank erosion has been recommended. This can be done by avoiding steep ditch slopes, leaving buffer strips along the streams and applying two-stage ditches (Kyllmar *et al*., 2013). Phosphorus ponds and wetlands have also been recommended since these collect nutrients from leachate and also increase the biodiversity of the agro-ecosystem (Uusi-Kämppä *et al*., 2000). These recent recommendations are also relevant when it comes to reducing DOM mobilization and transport since absorbed DOM and particulate phosphorus act somewhat similarly and can be mitigated using similar methods.

Nitrogen was shown to correlate inversely with the spectral slope, which describes the molecular weight of the FDOM (Helms *et al.*, 2008). This finding suggests that nitrogen concentrations can be reduced more effectively if mitigation measures are aimed at large DOM molecules. One example of a selective mitigation measure is, again, installation of ponds or constructed wetlands. In ponds and wetlands organic nitrogen can be removed by sedimentation (Braskerud, 2002). Filtration by plants can also remove suspended particles and large DOM molecules. Plants can also take up nutrients in ponds and wetlands while growing, which is another important removal mechanism. Denitrification is finally an important nitrogen removal mechanism in ponds and wetlands (Braskerud, 2002). However, incomplete denitrification may give rise to N_2O emissions, which is one of the main disadvantages with wetlands and ponds. Since dissolved particles will move with the soil water, improvement of the soil structure will not in the case of DOM have a big impact on the total leaching. However, large DOM molecules might be held in micro-pores if the main transport pathway of the soil water is through matrix flow. If the soil has a massive structure or is dominated by micro-pores, improvement of the soil structure could in fact increase the leaching of DOM.

The second significant nitrogen correlation found in the present study was the one between nitrogen and the C:A ratio, which suggests that humic-like fluorescents can hold more nitrogen than fulvic-like fluorescents. In the light of eutrophication control this finding suggests that fulvic-like fluorescents should be preferred in streams. How this could be done in practice is nothing this study can answer. It is important to remember that the properties of DOM may change along the flow path through photochemical oxidation and biological transformations (Hudson *et al*., 2007), which may lead to changed interactions between nutrients and DOM.

6 Conclusions

Dissolved organic matter (DOM) modulates various biogeochemical processes in agricultural streams. The role as a nutrient regulator was shown to relate mainly to the sorption capacity of the dissolved and particulate organic matter. Correlations between phosphorus and particulate properties were found and interpreted in terms of adsorption. When it comes to nitrogen a linkage was found to the molecular weight of the dissolved organic matter. This connection expresses the role of dissolved organic matter in anion adsorption and the importance of degradation of dissolved organic nitrogen as a nutrient source. Variations in organic matter properties across streams can affect the overall nutrient dynamics. Clear spatial variation could be demonstrated between the streams included in the present study. Catchment F26 differed from the other catchments in many aspects. The fluorescing dissolved organic molecules were significantly larger, more labile and to a higher degree plant derived than the molecules in the other catchments. Two of the catchments, O18 and E21, showed opposite properties compared to F26 in nearly all studied spectroscopic indices.

The results of the study demonstrate the importance of reducing particle leaching and leaching of large dissolved organic molecules to improve the water quality in streams. Examples of mitigation methods that advantageously could be used is the installation of ponds and wetlands on agricultural land, where suspended sediments and nutrients in leachate can be trapped. Actions against erosion along stream banks are also recommended, since reduced erodibility would lower the loss of soil particles and nutrients and DOM absorbed to it. These recommendations are in line with recent recommendations against nutrient leaching presented by other researchers. However, the study clearly demonstrated that catchment specific actions need to be taken since the catchments differed both when it comes to DOM and nutrient properties.

7 References

Amon, R. M. W. & Benner, R. (1996). Photochemical and microbial consumption of dissolved organic carbon and dissolved oxygen in the Amazon River system. *Geochimica et Cosmochimica Acta*, 60(10), pp 1783–1792.

Asmala, E., Autio, R., Kaartokallio, H., Pitkänen, L., Stedmon, C., & Thomas, D. N. (2013). Bioavailability of riverine dissolved organic matter in three Baltic Sea estuaries and the effect of catchment land use. *Biogeosciences*, *10*(11), 6969-6986.

Aufdenkampe, A. K., Mayorga, E., Raymond, P. A., Melack, J. M., Doney, S. C., Alin, S. R., Aalto, R. E. & Yoo, K. (2011). Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. *Frontiers in Ecology and the Environment*, 9(1), pp 53–60.

Baker, A. (2001). Fluorescence Excitation−Emission Matrix Characterization of Some Sewage-Impacted Rivers. *Environmental Science & Technology*, 35(5), pp 948–953.

Baker, A. (2002). Spectrophotometric discrimination of river dissolved organic matter. *Hydrological Processes*, 16(16), pp 3203–3213.

Baker, A. & Inverarity, R. (2004). Protein-like fluorescence intensity as a possible tool for determining river water quality. *Hydrological Processes*, 18(15), pp 2927–2945.

Baker, A. & Spencer, R. G. M. (2004). Characterization of dissolved organic matter from source to sea using fluorescence and absorbance spectroscopy. *Science of The Total Environment*, 333(1–3), pp 217–232.

Baker, A., Bolton, L., Newson, M. & Spencer, R. G. M. (2008). Spectrophotometric properties of surface water dissolved organic matter in an afforested upland peat catchment. *Hydrological Processes*, 22(13), pp 2325–2336.

Blombäck, K., Johnsson, H., Lindsjö, A., Mårtensson, K., Persson, K. & Schmieder, F. (2011). Läckage av näringsämnen från svensk åkermark för år 2009 beräknat med PLC5-metodik. Beräkningar av normalläckage av kväve och fosfor för 2009. SMED Rapport 2011:57.

Boerjan, W., Ralph, J. & Baucher, M. *LIGNIN BIOSYNTHESIS*. [online] (2003-11-28) (http://dx.doi.org/10.1146/annurev.arplant.54.031902.134938). Available from: http://www.annualreviews.org/doi/10.1146/annurev.arplant.54.031902.134938. [Accessed 2017-01-31].

Braskerud, B. C. (2002). Factors affecting nitrogen retention in small constructed wetlands treating agricultural non-point source pollution. *Ecological Engineering*, 18(3), pp 351–370.

Brown, M. (1977). Transmission spectroscopy examinations of natural waters. *Estuarine and Coastal Marine Science*, 5(3), pp 309–317.

Bushaw, K. L., Zepp, R. G., Tarr, M. A., Schulz-Jander, D., Bourbonniere, R. A., Hodson, R. E., Miller, W. L., Bronk, D. A. & Moran, M. A. (1996). Photochemical release of biologically available nitrogen from aquatic dissolved organic matter. *Nature*, 381(6581), pp 404–407.

Carstea, E. M., Baker, A., Bieroza, M., Reynolds, D. M. & Bridgeman, J. (2014). Characterisation of dissolved organic matter fluorescence properties by PARAFAC analysis and thermal quenching. *Water Research*, 61, pp 152–161.

