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Trihalomethane Formation in Drinking Water; Impact of Drought, Treatment Wetlands and Methods of Analysis

Ehbair, Abdassalam

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**Trihalomethane Formation in Drinking Water;
Impact of Drought, Treatment Wetlands
and Methods of Analysis**

A thesis submitted to the University of Bangor by:

Abdassalam Ehbair

In candidature for the degree of:

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School of Chemistry
University of Bangor
Bangor
Wales

Abstract

In England and Wales, two-thirds of drinking water comes from surface water. Rising dissolved organic carbon (DOC) concentrations in surface waters have been reported in the past few decades, commonly attributed to climate and land use change. Peatlands have historically been drained to create new grazing land and this has been suggested as one of the causes of the rising DOC trend.

The experimental work in this thesis was undertaken in North and West Wales catchments and primarily examines the variations in DOC concentrations and how these are linked with trihalomethane formation potential (THMFP) in different field and laboratory settings.

Chapter 2. From July 2012 – Feb 2013 water samples were collected from three contrasting sites in North Wales: Llyn Conwy (“lake”) a natural oligotrophic drinking water reservoir, Nant-y-Brwyn (“stream”) a natural Stream outside the catchment of the reservoir but analogous to other upland streams within the area and four man-made drainage ditches (“ditches”) within the predominately peat catchment of Llyn Conwy. The results of this study demonstrate that the drainage ditches have the potential to contribute more carbon per unit volume of water to the oligotrophic Llyn Conwy than the natural stream feeding into Llyn Conwy (equivalent to Nant-y-Brwyn). Mean DOC values were 6.32 mg/L higher in the drainage ditches when compared to Llyn Conwy. However, there were no statistically significant differences in the propensity to form trihalomethanes between the sites ($p>0.05$).

Chapter 3: From March 2014 until September 2014 monthly water sampling was performed to compare the effect of drought on a discontinuous peat-accumulating wetland experimental site in mid-Wales, UK. Porewater samplers were installed at both the droughted and control sites. The results demonstrated that peatland exhibited reduced porewater DOC and phenolics concentrations and a shift in quality to lower molecular weight and less aromaticity. The mean DOC concentration of the control being 14.1 ± 0.77 mg/L and the drought being 11.9 ± 0.45 mg/L ($F=4.93$, $p<0.05$). Despite this, a significant change in the mean standardised trihalomethane formation potential (THMFP) values were not observed ($p>0.05$).

Chapter 4: Twenty peat cores were collected from two locations in North Wales in June 2013; 10 each from Migneint bog and Cors Erddreiniog fen. The Mignent is the largest blanket bog in North Wales and Cors Erddreiniog is the largest area of fen on the island

of Anglesey. These peat cores were transferred to the laboratory and kept under controlled temperature and levels of water table over a period of 160 days.

The results indicated is that artificially exposing peat cores to a drought treatment reduced DOC concentrations compared to control cores (DOC: mean of all controls (71.68 ± 53.30 mg/L), mean of all droughts (39.69 ± 38.66 mg/L)). The values determined for the correlated THMFP values were mean drought 57.75 ± 20.33 $\mu\text{g CHCl}_3/\text{mg DOC}$ and mean control 54.59 ± 23.63 $\mu\text{g CHCl}_3/\text{mg DOC}$, but these differences were not significant

Chapter 5: The effect of constructed wetlands on DOC characteristics and THMFP of water sample from Llyn Cefni were measured twice a month from 2014 until March 2015. It was concluded that in the wintertime the constructed wetland is having a positive impact on water quality of the reservoir because it is still sequestering some nitrate and phosphate, but also reducing the DOC concentration of the water flowing into the reservoir by 18% on average.

Chapter 6: In order to investigate the effect of storage of water samples that contain THMs after disinfection treatment, samples were collected from two selected surface waters; Nant-y-Brwyn stream (oligotrophic) and Cefni reservoir (eutrophic) in North Wales, subject to experimental chlorination in the laboratory and then stored at 4°C and at room temperature for an 11 week period. The majority of the weekly measurements of the concentrations of key DBPs showed no statistical differences from the initial week 0 values, regardless of water source types or storage temperatures ($p < 0.05$).

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List of Abbreviations

AOM	Allogenic Organic Matter
CW	Constructed Wetland
DBPFP	Disinfection By-products Formation Potential
DBPs	Disinfection By-products
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
ECD	Electron Capture Detector
ESRI	Environmental Systems Research Institute
GC	Gas Chromatography
GIS	Geographic Information System
HAA	Haloacetic Acid
HSD	Honest Significant Difference
MM	Molecular Mass
NMR	Nuclear Magnetic Resonance
NOM	Natural Organic Matter
NW	North West
NYB	Nant-y-Brwyn
POM	Particular Organic Matter
RSW	Raw Surface Water
SE	South East
SEM	Standard Error of the Mean
SPME	Solid-phase Microextraction
STHMFP _{7d}	Standard Trihalomethane Formation Potential 7 days
SUVA	Specific Ultra-Violet Absorbance
TC	Total Carbon

TN	Total Nitrogen
THMFP	Trihalomethane Formation Potential
THMs	Trihalomethanes
TOC	Total Organic Matter
USEPA	United States Environmental Protection Agency
UV	Ultra-Violet Absorbance
WHO	World Health Organization
WTWs	Water Treatment Works

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Chapter 1: Introduction

1. Natural Organic Matter

1.1. Overview

Natural organic matter (NOM) is contained within all freshwater ecosystems. It is a mixture of heterogeneous chemicals originating from decaying animal and plant products and its size, composition and characteristics are influenced principally by the Earth's hydrological and carbon cycles (Schnitzer and Khan, 1972; Thurman, 1985; Murray *et al.*, 2004). NOM is mostly in a continual state of decomposition, because heterotrophic microorganisms slowly break it down, altering its chemical properties (Pusch *et al.*, 1998). NOM consists of a diverse pool of organic material, with compounds including low molecular weight short chain carbon compounds, carbohydrates and larger and more complex humic acids. The specific compounds that make up NOM are numerous and its precise nature is poorly understood. NOM is often separated into humic and non-humic compounds.

1.2. Humic and non-humic compounds

Humic compounds are a heterogeneous mix of compounds that together form humus, which is poorly soluble in water due to its overall hydrophobicity, even many of its constituents contain some polar functional groups (Figure 1.1). In addition humic substances are aromatic, high-molecular weight and black-brown in colour. They make up approximately 25–50% of freshwater NOM (Thurman, 1985; Zherebker *et al.*, 2016). The solubility properties of humic compounds at different pH's are not uniform, but there are trends in behaviour which allow sub-division into three sub-classes; humic acids, fulvic acids and humins (McDonald *et al.*, 2004). Humic acid components are not soluble at pH 2 or less but increase in solubility as pH increases. In general, the molecular weight of humic acids in stream water is between 1,500 and 5,000 Da, and between 50,000 and 500,000 in soils, whereas the molecular weights of fulvic acids are lower, ranging between 600 and 1000 Da in stream water and 1000 and 5000 Da in soils (McDonald *et al.*, 2004). Fulvic acids have higher oxygen content than humic acids and exhibit various colours from light yellow to yellow-brown (Kononova *et al.*, 2013; Hope *et al.*, 1994). The fulvic acid component is fully soluble between pH 1 to 14 whereas humins are insoluble in water at all pHs. Both humic and fulvic acids are described as hydrophilic in their natural state due to the strong hydrogen bonding between the molecular structures and water molecules. The major source of humic and fulvic acids is decaying plant material (Fukushima *et al.*, 1996; Kelleher and Andre 2006). The non-humic fraction is primarily consists of polysaccharides, non-polar proteins, and low-molecular weight, hydrophilic organic acids (Schnitzer and Khan, 1972; Grasso *et al.*, 1990).

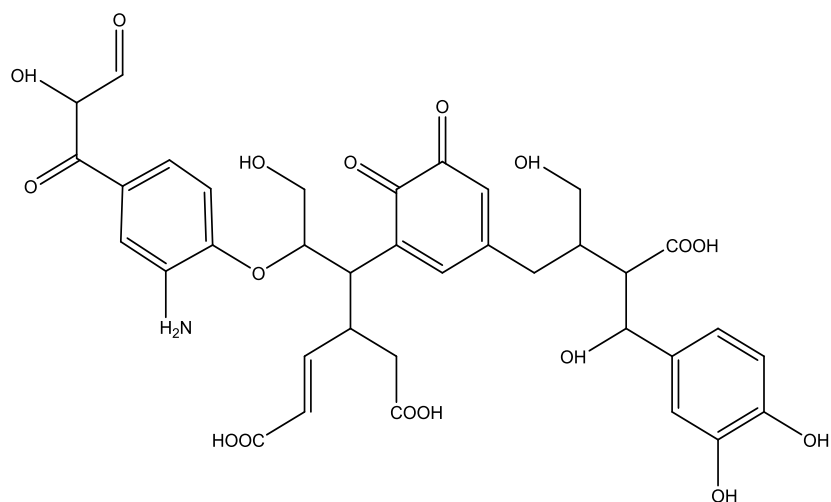


Figure 1.1. Proposed a humic acid building block (adapted from McDonald *et al.*, 2004)

A common and basic method to characterise NOM is to filter the freshwater solution through a 0.45 μM filter; the NOM retained on the filter is classed as particulate organic matter (POM), the NOM that passes through the filter and remains in solution is dissolved organic matter (DOM) (Thurman, 1985). Several analytical techniques can be employed to characterise NOM, including UV/Visible and fluorescence spectroscopies, resin fractionation, nuclear magnetic resonance (NMR) and time of flight mass spectrometry. Figure 1.2 shows the structure of a complex DOM molecule. Algogenic organic matter (AOM) may be an important non-humic DOC source, arising from passive and active exudation of extracellular organic matter from live algal cells and by intracellular organic matter released by algal cells breakage (Wetzel, 2001).