Chen, W., Westerhoff, P., Leenheer, J. A. & Booksh, K. (2003). Fluorescence Excitation−Emission Matrix Regional Integration to Quantify Spectra for Dissolved Organic Matter. *Environmental Science & Technology*, 37(24), pp 5701–5710.

Chkhikvishvili, I. D. & Ramazanov, Z. M. (2000). Phenolic substances of brown algae and their antioxidant activity. *Applied Biochemistry and Microbiology*, 36(3), pp 289–291.

Coble, P.G. et al., 2014. Aquatic organic matter fluorescence. *Cambridge university press*. 408 p.

Council Directive 1991/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources The Nitrates Directive: http://ec.europa.eu/environment/water/water-nitrates/index_en.html Visited 02.05.2017. Updated 17.10.2016

Council Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy: The EU Water Framework Directive – integrated river basin management for Europe: http://ec.europa.eu/environment/water/water-framework/index_en.html Visited 02.05.2017. Updated 08.06.2016.

Del Vecchio, R. & Blough, N. V. (2004). On the Origin of the Optical Properties of Humic Substances. *Environmental Science & Technology*, 38(14), pp 3885–3891.

Elmgren, R. & Larsson, U. (2001). Nitrogen and the Baltic Sea: Managing Nitrogen in Relation to Phosphorus. *The Scientific World Journal*, 1, pp 371–377.

Essington, M.E., 2015. Soil and water chemistry: an integrative process. *CRC Press*. 656 p.

Evans, C. D., Monteith, D. T. & Cooper, D. M. (2005). Long-term increases in surface water dissolved organic carbon: Observations, possible causes and environmental impacts. *Environmental Pollution*, 137(1), pp 55–71 (Recovery from acidificationin the UK: Evidence from 15 years of acid waters monitoringRecovery from acidificationin the UK: Evidence from 15 years of acid waters monitoring).

Fellman, J. B., Hood, E., Edwards, R. T. & D'Amore, D. V. (2009). Changes in the concentration, biodegradability, and fluorescent properties of dissolved organic matter during stormflows in coastal temperate watersheds. *Journal of Geophysical Research: Biogeosciences*, 114(G1), p G01021.

Ferrari, G. M., Dowell, M. D., Grossi, S. & Targa, C. (1996). Relationship between the optical properties of chromophoric dissolved organic matter and total concentration of dissolved organic carbon in the southern Baltic Sea region. *Marine Chemistry*, 55(3), pp 299–316.

Fleming-Lehtinen, V., Räike, A., Kortelainen, P., Kauppila, P. & Thomas, D. N. (2015). Organic Carbon Concentration in the Northern Coastal Baltic Sea between 1975 and 2011. *Estuaries and Coasts*, 38(2), pp 466–481.

Fölster, J., Kyllmar, K., Wallin, M. & Hellgren, S. (2012). Kväve- och fosfortrender i jordbruksvattendrag. Sveriges lantbruksuniversitet, Institutionen för vatten och miljö. Rapport 2012:1.

Graeber, D., Boëchat, I. G., Encina-Montoya, F., Esse, C., Gelbrecht, J., Goyenola, G., Gücker, B., Heinz, M., Kronvang, B., Meerhoff, M., Nimptsch, J., Pusch, M. T., Silva, R. C. S., Schiller, D. von & Zwirnmann, E. (2015). Global effects of agriculture on fluvial dissolved organic matter. *Scientific Reports*, 5, p 16328.

Hansen, A. M., Kraus, T. E. C., Pellerin, B. A., Fleck, J. A., Downing, B. D. & Bergamaschi, B. A. (2016). Optical properties of dissolved organic matter (DOM): Effects of biological and photolytic degradation. *Limnology and Oceanography*, 61(3), pp 1015–1032.

Hedges, J. I., Cowie, G. L., Richey, J. E., Quay, P. D., Benner, R., Strom, M. & Forsberg, B. R. (1994). Origins and processing of organic matter in the Amazon River as indicated by carbohydrates and amino acids. *Limnology and Oceanography*, 39(4), pp 743–761.

Heinz, M., Graeber, D., Zak, D., Zwirnmann, E., Gelbrecht, J. & Pusch, M. T. (2015). Comparison of Organic Matter Composition in Agricultural versus Forest Affected Headwaters with Special Emphasis on Organic Nitrogen. *Environmental Science & Technology*, 49(4), pp 2081–2090.

HELCOM Ministerial Meeting. 2007. HELCOM Baltic Sea Action Plan. Krakow 2007.

Helms, J. R., Stubbins, A., Ritchie, J. D., Minor, E. C., Kieber, D. J. & Mopper, K. (2008). Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnology and Oceanography*, 53(3), pp 955–969.

Hoikkala, L., Kortelainen, P., Soinne, H. & Kuosa, H. (2015). Dissolved organic matter in the Baltic Sea. *Journal of Marine Systems*, 142, pp 47–61.

Hood, E., Fellman, J. & Edwards, R. T. (2007). Salmon influences on dissolved organic matter in a coastal temperate brownwater stream: An application of fluorescence spectroscopy. *Limnology and Oceanography*, 52(4), pp 1580–1587.

Hood, E., McKnight, D. M. & Williams, M. W. (2003). Sources and chemical character of dissolved organic carbon across an alpine/subalpine ecotone, Green Lakes Valley, Colorado Front Range, United States. *Water Resources Research*, 39(7), p 1188.

Hudson, N., Baker, A. & Reynolds, D. (2007). Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters—a review. *River Research and Applications*, 23(6), pp 631–649.

Hudson, N., Baker, A., Ward, D., Reynolds, D. M., Brunsdon, C., Carliell-Marquet, C. & Browning, S. (2008). Can fluorescence spectrometry be used as a surrogate for the Biochemical Oxygen Demand (BOD) test in water quality assessment? An example from South West England. *Science of The Total Environment*, 391(1), pp 149–158.

Kalbitz, K., Geyer, W. & Geyer, S. (1999). Spectroscopic properties of dissolved humic substances — a reflection of land use history in a fen area. *Biogeochemistry*, 47(2), pp 219–238.

Kalbitz, K., Schmerwitz, J., Schwesig, D. & Matzner, E. (2003). Biodegradation of soil-derived dissolved organic matter as related to its properties. *Geoderma*, 113(3–4), pp 273–291 (Ecological aspects of dissolved organic matter in soils).

Karanfil, T., Erdogan, I. & Schlautman, M. (2005). The impact of filtrate turbidity on UV^sub 254^ and SUVA^sub 254^ determinations. *American Water Works Association. Journal; Denver*, 97(5), p 125–136,12.

Knapik, H. G., Fernandes, C. V. S., de Azevedo, J. C. R. & do Amaral Porto, M. F. (2014). Applicability of Fluorescence and Absorbance Spectroscopy to Estimate Organic Pollution in Rivers. *Environmental Engineering Science*, 31(12), pp 653–663.

Kothawala, D. N., Stedmon, C. A., Müller, R. A., Weyhenmeyer, G. A., Köhler, S. J. & Tranvik, L. J. (2014). Controls of dissolved organic matter quality: evidence from a large-scale boreal lake survey. *Global Change Biology*, 20(4), pp 1101–1114.

Kothawala, D. N., Ji, X., Laudon, H., Ågren, A. M., Futter, M. N., Köhler, S. J. & Tranvik, L. J. (2015). The relative influence of land cover, hydrology, and in-stream processing on the composition of dissolved organic matter in boreal streams. *Journal of Geophysical Research: Biogeosciences*, 120(8), p 2015JG002946.

Kyllmar, K., Carlsson, C., Gustafson, A., Ulén, B. & Johnsson, H. (2006). Nutrient discharge from small agricultural catchments in Sweden: Characterisation and trends. *Agriculture, Ecosystems & Environment*, 115(1–4), pp 15–26.

Kyllmar, K., Andersson, S., Aurall, A., Djodjik, F., Stjernman Forsberg, L., Gustafsson, J., Heeb, A. & Ulén, B. (2013). Riskfaktorer för fosforförluster samt förslag på motåtgärder i tre avrinningsområgen inom pilotprojektet Greppa Fosforn. Sveriges lantbruksuniversitet, Institutionen för mark och miljö. Ekohydrologi 137.

Kyllmar, K., Forsberg, L. S., Andersson, S. & Mårtensson, K. (2014). Small agricultural monitoring catchments in Sweden representing environmental impact. *Agriculture, Ecosystems & Environment*, 198, pp 25–35 (Nitrogen losses from agriculture in the Baltic Sea region).

Lakowicz, J.R. (2006). Principles of Fluorescence Spectroscopy, 3dr ed. New York: Springer Science+Business media. 938 p.

Ledesma, J. L. J., Grabs, T., Bishop, K. H., Schiff, S. L. & Köhler, S. J. (2015). Potential for long-term transfer of dissolved organic carbon from riparian zones to streams in boreal catchments. *Global Change Biology*, 21(8), pp 2963–2979.

McKnight, D. M., Boyer, E. W., Westerhoff, P. K., Doran, P. T., Kulbe, T. & Andersen, D. T. (2001). Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography*, 46(1), pp 38–48.

Monteith, D. T., Stoddard, J. L., Evans, C. D., de Wit, H. A., Forsius, M., Høgåsen, T., Wilander, A., Skjelkvåle, B. L., Jeffries, D. S., Vuorenmaa, J., Keller, B., Kopácek, J. & Vesely, J. (2007). Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, 450(7169), pp 537–540.

Naden, P. S., Old, G. H., Eliot-Laize, C., Granger, S. J., Hawkins, J. M. B., Bol, R. & Haygarth, P. (2010). Assessment of natural fluorescence as a tracer of diffuse agricultural pollution from slurry spreading on intensely-farmed grasslands. *Water Research*, 44(6), pp 1701–1712.

Ohno, T. (2002). Fluorescence Inner-Filtering Correction for Determining the Humification Index of Dissolved Organic Matter. *Environmental Science & Technology*, 36(4), pp 742–746.

Old, G. H., Naden, P. S., Granger, S. J., Bilotta, G. S., Brazier, R. E., Macleod, C. J. A., Krueger, T., Bol, R., Hawkins, J. M. B., Haygarth, P. & Freer, J. (2012). A novel application of natural fluorescence to understand the sources and transport pathways of pollutants from livestock farming in small headwater catchments. *Science of The Total Environment*, 417–418, pp 169–182.

Parlanti, E., Wörz, K., Geoffroy, L. & Lamotte, M. (2000). Dissolved organic matter fluorescence spectroscopy as a tool to estimate biological activity in a coastal zone submitted to anthropogenic inputs. *Organic Geochemistry*, 31(12), pp 1765–1781.

Peuravuori, J. & Pihlaja, K. (1997). Molecular size distribution and spectroscopic properties of aquatic humic substances. *Analytica Chimica Acta*, 337(2), pp 133–149.
Piccolo, A. (2002). The supramolecular structure of humic substances: A novel understanding of humus chemistry and implications in soil science. In: Agronomy, B.-A. in (Ed) pp 57–134. Academic Press.
Postnikova, P. V. (2015). Assessment of changes in the qualitative composition and properties of dissolved organic matter on hydro-optical characteristics when fractional filtration of natural water., 2015. p 96802Y–96802Y–6.
Reynolds, D. M. (2003). Rapid and direct determination of tryptophan in water using synchronous fluorescence spectroscopy. *Water Research*, 37(13), pp 3055–3060.
Rosario-Ortiz, F. L. & Korak, J. A. (2017). Oversimplification of Dissolved Organic Matter Fluorescence Analysis: Potential Pitfalls of Current Methods. *Environmental Science & Technology*, 51(2), pp 759–761.
Sleighter, R. L., Liu, Z., Xue, J. & Hatcher, P. G. (2010). Multivariate Statistical Approaches for the Characterization of Dissolved Organic Matter Analyzed by Ultrahigh Resolution Mass Spectrometry. *Environmental Science & Technology*, 44(19), pp 7576–7582.
Statistics Sweden, Agricultural Statistics Unit (2013). Jordbruksstatistik årsbok 2013 med data om livsmedel. *Sveriges officiella statistik*, Örebro 2013, 397 p.
Stedmon, C. A. & Markager, S. (2005). Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. *Limnology and Oceanography*, 50(2), pp 686–697.
Stedmon, C.A. & Cory, R.M. (2014). Biological origins and fate of fluorescent dissolved organic matter in aquatic environments. - in Coble et al. (2014). Aquatic organic matter fluorescence. *Cambridge university press*. 408 p.
Stepanauskas, R., Laudon, H. & Jørgensen, N. O. G. (2000). High DON bioavailability in boreal streams during a spring flood. *Limnology and Oceanography*, 45(6), pp 1298–1307.
Stepanauskas, R., Leonardson, L. & Tranvik, L. J. (1999). Bioavailability of wetland-derived DON to freshwater and marine bacterioplankton. *Limnology and Oceanography*, 44(6), pp 1477–1485.
Stjernman Forsberg, L., Kyllmar, K., Andersson, S., Johansson, G. & Blomberg, M. (2015). Växtnäringsförluster i små jordbruksdominerade avrinningsområden 2013/2014 – Årsredovisning för miljöövervakningsprogramet Typområden på jordbruksmark. Sveriges Lantbruksuniversitet. Ekohydrologi 141.
Suzuki, H. (2012). Electronic Absorption Spectra and Geometry of Organic Molecules: An Application of Molecular Orbital Theory. *Elsevier*. ISBN 978-0-323-14526-8.
Swedish Environmental Protection Agency (SEPA) webpage: http://www.swedishepa.se/Environmental-objectives-and-cooperation/Swedens-environmental-objectives/The-environmental-objectives-system/, visited 02.05.2017, updated 29.08.2016
Swedish Meteorological and Hydrological Institute (SMHI) webpage: http://www.smhi.se/kunskapsbanken/vinter-1.22843, visited 02.02.2017, updated 04.08.2015
Swedish Meteorological and Hydrological Institute (SMHI) webpage: http://opendata-download-metobs.smhi.se/explore/#, visited 16.05.2017

Ulén, B., von Brömssen, C., Johansson, G., Torstensson, G. & Forsberg, L. S. (2012). Trends in nutrient concentrations in drainage water from single fields under ordinary cultivation. *Agriculture, Ecosystems & Environment*, 151, pp 61–69.