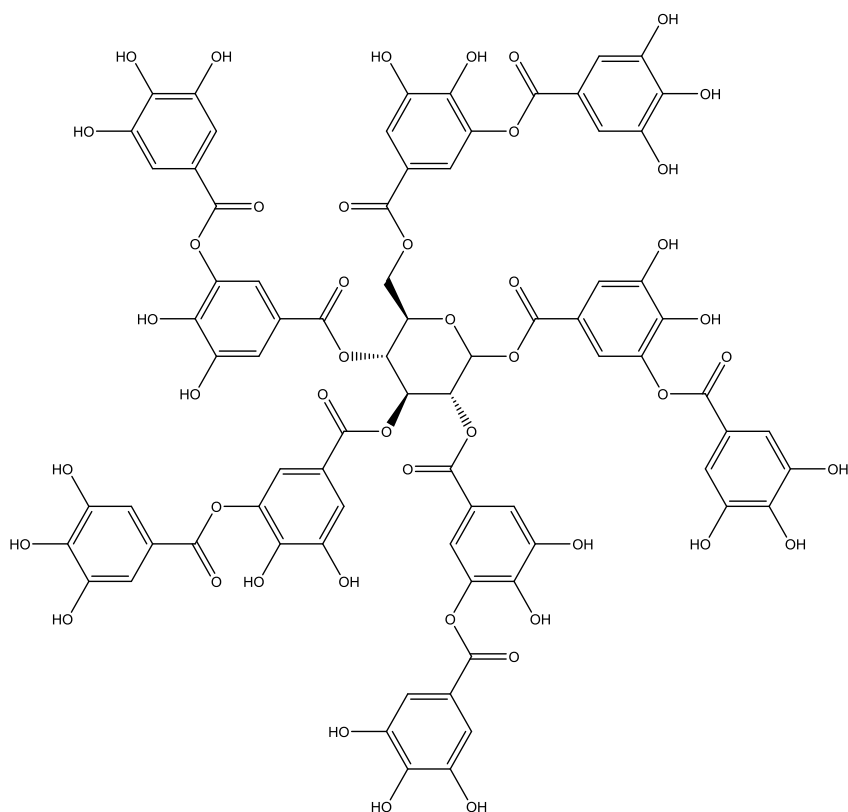


Figure 1.2. Structure of tannic acid (example of a complex organic compounds that exists as dissolved NOM).

1.3. Dissolved organic carbon (DOC)

1.3.1. Character and structure

Carbon is the key constituent of NOM; therefore, NOM may frequently categorised as either dissolved or particulate organic carbon (DOC/POC). The analytical techniques used to quantify and characterise organic matter in freshwaters and usually based on measuring the carbon in NOM. DOC is the main form of organic carbon in freshwaters (Jones and Mulholland, 1998), usually making up 90% of total organic carbon. It is derived from the cycling of carbon between the upper lithosphere and the atmosphere. Carbon dioxide gas is taken up from the atmosphere by vegetation during photosynthesis, used to form plant structures during the growing season, transferred to the soil during the autumn as vegetation dies and partly returned to the atmosphere as the organic matter is decomposed. As organic carbon resides in soil it can be transferred to freshwaters during rainfall (allochthonous source) and globally this is generally the dominant source of DOC in rivers, streams and lakes (Wetzel, 2001). It is common to observe strong correlations between lake DOC concentrations and the size of the lake's catchment (Schindler, 1971). DOC is also produced within freshwaters by algae and microorganisms (autochthonous sources). Freshwaters with a high

concentration of DOC will usually be coloured brown due to the presence of humic substances, which strongly absorb ultraviolet and visible light.

1.3.2. Changing Trends in DOC

In the United Kingdom, between 1988 and 2000, DOC was reported to have increased by an average of 65% across 22 streams and lakes (Freeman *et al.*, 2001a; Worrall *et al.*, 2003a). A similar rising trend has been measured in Central and Northern Europe and North America (Stoddard *et al.*, 2003; Skjelkvåle *et al.*, 2005; Monteith *et al.*, 2007; Evans *et al.*, 2006; Figure 1.3). The rising DOC trend has been linked to several factors, but the scientific community have yet to reach a consensus on the causes.

Freeman *et al.* (2001b) linked the trend to the rise in global temperatures between 1970 to 2000, stating the mechanism as being increased microbial activity and labile DOC export from peat soils. During simulations in the laboratory, the extracellular enzyme phenol oxidase activity increased by 36% for every 10°C rise in temperature (Freeman *et al.*, 2004b). Higher temperatures increase microbial and invertebrate decomposition processes in soils, resulting in increased DOC formation (Freeman *et al.*, 2001b; Cole *et al.*, 2002). Changes in hydrological flow regimes and precipitation patterns have also been suggested to cause rising DOC (Hejzlar *et al.*, 2003; Hongve *et al.*, 2004). Drought conditions can also lead to rapid increase in DOC from the catchment transferring into surface waters when rainfall occurs (Evans *et al.*, 2005). In addition, increased DOC production has been associated with a stimulation of plant production due to increased CO₂ in the atmosphere (Freeman *et al.* 2004a) and a soil microbial response to atmospheric nitrogen deposition (Pregitzer *et al.*, 2004; Findlay, 2005). Increased organic matter solubility is reported to be linked to decreased acidity and ionic strength of soil water (Evans *et al.*, 2006; Monteith *et al.*, 2007). Evans *et al.* (2012) demonstrated that pH is a key factor controlling the concentration of DOC in soils, and hypothesised that the decline in SO₂ dry deposition observed in many regions of the northern hemisphere over the last two decades is principally responsible for the DOC trend. Thus, these changes may lead to increased DOC export in late summer and autumn (Ritson *et al.*, 2014).