Uusi-Kämppä, J., Braskerud, B., Jansson, H., Syversen, N. & Uusitalo, R. (2000). Buffer Zones and Constructed Wetlands as Filters for Agricultural Phosphorus. *Journal of Environmental Quality*, 29(1), pp 151–158.

Weishaar, J. L., Aiken, G. R., Bergamaschi, B. A., Fram, M. S., Fujii, R. & Mopper, K. (2003). Evaluation of Specific Ultraviolet Absorbance as an Indicator of the Chemical Composition and Reactivity of Dissolved Organic Carbon. *Environmental Science & Technology*, 37(20), pp 4702–4708.

Wells, M.L. (2002). Marine colloids and trace metals. *Biogeochemistry of Marine Dissolved Organic Matter, Elsevier Science*, USA (2002), pp. 367–404.

Wetterlind, J. (2006). *Tidig höstplöjning på lerjordar : riskbedömning av kväveutlakning = Mouldboard ploughing in early autumn on clay soils : risk assessment of nitrogen leaching* [online]. Skara: Avdelningen för precisionsodling, Sveriges lantbruksuniversitet. Available from: http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-1-35. [Accessed 2017-02-01].

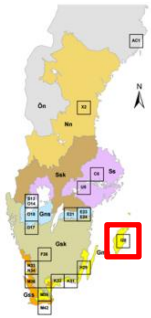
Wilson, H. F. & Xenopoulos, M. A. (2009). Effects of agricultural land use on the composition of fluvial dissolved organic matter. *Nature Geoscience*, 2(1), pp 37–41.

Withers, P. J. A., Jarvie, H. P., Hodgkinson, R. A., Palmer-Felgate, E. J., Bates, A., Neal, M., Howells, R., Withers, C. M. & Wickham, H. D. (2009). Characterization of Phosphorus Sources in Rural Watersheds. *Journal of Environmental Quality*, 38(5), pp 1998–2011.

Wu, F. C., Evans, R. D. & Dillon, P. J. (2003). Separation and Characterization of NOM by High-Performance Liquid Chromatography and On-Line Three-Dimensional Excitation Emission Matrix Fluorescence Detection. *Environmental Science & Technology*, 37(16), pp 3687–3693.

Zsolnay, A., Baigar, E., Jimenez, M., Steinweg, B. & Saccomandi, F. (1999). Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. *Chemosphere*, 38(1), pp 45–50.

Appendix 1. ***Catchment I28***

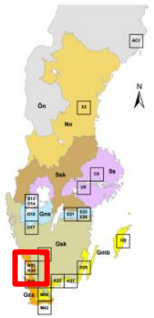


Temperature: 6.9 °C
Precipitation: 587 mm
Area: 4.8 ha
Soil texture: sandy loam
Arable land: 84%
Pasture: 2%
Drained area: 99%
Production: cereals, grass, potato
Livestock density: 0.3 AU/ha
Scattered households: 11 pers/km^2

Property	Flow-proportional sample		Grab sample	
	Average	Std dev	Average	Std dev
TOC (mg/l)	7.7	<0.1	8.6	0.7
DOC (mg/l)	6.7	0.3	7.2	1.5
DOC/TOC (%)	92.5	-	81.8	-
Turbidity (NTU), UF	7.1	3.2	2.0	0.4
Turbidity (NTU), F45	0.7	0.4	1.3	1.8
pH, UF	8.3	0.1	8.1	0.1
pH, F45	8.3	0.1	7.7	0.6

Property	Flow-proportional sample						Grab sample					
	UF		F45		F20		UF		F45		F20	
	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev
FIX	1.7	<0.1	1.7	<0.1	1.7	<0.1	1.7	<0.1	1.7	<0.1	1.7	<0.1
BIX	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1
HIX	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1
E2:E3	5.3	0.6	4.7	1.3	4.3	5.3	6.0	2.2	7.2	0.6	4.5	1.2
$SUVA_{254}$	2.6	0.4	2.9	0.5	2.9	0.5	2.4	0.3	2.6	0.8	2.8	0.8
Slope	2.0	0.1	2.0	0.1	2.0	0.1	2.0	0.1	2.0	0.1	2.0	0.1
Abs220	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.1
A:T ratio	5.5	0.3	4.9	0.4	5.1	0.4	5.5	1.4	5.5	0.5	4.8	0.9
C:A ratio	0.4	<0.1	0.4	<0.1	0.4	<0.1	0.4	0.1	0.4	0.1	0.4	0.1
C:M ratio	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1
C:T ratio	1.9	0.1	1.9	0.1	1.9	0.1	1.8	0.2	1.8	0.2	1.8	0.2
T:C ratio	0.5	<0.1	0.5	<0.1	0.5	<0.1	0.5	0.1	0.5	0.1	0.5	0.1

Catchment N34

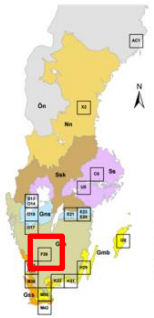


Temperature: 7.2 °C
Precipitation: 886 mm
Area: 13.9 ha
Soil texture: sandy loam and silt loam
Arable land: 85 %
Pasture: 2 %
Drained area: 93 %
Production: cereals, grass, potato
Livestock density: 0.3 AU/ha
Scattered households: 19 pers/km^2

Property	Flow-proportional sample		Grab sample	
	Average	Std dev	Average	Std dev
TOC (mg/l)	8.2	0.8	12.8	3.4
DOC (mg/l)	6.9	0.8	6.9	0.4
DOC/TOC (%)	80.3	-	69.0	-
Turbidity (NTU), UF	20.2	7.7	12.1	11.1
Turbidity (NTU), F45	0.8	0.7	0.6	0.5
pH, UF	7.8	0.1	7.7	0.3
pH, F45	7.8	0.1	7.7	0.3

Property	Flow-proportional sample						Grab sample					
	UF		F45		F20		UF		F45		F20	
	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev
FIX	1.6	0.1	1.6	0.1	1.6	0.1	1.6	0.1	1.6	0.1	1.6	0.1
BIX	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1
HIX	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1
E2:E3	6.2	2.2	5.6	2.6	4.2	1.7	3.6	1.7	5.3	2.1	4.2	1.1
$SUVA_{254}$	2.0	0.4	2.7	0.8	2.4	1.0	2.4	1.0	2.5	0.9	2.7	1.0
Slope	1.7	0.2	1.7	0.2	1.7	0.2	1.7	0.2	1.7	0.2	1.7	0.2
Abs220	0.3	<0.1	0.3	<0.1	0.3	<0.1	0.3	<0.1	0.3	<0.1	0.3	<0.1
A:T ratio	5.8	0.2	5.6	0.5	6.0	0.3	6.0	0.3	5.8	0.7	5.5	0.3
C:A ratio	0.4	<0.1	0.4	<0.1	0.4	0.1	0.4	0.1	0.4	0.1	0.4	0.1
C:M ratio	1.1	0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1
C:T ratio	2.1	0.1	2.1	0.1	2.1	0.1	2.1	0.1	2.1	0.1	2.1	0.1
T:C ratio	0.5	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.5	0.1