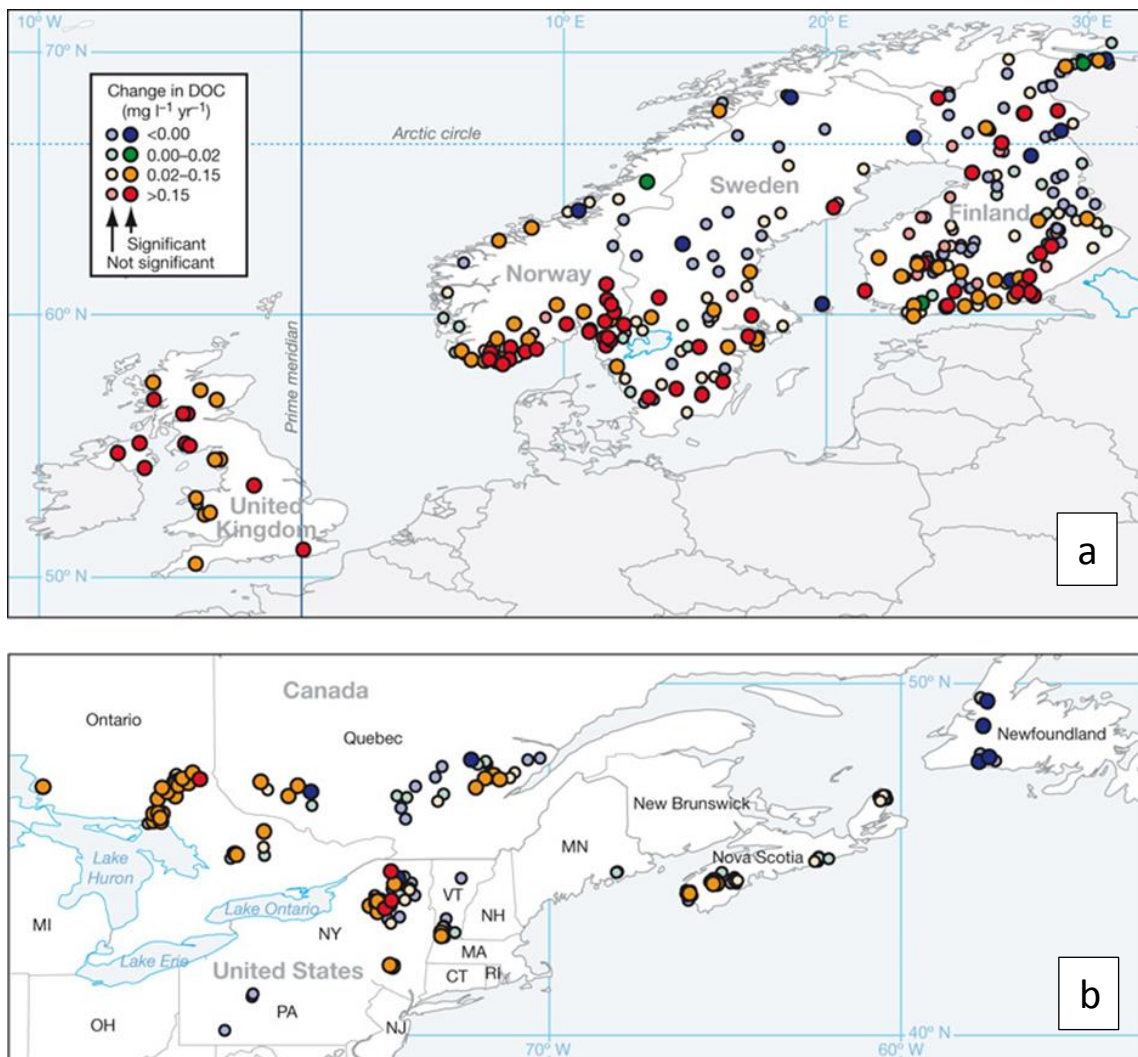


Figure 1.3. Trends in DOC concentration (mg/L, year) observed in Europe (a) and North America (b) for the period 1990-2004 (Monteith *et al.*, 2007).

1.3.3. Allochthonous vs. autochthonous sources

The contribution of DOC to freshwaters from different sources is highly variable on a seasonal basis. Allochthonous DOC inputs are greatest during the late summer/autumn period, at the end of the growing season when vegetation starts to die back and decompose (Holden & Adamson, 2002). Autochthonous DOC production peaks in the summer months, when temperatures and light levels are highest (Manny and Wetzel, 1973). Therefore, freshwater DOC concentrations tend to peak in autumn when the contribution from both sources are high (Royer and David, 2005). Globally, allochthonous sources tend to dominate DOC inputs over autochthonous sources (Manny and Wetzel, 1973). DOC from allochthonous sources is generally in higher molecular weight, more aromatic and absorbs more light than DOC from autochthonous sources (Hedges *et al.*, 1994; Gergel *et al.*, 1999).

1.3.4. Phenolic Compounds in DOC

Phenolic compounds are a specific group of organic chemicals common in nature (Ribéreau, 1972). They are characterised by the presence of at least one aromatic ring carrying one or more hydroxyls (Appel, 1993). Phenolic compounds comprise a significant part of freshwater DOC, especially in areas which drain organic soils. Phenolic compound concentrations in water samples can be determined using a colorimetric technique described by Box (1983).

1.4. Carbon Balance

1.4.1. Peatlands

Peatland soils are one of the most important sources of DOC to freshwaters. Peat soils are known to export high concentrations of DOC, because the soil is carbon rich and precipitation tends to be high in areas where peat is found, so there is a continual flush of DOC-rich waters. Figure 1.4 shows the distribution of peatlands globally, with approximately 400 million hectares throughout the world. Peatlands form when the climate and topography result in a high water table; this causes anaerobic conditions in the soil and suppresses the decomposition of organic matter. The suppression of decomposition has been attributed to significant lowering of activity of the enzyme phenol oxidase (Freeman *et al.*, 2004b), which requires oxygen to function but this is virtually absent from peat. This leads to a build-up of inhibitory phenolic compounds, such as tannic acid, and these compounds are known to inhibit hydrolase enzymes, the main decomposers of organic material in soils. Therefore, organic matter builds up slowly over time such that peatlands may be many metres deep.

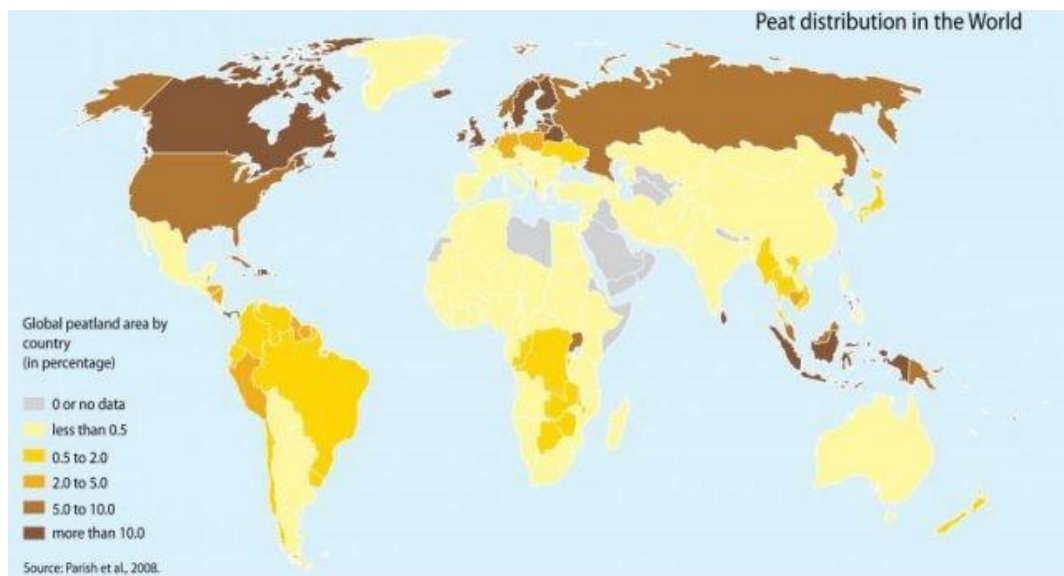


Figure 1.4. Global peatland distribution (Riccardo Pravettoni, UNEPGRID-Arendal).

Previous studies have demonstrated the influence peatlands have on DOC concentrations in freshwaters. Dillon and Molot (1997) described how DOC export rates are best modelled by a simple linear function based on the percentage of peat coverage in the catchment. Aitkenhead *et al.* (1999) observed that Scottish rivers with the highest DOC concentrations have extensive peatlands in their catchments. Research by Urban *et al.* (1989) highlighted that the most important variable controlling the DOC yield from a catchment is the proportion of the area occupied by peatlands. The role of peatlands in influencing the DOC concentration of freshwaters is therefore critical, particularly in the temperate regions of the northern hemisphere where most peatlands exist.

There are two contrasting types of peatland; bogs and fens, as defined below:

1.4.1.1. Bogs

Ombrotrophic (entirely rain-fed) or oligotrophic (nutrient-poor) bogs are acidic peatlands with a pH of 4.0 to 4.8, with no significant inflow or outflow of surface water or groundwater, which support acidophilic vegetation, particularly mosses. Bogs are dependent on rainfall for their water and nutrition inputs. They are often dominated by *Sphagnum*, with acidity boosted by the organic acids that form as a result of their decomposition (Warner and Rubec, 1997).

1.4.1.2. Fens

A fen is a minerotrophic, meso- or eutrophic open peatland system that generally receives some drainage from surrounding mineral soils and is often covered by grasses, sedges or reeds (Mitsch and Gosselink, 2000).

1.4.2. Wetlands as carbon reserves

Wetlands store a large proportion of the world's organic carbon. Bridgham *et al.* (2008) estimated that peatlands contain between 329 and 525 x 10¹⁵ Tg of C. There are a number of carbon reservoirs within wetlands, such as plant biomass carbon, microbial biomass carbon, particulate organic carbon, dissolved organic carbon, carbon dioxide and methane. Figure 1.5 illustrates the carbon cycle within a wetland. Worrall *et al.* (2009) calculated the carbon budget of a 11.4 km² peatland catchment in northern England. The study included both fluvial and gaseous carbon fluxes and net ecosystem respiration of CO₂. During the 13-year period of data collection, the carbon balance averaged 56 g C/km²/yr. Scaling up to the whole of the UK; this equates to a national peatland carbon balance of approximately 1.2 Tg ± 0.4 Pg C/year.

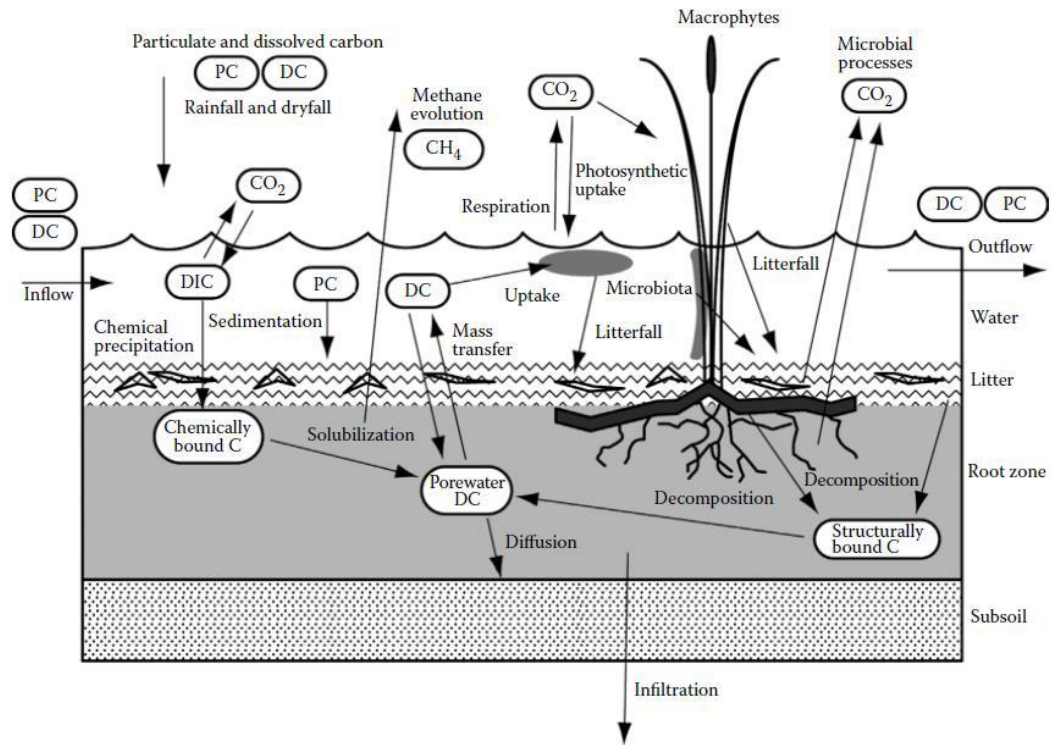


Figure 1.5. The carbon cycle within a wetland (Kayranli *et al.*, 2010).

1.4.3. Decomposition

Decomposing plant materials are the main source of DOC in surface waters (Thurman, 1985). Peatlands provide a large pool of carbon due to the accumulation of deep layers of peat in wetland environments as a result of low decomposition rates (Mitsch and Gosselink, 2000; Davidson and Janssens, 2006). During the summer DOC accumulates in the peat matrix due to higher rates of decomposition and increased plant production and senescence (Tegen and Dorr, 1996). This DOC is then leached out later during rainfall events. Scott *et al.* (1998) and Clymo (1965) reported that microbial processes are predominantly responsible for decomposition within wetlands. Highest rates of decomposition of organic matter occur in at or near the surface of the wetland where fresh litter is present and there may be more oxygen. The anaerobic conditions lower in the peat profile result in the inhibition of microbial decomposition and the accumulation of organic matter (Gorham, 1991).

1.4.4. Human disturbance of peatlands

Peatlands store huge amounts of carbon but anthropogenic activities are releasing that carbon (Holden *et al.*, 2004; Charman *et al.*, 2013; Swindles *et al.*, 2015). There is growing evidence that the peatlands of North Western Europe, South East Asia and North America are being damaged during drainage and harvesting of peat (Holden *et al.*, 2004; Hooijer *et al.*, 2010, 2012). It also appears that

climate change is a particular risk to UK Peatlands (Gallego-Sala *et al.*, 2010; Li *et al.*, 2016) and these areas are damaged by atmospheric pollution (Smart *et al.*, 2010), peat extraction, drainage for agriculture, over-grazing, wild-fire and construction of buildings (Holden *et al.*, 2006; Davies *et al.*, 2010; Wellock *et al.*, 2011). During the 1940s, vast areas of peat were drained by excavation of ditches that aimed to reduce the water table level and in order to increase the proportion of land suitable for agricultural use (Holden *et al.*, 2006). The excavation of ditches in peatlands leads to changes in ecosystem-level biodiversity, hydrology and carbon sequestration, with some studies reporting an increase in the flux of dissolved organic carbon (DOC) (Ramchunder *et al.*, 2012; Parry *et al.*, 2014). To attempt to reverse these effects, ditch blocking with dams has been widely employed, with the aim to raise the water table and have a positive effect on carbon sequestration (Beadle *et al.*, 2015; Swindles *et al.*, 2016).

1.4.5. Drought and carbon fluxes

Climate change is reported to be resulting in an increased risk of drought, which may influence peat carbon stores. Laboratory simulations indicate that drought and the resultant lowering of the water table is likely to increase the release of CO₂ from peat (Fenner and Freeman, 2011; Chen *et al.*, 2012). In some cases, an increase in CO₂ emissions is observed during rewetting (Fenner and Freeman 2011). During drought, oxygen ingress increases the enzymic activity of phenol oxidase leading to a correlated increase in degradation of phenolic compounds. This in turn stimulates hydrolase enzymes which break down organic matter, thereby releasing carbon (Freeman *et al.*, 2001a). As such phenolic compounds are considered key to the storage and release of carbon in peatlands (Freeman *et al.*, 2001a). Climate change may also lead to an increased export of carbon from peatland ecosystems to freshwaters and increased drought events may explain why DOC concentration have been increasing in many northern hemisphere freshwater systems (Worrall, *et al.* 2004b; Evans, *et al.* 2005).

1.5. Potable water treatment

1.5.1. Potable water treatment and NOM removal

The production of clean safe drinking water involves two main stages: reducing the concentration of DOC and adding a disinfectant. DOC is removed to improve the taste, colour and odour of the water (Lambert and Graham, 1995) and to minimise the formation of disinfection by-products (DBPs) (Rook, 1974). DOC itself is not harmful to humans, however DBPs resulting from the reaction between DOC and the disinfectant may have several negative effects on human health (Bull *et al.*, 1995; Sketchell *et al.*, 1995; Nieuwenhuijsen *et al.*, 2000; Palacios *et al.*, 2000). In the UK, reservoirs used as sources of drinking water are often located in upland environments, where the catchment soils are

predominantly organic and may leach high concentrations of DOC to surface waters (Tang *et al.*, 2013). Therefore, treatment works in the UK often have to employ several DOC removal processes to ensure the water is sufficiently clean for disinfection. This is commonly the most expensive stage of water treatment (Matilainen *et al.*, 2010). Coagulation is the most frequently used process to remove DOC. It involves the addition of a positively charged Al^{3+} (from aluminium sulphate) or Fe^{3+} (from ferric chloride) bonds to negatively charged organic matter to form large 'flocs' of material which can be scraped off the water's surface (Matilainen *et al.*, 2010). DOC compounds which are more negatively charged form flocs most easily. See section 1.5.4 for further discussion of coagulation. Other types of DOC removal include rapid gravity filtration and dissolved air flotation. Chlorine is the most frequently used disinfectant globally (Richardson, 2003), however its addition to water containing DOC can result in the formation of a class of DBPs called trihalomethanes (THMs), which are generally the most commonly occurring DBP in chlorinated waters.

1.5.2. Trihalomethanes (THMs)

The most common type of DBPs are trihalomethanes (Figure 1.6). They are simple derivatives of methane formed when three of the hydrogen atoms are replaced by a halogen (Peterson *et al.*, 1993) and include chloroform (CHCl_3) bromodichloromethane (CHCl_2Br), dibromochloromethane (CHClBr_2) and bromoform (CHBr_3).

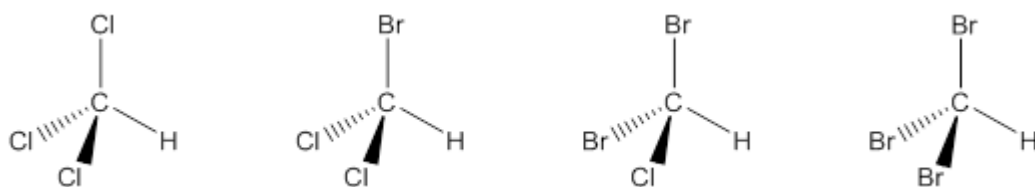


Figure 1.6. Structure of (left to right) chloroform, bromodichloromethane, dibromochloromethane and bromoform.

These are formed during the reaction between the chlorine the aqueous organic matter (Sketchell *et al.*, 1995). These compounds are collectively termed trihalomethanes and abbreviated as THM or TTHM (for total trihalomethanes). Chloroform (CHCl_3) is the most common of these compounds and the most abundant organohalide in the environment. The formation of THMs that containing bromine are usually lower than chloroform during water treatment. The discovery that chlorinating drinking water produced THMs was made in the 1970s, the higher molecular weight organic compounds such as humic acids, are generally most reactive with chlorine (Oliver and Visser, 1980; Uyguner *et al.*, 2004).

1.5.3. THM amelioration

WHO (2005) reported that THMs are carcinogenic. Therefore, water treatment companies work to minimise THM concentration in the water supply. THMs can be controlled using a number of methods including removal of precursors prior to disinfection, removal of THMs themselves prior to water supply to the public, or by reducing their formation using alternative chemical disinfectants or non-chemical disinfection processes (WHO, 2011). The most widely used method, because of cost effectiveness and efficiency is removal of the precursors prior to chlorination (US EPA, 1999). This is discussed in more detail in section 1.5.4. Ozone is an example of alternative chemical disinfectants. Chloramination has also been used to minimise THM formation and is normally used as secondary disinfection stage (DWI, 2010). It is technically possible to remove THMs before disinfected water is part of the general supply, however it is not generally the preferred method. Other techniques for removing THMs include activated UV radiation and carbon filtration-adsorption (WHO, 2011).