Catchment F26

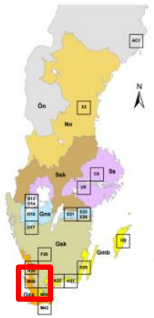


Temperature: 6.2 ºC
Precipitation: 1066 mm
Area: 1.8 ha
Soil texture: loamy sand
Arable land: 70 %
Pasture: 3 %
Drained area: -
Production: grass
Livestock density: 1.3 AU/ha
Scattered households: 33 pers/km^2

Property	Flow-proportional sample		Grab sample	
	Average	Std dev	Average	Std dev
TOC (mg/l)	12.7	3.4	19.2	2.4
DOC (mg/l)	19.1	0.8	16.8	0.2
DOC/TOC (%)	105.9	-	88.4	-
Turbidity (NTU), UF	30.1	12.4	6.2	1.5
Turbidity (NTU), F45	1.0	0.7	1.4	1.5
pH, UF	7.6	0.2	7.3	0.2
pH, F45	7.4	0.3	7.3	0.2

Property	Flow-proportional sample						Grab sample					
	UF		F45		F20		UF		F45		F20	
	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev
FIX	1.5	0.1	1.5	0.1	1.5	0.1	1.5	0.1	1.5	0.1	1.5	0.1
BIX	0.6	<0.1	0.6	<0.1	0.6	<0.1	0.6	<0.1	0.6	<0.1	0.6	<0.1
HIX	1.0	0.1	1.0	0.1	1.0	0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1
E2:E3	1.5	0.5	1.5	0.5	1.5	0.5	1.4	0.5	1.4	0.5	1.4	0.5
$SUVA_{254}$	3.1	1.0	3.5	1.0	3.4	1.0	3.2	0.5	3.4	0.7	3.0	0.4
Slope	1.4	0.1	1.4	0.1	1.4	0.1	1.3	0.1	1.3	0.1	1.3	0.1
Abs220	0.6	0.1	0.6	0.1	0.6	0.1	0.7	0.1	0.7	0.1	0.7	0.1
A:T ratio	6.7	0.3	6.9	0.4	6.6	0.4	6.4	0.4	6.5	0.4	7.9	3.2
C:A ratio	0.4	<0.1	0.4	<0.1	0.4	<0.1	0.4	0.1	0.4	0.1	0.4	0.1
C:M ratio	1.1	<0.1	1.0	0.1	1.0	0.1	1.0	0.1	1.0	0.1	1.0	0.1
C:T ratio	2.6	0.1	2.6	0.1	2.6	0.1	2.6	0.2	2.6	0.2	2.6	0.2
T:C ratio	0.4	0.1	0.4	0.1	0.4	0.1	0.4	<0.1	0.4	<0.1	0.4	<0.1

Catchment M36



Temperature: 7.6 ºC
Precipitation: 719 mm
Area: 7.8 ha
Soil texture: clay, sandy loam
Arable land: 86 %
Pasture: 1 %
Drained area: 88 %
Production: cereals, grass, potato
Livestock density: 0.3 AU/ha
Scattered households: 37 pers/km^2

Property	Flow-proportional sample		Grab sample	
	Average	Std dev	Average	Std dev
TOC (mg/l)	6.6	0.7	8.2	0.7
DOC (mg/l)	11.5	4.6	7.3	0.2
DOC/TOC (%)	82.0	-	82.9	-
Turbidity (NTU), UF	30.4	21.8	24.5	20.8
Turbidity (NTU), F45	0.6	0.5	0.5	0.4
pH, UF	8.2	<0.1	8.1	0.1
pH, F45	7.8	0.7	8.0	0.1

Property	Flow-proportional sample						Grab sample					
	UF		F45		F20		UF		F45		F20	
	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev
FIX	1.6	0.1	1.6	0.1	1.6	0.1	1.6	0.1	1.6	0.1	1.6	0.1
BIX	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1
HIX	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1
E2:E3	4.0	1.3	5.2	2.5	4.9	2.5	4.8	3.1	5.0	2.7	3.9	1.1
$SUVA_{254}$	2.7	0.1	2.7	0.7	2.7	0.7	2.5	0.5	2.5	0.7	2.7	0.6
Slope	1.7	0.2	1.7	0.2	1.7	0.2	1.8	0.2	1.8	0.2	1.8	0.2
Abs220	0.3	<0.1	0.3	<0.1	0.3	<0.1	0.3	0.1	0.3	0.1	0.3	0.1
A:T ratio	5.4	0.1	5.9	0.2	5.5	0.2	5.7	0.2	5.6	0.2	5.5	0.2
C:A ratio	0.4	<0.1	0.4	<0.1	0.4	<0.1	0.4	0.1	0.4	0.1	0.4	0.1
C:M ratio	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1
C:T ratio	2.1	0.1	2.1	0.1	2.1	0.1	2.1	0.1	2.1	0.2	2.1	0.1
T:C ratio	0.5	0.1	0.5	0.1	0.5	0.1	0.5	<0.1	0.5	<0.1	0.5	<0.1

Catchment C6

Temperature: 5.5 ºC
Precipitation: 623 mm
Area: 33.1 ha
Soil texture: clay loam
Arable land: 59 %
Pasture: 2 %
Drained area: 95%
Production: cereals
Livestock density: <0.1 AU/ha
Scattered households: 10 pers/km^2

Property	Flow-proportional sample		Grab sample	
	Average	Std dev	Average	Std dev
TOC (mg/l)	10.3	1.8	8.2	0.7
DOC (mg/l)	7.1	0.4	7.3	0.2
DOC/TOC (%)	81.4	-	82.9	-
Turbidity (NTU), UF	19.1	11.0	47.7	62.3
Turbidity (NTU), F45	0.5	0.6	0.8	0.6
pH, UF	8.3	0.1	8.0	0.2
pH, F45	8.2	0.3	7.5	0.9

Property	Flow-proportional sample						Grab sample					
	UF		F45		F20		UF		F45		F20	
	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev
FIX	1.7	0.1	1.7	0.1	1.7	0.1	1.6	0.1	1.6	0.1	1.6	0.1
BIX	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	0.1	0.7	0.1	0.7	0.1
HIX	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1
E2:E3	5.0	3.3	6.8	2.7	4.6	1.8	4.0	3.4	5.4	3.4	4.3	1.1
$SUVA_{254}$	2.3	0.3	2.4	0.7	2.5	0.8	2.6	0.9	2.4	0.8	2.5	0.9
Slope	2.0	0.2	2.0	0.2	2.0	0.2	1.9	0.2	1.9	0.2	1.9	0.2
Abs220	0.2	0.1	0.2	0.1	0.2	0.1	0.4	0.3	0.2	0.1	0.2	0.1
A:T ratio	5.5	0.7	5.1	0.4	5.6	0.4	9.2	6.0	5.6	0.2	5.5	0.2
C:A ratio	0.4	0.1	0.4	0.1	0.4	0.1	0.3	0.1	0.3	0.1	0.3	0.1
C:M ratio	1.0	0.1	1.0	0.1	1.0	0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1
C:T ratio	2.0	0.1	2.0	0.1	2.0	0.1	1.9	0.1	1.9	0.1	1.9	0.1
T:C ratio	0.5	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.5	0.1

Catchment E21

Temperature: 6.0 ºC
Precipitation: 506 mm
Area: 16.3 ha
Soil texture: sandy loam
Arable land: 89 %
Pasture: 1 %
Drained area: 95%
Production: cereals
Livestock density: 0.2 AU/ha
Scattered households: 9 pers/km^2

Property	Flow-proportional sample		Grab sample	
	Average	Std dev	Average	Std dev
TOC (mg/l)	5.4	0.5	6.1	1.1
DOC (mg/l)	5.1	0.4	5.0	0.9
DOC/TOC (%)	81.0	-	69.1	-
Turbidity (NTU), UF	13.5	11.4	5.7	1.3
Turbidity (NTU), F45	1.6	2.4	1.5	2.1
pH, UF	8.2	<0.1	8.2	0.1
pH, F45	7.9	0.5	8.0	0.4

Property	Flow-proportional sample						Grab sample					
	UF		F45		F20		UF		F45		F20	
	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev
FIX	1.7	0.1	1.7	0.1	1.7	0.1	1.7	0.1	1.7	0.1	1.7	0.1
BIX	0.8	<0.1	0.8	<0.1	0.8	<0.1	0.8	<0.1	0.8	<0.1	0.8	<0.1
HIX	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1
E2:E3	9.9	4.0	5.6	1.8	5.5	1.4	10.7	4.0	7.4	5.0	6.2	2.0
$SUVA_{254}$	1.8	0.8	2.7	0.8	2.7	0.7	1.7	0.6	2.8	1.3	2.6	0.8
Slope	2.1	0.2	2.1	0.2	2.1	0.2	2.0	0.2	2.0	0.2	2.0	0.2
Abs220	0.2	<0.1	0.2	<0.1	0.2	<0.1	0.2	0.1	0.2	0.1	0.2	0.1
A:T ratio	5.1	0.3	5.5	0.4	5.3	0.7	5.1	0.2	5.9	0.5	5.8	1.2
C:A ratio	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.1
C:M ratio	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1
C:T ratio	1.7	0.1	1.7	0.1	1.7	0.1	1.8	0.1	1.8	0.1	1.8	0.1
T:C ratio	0.6	0.1	0.6	0.1	0.6	0.1	0.5	0.1	0.5	0.1	0.5	0.1

Catchment O18

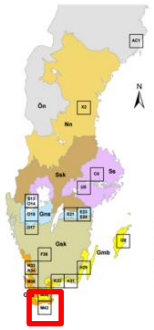
Temperature: 6.1 °C
Precipitation: 655 mm
Area: 7.7 ha
Soil texture: clay
Arable land: 92 %
Pasture: 0 %
Drained area: 100 %
Production: cereals
Livestock density: <0.1 AU/ha
Scattered households: 8 pers/km^2

Property	Flow-proportional sample		Grab sample	
	Average	Std dev	Average	Std dev
TOC (mg/l)	8.1	-	5.0	0.4
DOC (mg/l)	4.4	0.2	3.0	0.6
DOC/TOC (%)	54.5	-	64.7	-
Turbidity (NTU), UF	59.1	-	18.7	4.9
Turbidity (NTU), F45	1.3	1.0	0.6	0.4
pH, UF	8.3	0.1	8.2	0.1
pH, F45	8.3	0.1	8.2	0.1

Property	Flow-proportional sample						Grab sample					
	UF		F45		F20		UF		F45		F20	
	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev
FIX	1.7	0.1	1.7	0.1	1.7	0.1	1.7	0.1	1.7	0.1	1.7	0.1
BIX	0.8	0.1	0.8	0.1	0.8	0.1	0.8	0.1	0.8	0.1	0.8	0.1
HIX	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1
E2:E3	0.9	-	3.7	0.5	4.2	0.1	5.5	0.8	6.0	1.4	4.3	0.1
$SUVA_{254}$	3.6	-	2.6	1.6	2.4	1.4	1.8	0.4	2.7	1.5	2.8	1.2
Slope	1.6	0.1	1.6	0.1	1.6	0.1	1.8	0.1	1.8	0.1	1.8	0.1
Abs220	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1
A:T ratio	6.0	1.5	6.0	1.5	6.0	1.5	6.4	0.5	5.7	0.1	6.3	0.4
C:A ratio	0.4	0.1	0.4	0.1	0.4	0.1	0.3	0.1	0.3	0.1	0.3	0.1
C:M ratio	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1
C:T ratio	1.8	0.1	1.8	0.1	1.8	0.1	1.8	0.1	1.8	0.1	1.8	0.1
T:C ratio	0.5	<0.1	0.5	<0.1	0.5	<0.1	0.6	0.1	0.6	0.1	0.6	0.1

- Not enough samples to calculate standard deviation

Catchment M42



Temperature: 7.7 ºC
Precipitation: 709 mm
Area: 8.2 ha
Soil texture: sandy loam, loam
Arable land: 93 %
Pasture: 0 %
Drained area: 100 %
Production: cereals
Livestock density: 0.1 AU/ha
Scattered households: 10 pers/km^2

Property	Flow-proportional sample		Grab sample	
	Average	Std dev	Average	Std dev
TOC (mg/l)	8.5	2.6	14.5	2.3
DOC (mg/l)	11.8	0.2	12.7	0.2
DOC/TOC (%)	99.0	-	84.3	-
Turbidity (NTU), UF	8.5	3.0	4.7	3.7
Turbidity (NTU), F45	0.6	0.5	0.6	0.4
pH, UF	8.3	0.2	8.0	0.2
pH, F45	8.3	0.2	8.0	0.2

Property	Flow-proportional sample						Grab sample					
	UF		F45		F20		UF		F45		F20	
	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev
FIX	1.6	<0.1	1.6	<0.1	1.6	<0.1	1.6	<0.1	1.6	<0.1	1.6	<0.1
BIX	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1
HIX	1.0	0.1	1.0	0.1	1.0	0.1	1.0	0.1	1.0	0.1	1.0	0.1
E2:E3	2.7	1.4	3.2	1.0	3.1	0.5	3.1	0.1	3.1	0.1	3.1	0.1
$SUVA_{254}$	3.1	0.9	3.2	0.8	2.8	0.7	2.8	0.7	2.7	0.7	3.0	0.6
Slope	1.9	0.1	1.9	0.1	1.9	0.1	1.9	0.1	1.9	0.1	1.9	0.1
Abs220	0.5	0.1	0.5	0.1	0.5	0.1	0.5	<0.1	0.5	<0.1	0.5	<0.1
A:T ratio	5.5	0.1	5.4	0.3	5.4	0.3	5.4	0.2	5.4	0.2	5.4	0.2
C:A ratio	0.4	<0.1	0.4	<0.1	0.4	<0.1	0.4	0.1	0.4	0.1	0.4	0.1
C:M ratio	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1
C:T ratio	2.1	0.1	2.1	0.1	2.1	0.1	2.1	0.1	2.1	0.1	2.1	0.1
T:C ratio	0.5	<0.1	0.5	<0.1	0.5	<0.1	0.5	0.1	0.5	0.1	0.5	0.1