1.5.4. Coagulation and Flocculation Process Fundamentals

Coagulation and flocculation are used principally to remove organic compounds in raw surface waters. These compounds are kept suspended and stabilised in the water column due to their surface charge. There are three mechanisms involved in coagulation: charge neutralization, sweeping and bridging (Li *et al.*, 2006). Charge neutralization refers to the destabilisation of particulates by adding a hydrolysable metal salt, for example aluminium sulphate $\text{Al}_2(\text{SO}_4)_3$, this then hydrolyses to form cationic species which adsorbed onto the surface of negatively charged compounds. The coagulant is dispersed by mixing the solution. Collision between organic compounds and the coagulant result in the formation of micro-flocs. Further mixing results in the aggregation of micro-flocs to form larger flocs which can be easily removed by sedimentation. Sweep flocculation refers to the use of high concentrations of an amorphous metal hydroxide which precipitates as it falls through the water column, capturing the suspended particles. Particle bridging occurs when a colloidal particle acts as a chemical bridge between two or more coagulant molecules.

1.5.5. Factors affecting THM formation

Final THM concentrations depend on concentration of DOC and chlorine, reaction time, water temperature and pH (Rodrigues *et al.*, 2007). THM formation is reported to have a positive correlation with temperature (Peters *et al.*, 1980), as evidenced the summer months when higher THM concentrations are observed. There is a demonstrated pH effect on the reaction kinetics of THM

formation (Adin *et al.*, 1991). For a related DBP haloacetic acid (HAA) there is inverse correlation between pH and HAA formation potential (Kim *et al.*, 2003). In addition, there is a positive relationship between contact time and THM concentration. In the UK the minimum period of contact with chlorine is 30 min at pH < 8.0 and residual chlorine must be > 0.5 mg/L after that time is required before distribution (WHO, 2011). The reaction between chlorine and DOC also depends on the length of time of water spends in the distribution system. Also greater DOC concentrations in the raw water will lead to greater amounts of DBPs being formed. In addition, DOC type influences THM formation (Liang and Singer, 2003), with humic and fulvic acids generally considered to be the principal precursor to THM formation (Bond *et al.*, 2009). DBPs form either by the oxidation across carbon-carbon double bonds or by substitution reactions (Westerhoff *et al.*, 2004). Derivatives of chloro-bromo DBPs are formed when the bromide ion is present (e.g. bromoform) (Pourmoghaddas *et al.*, 1995). Algal organic matter is a particular problem during the treatment of water as it is very difficult to remove increase the level of total organic carbon and hence THM formation (Gough *et al.*, 2015). (Gough *et al.*, 2015; Li *et al.*, 2012). Furthermore, with DOC concentrations of freshwaters increasing, it will be harder for water companies to minimise the formation of DBPs.

1.5.6. The health risk posed by DBPs

Studies have reported that DBPs pose only a low-magnitude risk in treated water, although these risks must be considered in the context of a lifetime of exposure (Singer, 2006). Humans can be exposed to these compounds either through drinking water (Nazir and Khan, 2006) and bathing (Xu, *et al.*, 2002; Chu and Nieuwenhuijsen, 2002). Exposure to these compounds has been associated with increased risk of cancer (Peterson, *et al.*, 1993; Bull, *et al.*, 1995; Singer, 1999). In 1974, chloroform was identified in chlorinated water supplies (IARC, 1991). Since then a strong relationship has been found between bladder cancer and exposure to THMs. THMs and HAAs have a carcinogenic effect on humans. (Chu and Nieuwenhuijsen, 2002). There is evidence that many of the DBPs found in chlorinated drinking water supplies are toxic to laboratory animals (Nieuwenhuijsen *et al.*, 2000). Women who drank water containing more than 75 µg/L THMs had a miscarriage rate of 15.7%, compared to 9.5% for women that drink water containing low THM concentrations (Capece, 1998). The level of DBPs in drinking water (generally between 10 to 100 of µg/L) is considered to pose a carcinogenic risk (Waller, *et al.*, 1998). As a result of such research, guideline maximum THM levels have been set; in USA the maximum limit for THMs in drinking water is 80 µg/L (Yoon *et al.*, 2003). In the UK is 100 µg/L in UK (DWI, 1998) and in Denmark is just 10-15 µg/L (DWI, 2010).

1.6. Constructed Wetland (CW)

A constructed wetland (CW) is an artificial wetland created to clean a source of water. The system uses the natural functions of vegetation, soil, and organisms to remove contaminants from water. Constructed wetlands also work like filtration systems to remove sediments and pollutants such as heavy metals from the water. There are two types of constructed wetlands; free water surface wetlands and subsurface flow wetlands (reference). Free water surface flow wetlands have the water surface exposed to the atmosphere throughout the year and this type of constructed wetland is like a marsh and bog, with some emergent vegetation. Water near to the bottom of the wetlands is anoxic, which inhibits nitrification, while the water close to the surface is where oxygen may be available, in addition to microsites on living plants such as roots and rhizomes. The second type of constructed wetland is a subsurface flow wetland, which is where the water level is completely contained below the soil surface (Díaz *et al.*, 2009).

Wetlands tend to increase the concentrations and change characteristics of DOC (Rostad *et al.*, 2001). Consequently, the impact of constructed wetlands on the potential formation of DBPs an important issue relating to human health, environmental hazards and wetland treatment technology. A relatively small number of studies have examined the impact of constructed wetlands on DOC characteristics and propensity to form DBPs. One of the most comprehensive was that of Rostad *et al.* (2000) who reported significantly higher DBP formation potential (DBPFP) at the outlet of a constructed wetland system compared to the inlet. The reason for this was an increase in the aromatic content of the DOC as it moved through the wetland.

1.6.1. Eutrophic and oligotrophic waters

In the UK, 66% of drinking water comes from surface water such as reservoirs and lakes and 34% comes from groundwater. In lowland reservoirs, eutrophication is a major problem, and can increase the DOC concentration of the water (Nixon, 1995). Eutrophication is characterized by excessive plant and algal growth, which can be due to a stimulation of the factors controlling the rate of photosynthesis (Schindler, 2006), such as sunlight, carbon dioxide concentration, and nutrient fertilizers. Over centuries, eutrophication naturally occurs when lakes fill in with sediments (Carpenter, 1981; Scholz *et al.*, 2016). Human activities such as farming have increased the rate of eutrophication through discharges of nutrients, such as nitrogen and phosphorus (Carpenter *et al.*, 1998; Marcos *et al.*, 2013). Eutrophication is associated with harmful algal blooms that cause water pollution in aquatic ecosystems (Smith and Schindler, 2009). This can increase the input of autochthonous DOC and increase DBP formation in treated water (Pierson *et al.*, 2010).

1.7. Scope of study

The experimental work in this thesis was undertaken in North and West Wales catchments that linked to their WTWs, where DOC concentrations have been increasing. This increasing trend is a concern for WTWs, because of the associated increase in THM levels in the finished water. This research examines the variations in DOC concentration and how these are linked with THMFP in different places and times. The main aims of this thesis are summarised below:

Chapter 2: To assess whether man-made drainage ditches are contributing more DOC to Llyn Conwy than natural streams.

Chapter 3: To determine whether peatland drought conditions, simulated in the field, change the characteristics of the DOC to make it more or less likely to form DBPs in a water treatment plant.

Chapter 4: To determine whether peatland drought conditions, simulated in the laboratory, change the characteristics of the DOC to make it more or less likely to form DBPs in a water treatment plant.

Chapter 5: To understand whether a constructed wetland used to remove nutrients from a stream flowing into a eutrophic reservoir affects the quantity and quality of DOC in the stream.

Chapter 6: To assess long term stability of trihalomethane compounds in water samples and to test for differences in storage temperature (4°C vs. 25°C) and water type (nutrient poor vs. nutrient rich).

Thesis structure

Chapter 1: Literature review

Chapter 2: Characteristics of dissolved organic carbon (DOC) exported from drained peat with respect to disinfection by-product formation.

Chapter 3: Field experiment investigating effects of peatland drought on dissolved organic carbon (DOC) release and implications for trihalomethane (THM) formation.

Chapter 4: Laboratory experiment investigating the effects of Peatland Drought on Dissolved Organic Carbon (DOC) and Trihalomethane (THM) Formation (submitted to 'Scientific Reports').

Chapter 5: Impact of constructed wetlands on DOC characteristics and THMFP.

Chapter 6: Simultaneous analysis of water quality and temperature on the stability of trihalomethanes in water samples during prolonged storage.

Chapter 7: Final Discussion

Each of the above chapters 2-6 has been prepared in the style of a paper that is suitable for submission to a peer reviewed journal.

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Chapter 2: Characteristics of dissolved organic carbon (DOC) exported from drained peat with respect to disinfection by-product formation.