Catchment U8

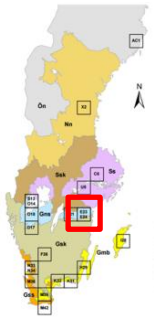
Temperature: 5.9 °C
Precipitation: 539 mm
Area: 5.7 ha
Soil texture: clay
Arable land: 56 %
Pasture: 2 %
Drained area: -
Production: cereals, grass
Livestock density: 0.2 AU/ha
Scattered households: -

Property	Flow-proportional sample		Grab sample	
	Average	Std dev	Average	Std dev
TOC (mg/l)	10.11	25.0	14.0	-
DOC (mg/l)	18.1	6.7	10.5	1.2
DOC/TOC (%)	152.6	-	74.8	-
Turbidity (NTU), UF	56.8	60.3	19.6	-
Turbidity (NTU), F45	2.4	3.4	1.6	1.9
pH, UF	7.4	0.2	-	-
pH, F45	7.9	<0.1	7.5	-

Property	Flow-proportional sample						Grab sample					
	UF		F45		F20		UF		F45		F20	
	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev
FIX	1.7	0.1	1.7	0.1	1.7	0.1	1.6	<0.1	1.6	<0.1	1.6	<0.1
BIX	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1	0.7	<0.1
HIX	0.9	0.1	0.9	0.1	0.9	0.1	0.9	<0.1	0.9	<0.1	0.9	<0.1
E2:E3	2.5	1.8	3.8	2.2	3.4	3.1	1.0	-	4.2	2.3	2.6	-
$SUVA_{254}$	2.5	1.7	2.2	0.9	2.2	0.9	2.6	-	2.5	1.1	1.7	-
Slope	2.0	0.2	2.0	0.2	2.0	0.2	1.9	<0.1	1.9	<0.1	1.9	<0.1
Abs220	0.4	0.3	0.4	0.3	0.4	0.3	0.5	-	0.3	<0.1	0.3	<0.1
A:T ratio	4.6	1.8	4.3	2.2	4.3	2.2	5.4	0.3	5.4	0.3	5.4	0.3
C:A ratio	0.4	0.1	0.4	0.1	0.4	0.1	0.4	<0.1	0.4	<0.1	0.4	<0.1
C:M ratio	1.0	0.1	1.0	0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1	1.0	<0.1
C:T ratio	1.6	0.1	1.6	0.1	1.6	0.1	2.1	0.1	2.1	0.1	2.1	0.1
T:C ratio	0.6	0.2	0.9	0.9	1.2	1.4	0.5	<0.1	0.5	0.1	0.5	<0.1

- Not enough samples to calculate standard deviation

Catchment E23



Temperature: 6.3 ºC
Precipitation: 587 mm
Area: 7.4 ha
Soil texture: clay
Arable land: 54 %
Pasture: 8 %
Drained area: -
Production: cereals, grass
Livestock density: 0.6 AU/ha
Scattered households: -

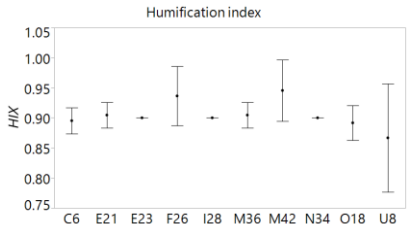
Property	Flow-proportional sample		Grab sample	
	Average	Std dev	Average	Std dev
TOC (mg/l)	-	-	8.9	4.3
DOC (mg/l)	8.4	-	8.6	1.4
DOC/TOC (%)	-	-	75.7	-
Turbidity (NTU), UF	-	-	48.8	48.9
Turbidity (NTU), F45	1.1	-	0.5	0.4
pH, UF	-	-	7.8	0.4
pH, F45	7.5	-	8.0	0.2

Property	Flow-proportional sample						Grab sample					
	UF		F45		F20		UF		F45		F20	
	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev	Average	Std dev
FIX	1.6	-	1.6	-	1.6	-	1.7	0.1	1.7	0.1	1.7	0.1
BIX	0.7	-	0.7	-	0.7	-	0.7	<0.1	0.7	<0.1	0.7	<0.1
HIX	0.9	-	0.9	-	0.9	-	0.9	<0.1	0.9	<0.1	0.9	<0.1
E2:E3	-	-	4.8	-	2.6	-	2.6	0.6	3.9	0.6	3.7	2.6
$SUVA_{254}$	-	-	3.5	-	2.8	-	2.8	0.9	2.6	0.9	2.4	0.9
Slope	1.7	-	1.7	-	1.8	-	1.8	0.2	1.8	0.2	1.8	0.2
Abs220	0.3	-	0.3	-	0.3	-	0.5	0.1	0.3	0.1	0.3	0.1
A:T ratio	-	-	5.7	-	5.1	-	5.0	0.4	5.1	0.4	5.3	0.1
C:A ratio	0.4	-	0.4	-	0.4	-	0.4	0.1	0.4	0.1	0.4	0.1
C:M ratio	1.0	-	1.0	-	1.0	-	1.0	<0.1	1.0	<0.1	1.0	<0.1
C:T ratio	2.1	-	2.1	-	2.1	-	2.1	0.1	2.1	0.1	2.1	0.1
T:C ratio	0.5	-	0.5	-	0.5	-	0.5	<0.1	0.5	<0.1	0.5	<0.1

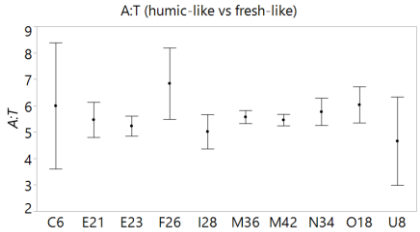
- Not enough samples to calculate standard deviation

Appendix 2.

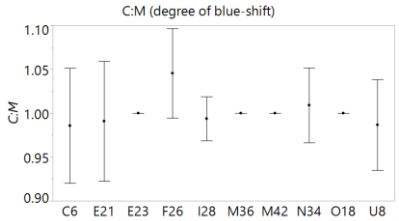
Spatial variation in DOM properties is presented in the figures below. The error bars represent standard deviation.