2.1. Abstract

For the last two decades, the freshwater concentration of dissolved organic carbon (DOC) has increased in many parts of the northern hemisphere, particularly in rivers and streams draining peat soils. This has been attributed to reduced acidic deposition and climatic and land use changes. Concentrations of DOC in freshwaters used as sources of drinking water must be reduced prior to the addition of a disinfectant to minimise the formation of potentially harmful carcinogenic compounds (disinfection by-products; DBPs); hence, the rising DOC trend poses a threat to human health as it may lead to increased DBP production. One land use change that has been linked to rising DOC, is peatland drainage undertaken in the UK extensively during the 1960s and 1970s to improve upland areas for agricultural grazing. Recent evidence has demonstrated that this can increase DOC concentration in drainage waters due to the increased decomposition of the peat matrix, however relatively few studies have examined the characteristics of this extra carbon and its likelihood to form DBPs compared to DOC supplied by natural Streams. In this study we present data demonstrating that the DOC concentration of drainage ditches is higher than a natural stream, but there are no differences in the propensity of DOC to form trihalomethanes.

2.2. Introduction

Natural organic matter (NOM) is contained within all freshwater ecosystems. It is a mixture of heterogeneous compounds derived from decaying animal and plant products and the concentration and characteristics are influenced by the Earth's hydrological and carbon cycles (Khan and Schnitzer, 1972; Marray and Parsons, 2004; Thurman, 1985). NOM is usually divided into two broad categories through filtration properties; that which is retained on a 0.45 μM filter is Particulate Organic Matter (POM) and that which passes through the filter and remains in solution is Dissolved Organic Matter (DOM) (Thurman, 1985). Carbon is the key constituent of NOM, therefore NOM is often referred to as either dissolved or particulate organic carbon (DOC/POC) and the analytical techniques used to quantify and characterise NOM/DOM in freshwaters are usually based on measuring the carbon component. DOC is the dominant form of organic carbon in freshwaters (Jones and Mulholland, 1998; Palmer *et al.*, 2015), usually comprising 90% of total organic carbon, and is considered a dynamic, intermediate stage in the cycling of carbon between the terrestrial environment and the atmosphere (Jones *et al.*, 2016). As organic carbon resides in soil it can be transferred to freshwaters during rainfall with this allochthonous source being generally the dominant origin of DOC in rivers, streams and lakes globally. It is common to observe strong correlations between a lake's DOC concentration and the proportion of peat in its catchment (Arvola *et al.*, 2016; Palmer *et al.*, 2015). DOC is also produced within freshwaters by algae and microorganisms; i.e., the autochthonous source. Freshwaters with a high concentration of DOC are usually coloured brown due to the presence of humic substances that strongly absorb ultraviolet and visible light (Jones *et al.*, 2016). The contribution from each source varies on a seasonal basis; allochthonous DOC inputs are greatest during the late summer/autumn period whilst autochthonous DOC production peaks in the summer months, when temperatures and light levels are at their highest. Therefore freshwater DOC concentrations tend to peak in September-October when the contribution from both sources are high (Jones *et al.*, 20016). Globally, allochthonous sources tend to dominate DOC inputs over autochthonous sources (Manny and Wetzel, 1973). DOC from allochthonous sources is generally a higher molecular weight, with a tendency to have more aromatic moieties and absorbs more light than the DOC from autochthonous sources (Gergel *et al.*, 1999; Hedges *et al.*, 1994).

Phenolic compounds are a specific group of organic chemicals common in nature (Ribéreau, 1972) and are characterised by the presence of one or more aromatic rings carrying at least one

hydroxyl group (Appel, 1993). Phenolic compounds can be a significant fraction of freshwater DOC, especially in areas draining organic soils such as peats.

The presence of DOC in freshwaters may cause issues for the production of clean, safe drinking water. The DOC must be removed to improve the taste, odour and aesthetics of the water and to minimise the formation of disinfection by-products (DBPs). DBPs result from the reaction between chlorine, added as a disinfectant, and the NOM present in drinking water (Sketchell *et al.*, 1995). Although brominated DBPs such as bromoform can also be formed (Peterson *et al.*, 1993) in the presence of bromide. Halogenation of NOMs occurs either via the oxidation of carbon-carbon double bonds or by substitution, where a functional group is replaced by a halogen (Rodrigues *et al.*, 2007). DBPs are organo-halide compounds with trihalomethanes (THMs) representing the most common sub-group. These are simple derivatives of methane formed when three of the hydrogen atoms are replaced by a halogen (Bond *et al.*, 2009). The rate of DBP formation depends on the concentration of DOC, chlorine, water temperature and pH (Westerhoff *et al.*, 2004). The humic and fulvic acids component of DOC has been found to be the principal reaction precursor to THMs (Williams *et al.*, 1997). Higher temperatures lead to greater DBP formation, so the concentration of DBPs in summer are usually higher than in winter (Pourmoghaddas and Stevens, 1995).

A concern within the water industry is that there is significant evidence that the concentration of DOC in many freshwater environments in the northern hemisphere is increasing. It has been reported that between 1988 and 2000, DOC increased by 65% in streams and lakes in the UK (Freeman *et al.*, 2001a). There is also evidence of this increasing trend in other parts of Europe and North America (Evans *et al.*, 2006; Monteith *et al.*, 2007). This has been attributed to increasing temperature (Freeman *et al.*, 2001b) and atmospheric CO₂ concentrations (Freeman *et al.*, 2004a), more frequent droughts (Fenner *et al.*, 2011) and reduced acid deposition (Evans *et al.*, 2012). Increased temperatures (+0.66°C between 1970-2000) may have led to greater microbial activity and decomposition in soils, thus leading to greater DOC release (Freeman *et al.*, 2001b). According to (Evans *et al.*, 2012) pH may also influence the concentration of DOC in soils and they hypothesised that the decline in sulphur deposition observed in many regions of the northern hemisphere over the last two decades is connected with the rising DOC trend. However, there are likely to be several drivers influencing this increasing trend. One that is particularly relevant in the UK is peatland drainage (Strack *et al.*, 2006).

All peatlands export DOC to freshwaters, regardless of their status, typically in the range 5-40 g DOC m⁻² yr⁻¹ (Moor *et al.*, 1997; Price, 2003). DOC export also tends to be related to the area of peatland within the catchment (Roulet *et al.*, 1992; Tahvanainen, 2011). The export of DOC from peatland areas is affected by processes that lower the water table, such as climate change and drainage (Freeman *et*

al., 2004b; Laine *et al.*, 1992; Prévost *et al.*, 1999). Drainage of peatland areas can induce significant changes to hydrology (Huotari *et al.*, 2013) with a lowering of the water table by 20–60 cm depending on distance from the ditch, reported in some studies (Prévost *et al.*, 1999; Räike *et al.*, 2012; Sarkkola *et al.*, 2009). Such changes are reportedly responsible for increased riverine and pore water DOC concentrations in a number of areas in the northern hemisphere (Aitkenhead *et al.*, 1999; Koprivnjak and Moore 1992; Moore *et al.*, 1998; Strack *et al.*, 2008). The increased loss of DOC from peatland ecosystems has important implications for their carbon budgets and will add CO₂ to the atmosphere as a significant proportion of freshwater DOC is completely mineralised in fluvial systems (Mattsson *et al.*, 2005). Peatland drainage is increasingly being recognised as having a negative impact on atmospheric CO₂ concentration, and as a result ditch blocking has been a commonly used technique to restore the peatland water table and eventually reduce CO₂ emission (Turner *et al.*, 2013).

The aim of this study was to determine whether man-made drainage ditches are contributing a greater quantity of DOC to Llyn Conwy than natural Streams and whether this DOC has a different propensity to form THMs during chlorination. The work will help inform water companies as to whether drainage ditches within peat-dominated reservoir catchments represent a threat to water quality. We hypothesise that the ditches will have a greater DOC concentration than the natural stream and the DOC will have a higher reactivity with chlorine.

2.3. Methods

2.3.1. Study Site

Sampling was performed at three contrasting sites in north Wales (Figure 2.1 & Table 2.1); Llyn Conwy (“lake”) a natural oligotrophic drinking water reservoir, Nant-y-Brwyn (“stream”) a natural Stream outside the catchment of the reservoir but analogous to other upland streams within the area (Jones, unpublished data) and four man-made drainage ditches (“ditches”) within the predominately peat catchment of Llyn Conwy.



Figure 2.1. The location of Llyn Conwy in south eastern Snowdonia. White markers show locations of the six sampling sites

Site name	Site type	UK grid reference
Llyn Conwy	Lake	SH 78150 45586
Nant-y-Brwyn	Natural stream	SH 79147 45267
Ditch A	Man-made drainage ditch	SH 78367 45855
Ditch B	Man-made drainage ditch	SH 78349 45873
Ditch C	Man-made drainage ditch	SH 78354 45931
Ditch D	Man-made drainage ditch	SH 78333 45892

Table 2.1. Site identity (ID), type and grid reference location.

2.3.2. Sampling regime

Sampling was performed approximately twice monthly from May 2013 to May 2014 (see supplementary data Table 2.S1 for exact dates). Data is also included for the Lake, Stream and some Ditches for various months from July 2012 – Feb 2013 which was collected as part of another project (Hughes, 2013). Approximately 250 mL of water sample was collected at each site in acid washed (using 3% HCl) plastic bottles. The temperature of the water was recorded in situ. pH and conductivity were measured in the laboratory on unfiltered samples on the day of

sampling. The pH was measured using a Seven Easy pH meter (Mettler Toledo, Leicester, UK). The probe was calibrated using buffers of pH 4 and 7 at 25°C (Sigma, Dorset, UK). Conductivity was measured with a Primo 5 conductivity meter (Hanna Instruments, Bedfordshire, UK). All samples were filtered through 0.45 µm cellulose acetate filters and refrigerated at 4°C until further analysis was undertaken.

2.3.3. Laboratory Analyses

2.3.3.1. Dissolved organic carbon (DOC)

Concentrations of DOC were determined using a Thermalox TC/TN analyser (Analytical Sciences Ltd, Cambridge, UK), which is a combustion and infrared detection-based technique. For DOC, samples were acidified to pH <3 prior to analysis to remove inorganic carbon using 10 M HCl. The instrument was calibrated with potassium hydrogen phthalate standards. The injection volume was 15 µL and oven temperature 680°C. Results were corrected if standard solutions deviated by 10% or more from the correct concentration.

2.3.3.2. Phenolic compounds

The phenolic component of DOC was determined using a method modified from Box (1983), adapted for 300 µL microplate wells. A calibration curve was generated using a series of phenol standard solutions ranging in concentration from 0.5 - 10 mg/L. The assay product was detected by measuring the absorbance at 750 nm on a Spectramax M2e spectrophotometer (Molecular Devices, Wokingham, UK).

2.3.3.3. UV/visible absorption

SUVA is used as an indicator of overall DOC quality i.e. molecular weight and aromaticity, with most freshwaters having values in the range 2-4 (Leenheer *et al.*, 2003). SUVA was calculated by dividing the absorbance value at 254 nm (measured on the Spectramax M2e spectrophotometer) by the DOC concentration (mg/L).

2.3.3.4. Trihalomethane formation potential (THMFP)

The standard trihalomethane formation potential (THMFP) analysis was used to determine the concentration of trihalomethanes that can be formed per unit DOC under ideal conditions, to give an indication of the reactivity of DOC with chlorine and its propensity to form THMs. A modified version of the THMFP test detailed in Leenheer *et al.* (2000) was used. In this study samples were

diluted to 1 mg/L DOC in order to derive a standardised THMFP_{7d} value (STHMFP_{7d}) which provides a measure of DOC reactivity. A THMFP_{7d} value was then calculated by multiplying STHMFP_{7d} by DOC concentration. 97.5 mL of diluted sample was dosed with 2.0 mL of 0.5 M KH₂PO₄ to buffer the solution to pH 6.8. Samples were then dosed with 0.5 mL of NaOCl to provide 5 mg of free Cl per mg of DOC. The solution was transferred to a 100 mL amber glass bottle and incubated in the dark at 25°C for 7 days to allow the reaction between organic carbon and chlorine to occur. After 7 days incubation, the reaction was quenched by adding 0.4 mL of 0.8 M Na₂SO₃ to 14.6 mL of incubated sample. This solution was then transferred to a 22 mL amber headspace vial and analysed by the following method. The concentration of THMs was measured using a solid phase micro-extraction (SPME) technique similar to that described by Sarrión *et al.* (2000). The technique uses a Varian GC 450, a Restek MX 1 column (diphenyl/dimethyl polysiloxane phase, 30 m length, 0.53 mm i.d.), N₂ carrier gas at a flow rate of 10mL/min and a ⁶³Ni Electron Capture Detector. The oven temperature was held at 35°C for 9 minutes and then increased at a rate of 10 °C /minute to 140°C and held for 2 minutes and finally increased to 180°C and held for 3 minutes. The injector temperature was 290°C and the detector temperature 300°C.

Trihalomethane calibration solutions were made from a stock solution containing 2000 µg/mL of each of the 4 THMs; chloroform (CHCl₃), bromodichloromethane (CHBrCl₂), dibromochloromethane (CHBr₂Cl), and bromoform (CHBr₃) (Sigma). A series of standard solutions were then prepared ranging from 1-500 µg/L.

2.3.4. Statistical Analysis

The effect of site (three levels; Lake, Stream, ditch) on each measured parameter were determined using one-way ANOVA and Tukey HSD post-hoc tests in R v3.3.1. Pearson correlation was used to test for significant relationships between all dependent variables at each factor level. Most data met the homogeneity and normality assumptions, which were tested using the Bartlett and Shapiro Wilk tests, but those that did not were log-transformed. Data points were removed if they were outliers. A p value of 0.05 was used to determine significance.

2.4. Results

All samples from the three sites were acidic throughout the sampling period (Figure 2.2a) and there was a significant effect of the site on pH ($F=33.598$, $p<0.001$; Figure 2.2b, Table 2.2). The lowest mean pH was measured in the ditches, at 4.09 ± 0.05 . Both the stream and the lake had a significantly higher mean pH values, at 5.20 ± 0.76 and 5.26 ± 0.41 (both $p<0.001$), but the stream and the lake did not differ significantly ($p>0.05$). There was a large temporal variability for each site between the 3 sites, but no clear seasonal trend. For the lake, the highest pH was recorded in October 2013, at 6.8, and lowest in December 2013, at 4.4. For the stream the highest pH was recorded in July 2013 at 6.6 and lowest in January 2014 at 3.5. For the ditches the highest value was measured in March 2014 at 5.7 for ditch B and lowest in November 2013, at 3.8 for ditch C.

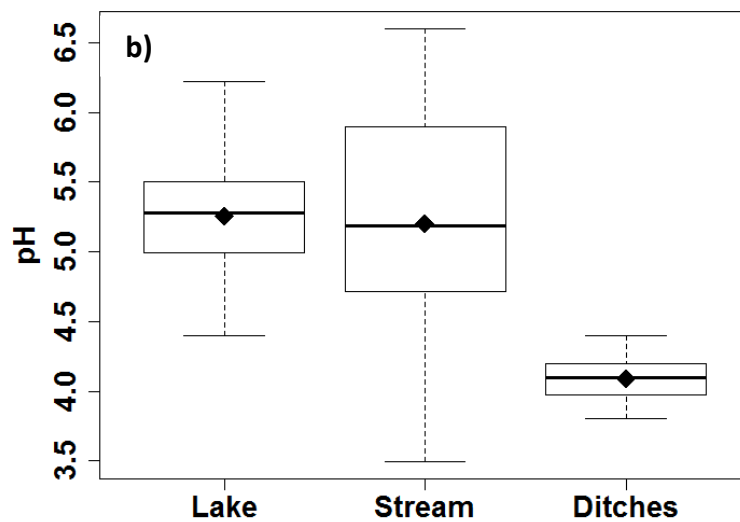
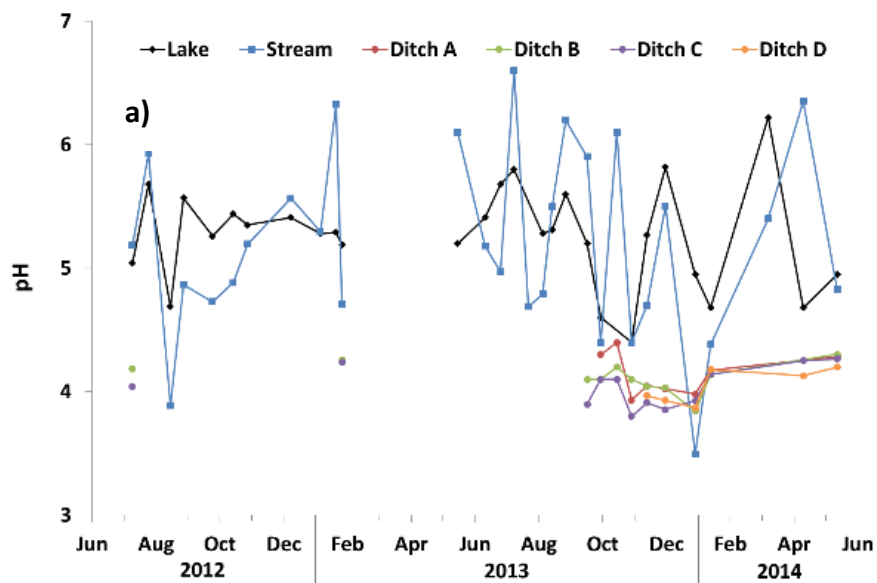


Figure 2.2 a) time series and b) mean pH of all study sites for the period July 2012 to May 2014. Diamond symbols are mean values, bold horizontal lines are median values, the box represents the interquartile range and whiskers the 5th and 95th percentiles

	pH	Conductivity	Phenolics	DOC	SUVA	THMs
F value	33.5	32.580	48.977	37.462	51.158	1.180
P value	<0.001	<0.001	<0.001	<0.001	<0.001	p>0.05

Table 2.2. Results of one-way ANOVA analysis two test effect of sites on measured parameters.