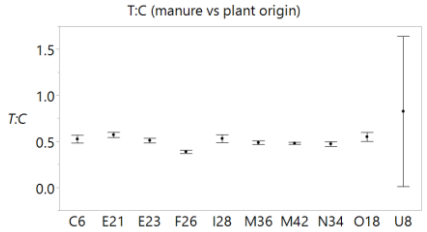
Humification index with standard deviation given by the bars. None of the catchments differ from all other catchments, but M42 differs from most ($p<0.001$)



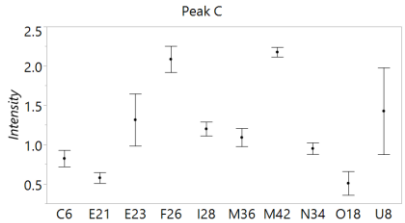
A:T ratio with standard deviation given by the bars. F26 differ from all other catchments ($p<0.01$) and O18 and I28 from most.



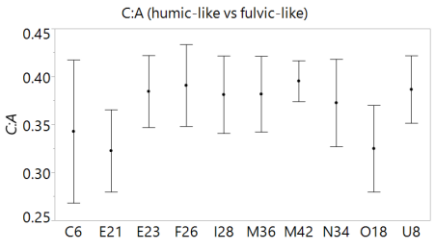
C:M ratio with standard deviation given by the bars. F26 differ from all other catchments ($p<0.01$). No other differences.



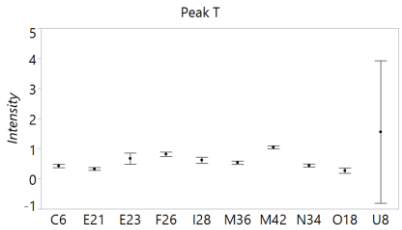
T:C ratio with standard deviation given by the bars. Significant differences ($p<0.05$) between most catchments.



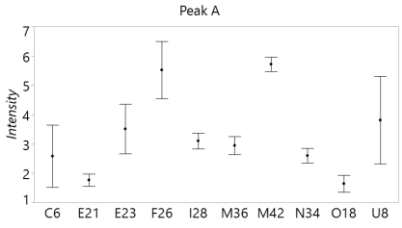
Peak C intensity, with standard deviation given by the bars. Significant differences ($p<0.05$) between all catchments except U8 – I28, U8 – E23, I28 – E23 and O18 – E21.



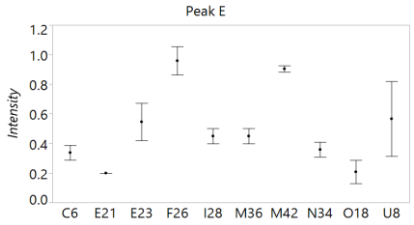
C:A ratio with standard deviation given by the bars. O18 and E21 differ from most other catchments ($p<0.05$).



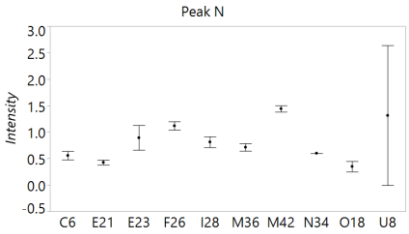
Peak T intensity, with standard deviation given by the bars. Significant differences ($p<0.05$) between all catchments except U8 – I28, U8 – E23, I28 – E23 and N34 – C6.



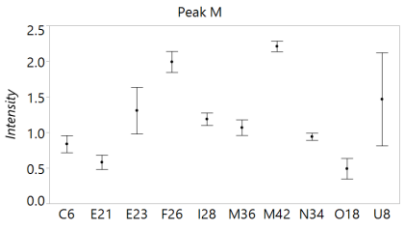
Peak A intensity, with standard deviation given by the bars. Significant differences ($p<0.05$) between all catchments except U8 – I28, U8 – E23, I28 – E23, O18 – E21, M36 – I28 and M36 – E23.



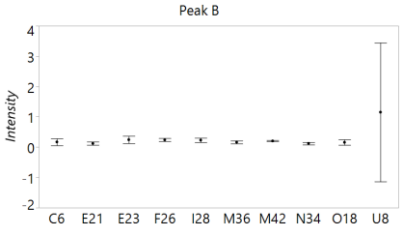
Peak E intensity, with standard deviation given by the bars. Significant differences ($p<0.05$) between all catchments except U8 – I28, U8 – M36, N34 – C6, I28 – E21, M36 – I28 and U8 – E23.



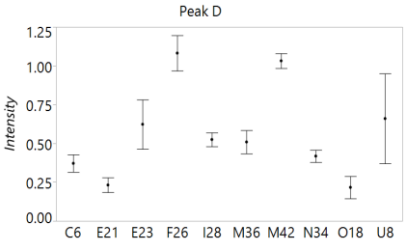
Peak N intensity, with standard deviation given by the bars. Significant differences ($p<0.05$) between all catchments except U8 – I28, U8 – E23 and I28 – E23.



Peak M intensity, with standard deviation given by the bars. Significant differences ($p<0.05$) between all catchments except U8 – I28, U8 – E23, I28 – E23 and O18 – E21.



Peak B intensity with standard deviation given by the bars. All catchments differ from some of the others ($p<0.05$) but no catchment differs from all



Peak D intensity, with standard deviation given by the bars. Significant differences ($p<0.05$) between all catchments except U8 – M36, U8 – I28, U8 – E23, I28 – E23, M36 – I28, M42 – F26 and O18 – E21.

Appendix 3. Temporal nutrient concentrations

Temporal variation in phosphorus fractions. Standard deviation given.

	Total P (mg/l)	***Dissolved P (mg/l)***	***PO4 (mg/l)***	***TOC (mg/l)***
Week 2	0.30 ± 0.25	0.39 ± 0.29	0.045 ± 0.045	10.97 ± 7.49
Week 4	0.40 ± 0.27	0.31 ± 0.22	0.051 ± 0.041	10.54 ± 4.79
Week 6	0.42 ± 0.27	0.32 ± 0.25	0.043 ± 0.039	9.12 ± 4.71
Week 8	0.35 ± 0.25	0.39 ± 0.31	0.052 ± 0.057	9.03 ± 4.75
Long term	0.15	0.07		9.43

Temporal variation in orthophosphate. Standard deviation given.

	Ortho-P (mg/l), UF	***Ortho-P (mg/l), F45***	***Ortho-P (mg/l), F20***
Week 2	0.21 ± 0.54	0.09 ± 0.26	0.11 ± 0.25
Week 4	-	0.10 ± 0.06	0.06 ± 0.05
Week 6	0.13 ± 0.23	0.04 ± 0.04	0.04 ± 0.04
Week 8	0.21 ± 0.28	0.12 ± 0.26	0.13 ± 0.26

Temporal variation in nitrogen fractions and suspended solids. Standard deviation given.

	Total N (mg/l)*	***NO3+NO2** (mg/l)***	***NH4 (mg/l)***	***Suspended solids (mg/l)***
Week 2	11.63 ± 8.63	11.12 ± 8.52	0.085 ± 0.072	43.4 ± 58.2
Week 4	11.94 ± 8.57	10.99 ± 8.10	0.089 ± 0.111	54.5 ± 85.4
Week 6	11.70 ± 8.34	11.25 ± 8.09	0.080 ± 0.113	44.7 ± 74.0
Week 8	11.23 ± 9.16	10.71 ± 8.09	0.078 ± 0.088	43.3 ± 59.3
Long term	6.71	5.78	0.08	45.63