The conductivity varied significantly when comparing the mean values for the lake ($34.67 \pm 9.33 \mu\text{S/cm}$) and stream ($39.3 \pm 13.2 \mu\text{S/cm}$) versus the ditches ($66.68 \pm 4.81 \mu\text{S/cm}$) ($F=32.580$, $p<0.001$; Figure 2.3b). The lake and the stream had statistically similar mean conductivity values,

($p > 0.05$). The conductivity of each of the four ditch sites (A-D) varied in a very similar way over the period June 2013 – June 2014 (Figure 2.4a). There was a dramatic increase in the conductivity for the ditches from September 2013 (33 $\mu\text{S}/\text{cm}$ for ditch B) to January 2014 (115 $\mu\text{S}/\text{cm}$ for Ditch d). The lake and stream generally had much less variance in their conductivities compared to the ditches during the period June 2013 – June 2014 (min-max: lake, 21-59 $\mu\text{S}/\text{cm}$; stream 22-82 $\mu\text{S}/\text{cm}$).

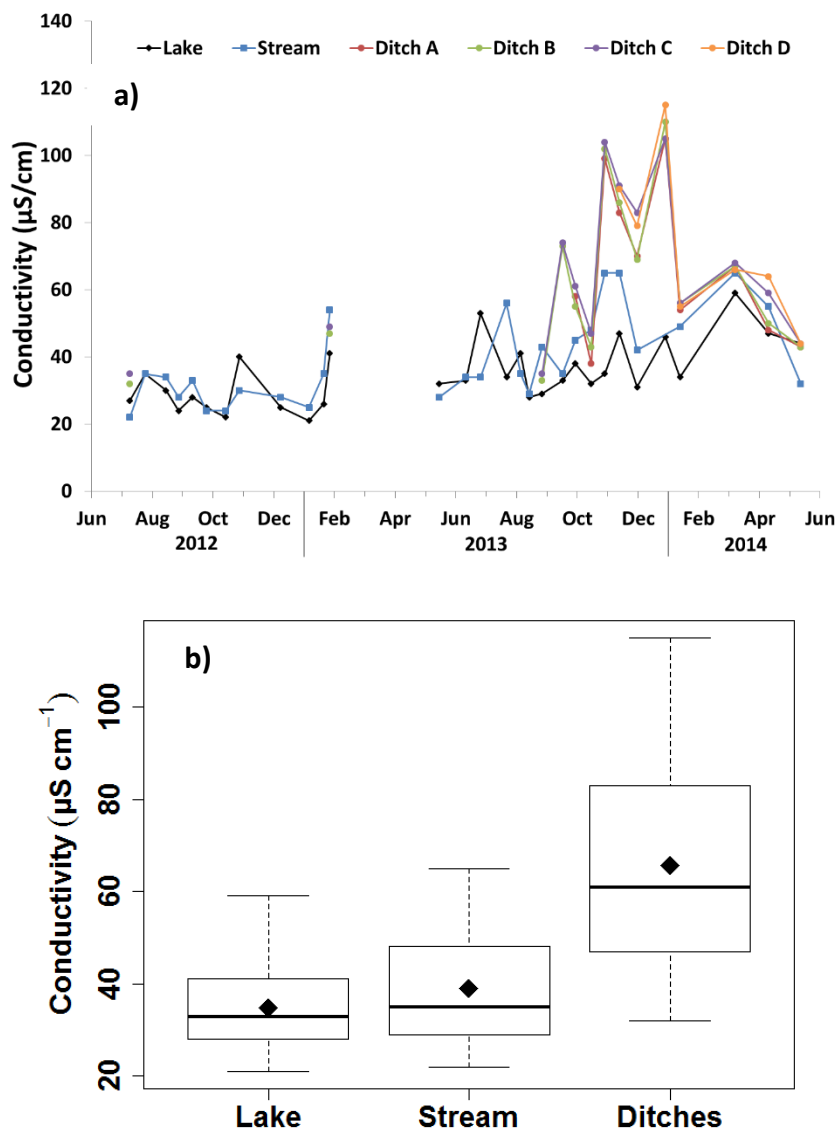


Figure 2.3 a) time series and b) mean conductivity of all study sites for the period July 2012 to May 2014. Diamond symbols are mean values, bold horizontal lines are median values, the box represents the interquartile range and whiskers the 5th and 95th percentiles.

		pH	Conductivity	Phenolics	DOC	SUVA	THMs
Lake	Stream	.577	.423	.000	.000	.000	.997
Lake	Ditch	.000	.000	.000	.000	.000	.400
Stream	Ditch	.000	.000	.403	.000	.000	.476

Table 2.3. Results of Tukey HSD post-hoc tests for each level of the Site factor.

DOC concentration varied when comparing the mean values for the lake (4.53 ± 0.75 mg/L) and stream (12.69 ± 7.15 mg/L) versus the ditches 19.01 ± 0.43 mg/L ($F=37.462$, $p<0.001$; figure 2.4b) . The DOC in the ditches was on average 6.32 mg/L greater than stream and the stream 8.16mg/L greater than the lake. The ditches had a statistically significant higher DOC concentration than lake and the stream, at 19.01 ± 0.43 mg/L ($p<0.001$) and the mean DOC concentration of the ditches was higher than both the stream and lake. There was little evidence of a seasonal trend in the DOC data for the lake, but both the stream and ditches exhibited large variation with the season, being highest in the summer and autumn and lowest in the winter and spring for all years recorded. For the stream the highest concentration was recorded in 16th of August 2012, at 31.30 ± 0.85 mg/L, and lowest on 19th of February 2013, at 3.79 ± 0.08 mg/L. For the ditches the highest value was measured in May 2014, at 40.80 ± 0.45 mg/L for Ditch A, and lowest in January 2014 at 6.34 ± 0.16 mg/L for Ditch D.

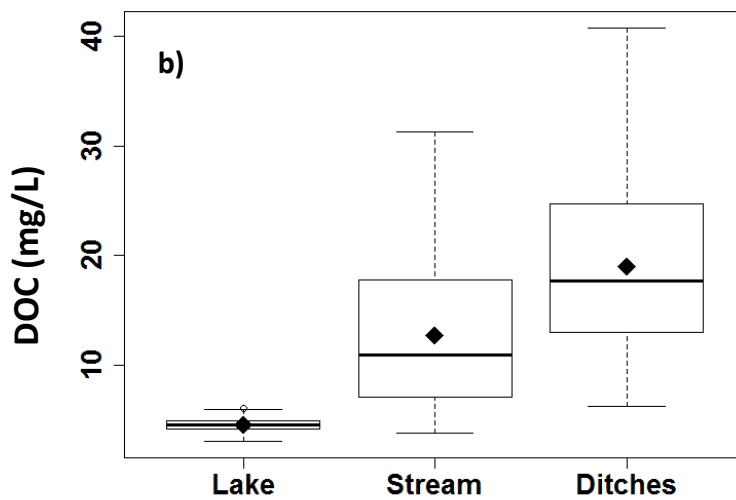
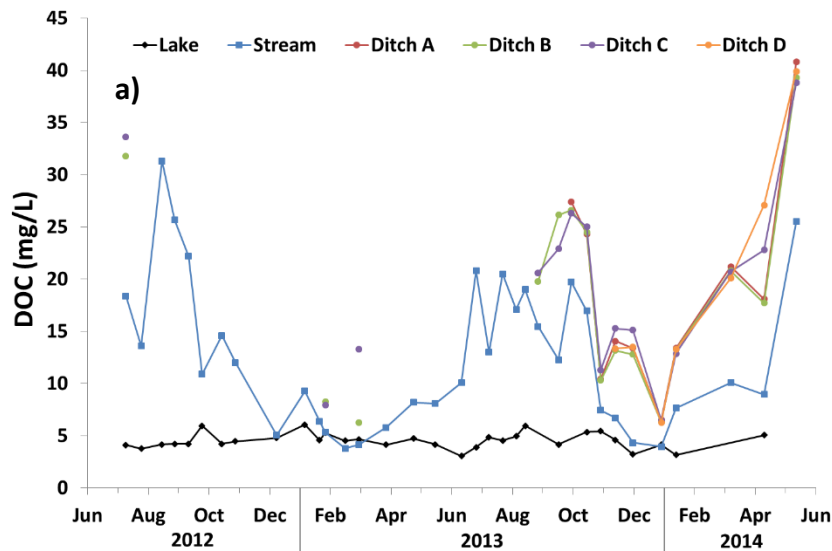


Figure 2.4a) time series and b) mean DOC concentration mg/L of all study sites for the period July 2012 to May 2014. Diamond symbols are mean values, bold horizontal lines are median values, the box represents the interquartile range and whiskers the 5th and 95th percentiles.

There was a significant difference in SUVA ($F=51.158$, $p<0.001$; Figure 2.5a) with respect to site. The lowest mean SUVA was measured for the Lake, at 3.35 ± 0.41 L/mg/m. The stream had a significantly higher SUVA, at 4.06 ± 0.37 L/mg/m ($p<0.001$), and the ditches a significantly higher than both, at 4.15 ± 0.06 L/mg/m ($p<0.001$).

The phenolics concentration varied when comparing the mean values for the lake (0.84 ± 0.42 mg/L) versus the stream (2.35 ± 1.33 mg/L) and ditches (2.27 ± 0.19 mg/L) ($F=48.977$, $p<0.001$; Figure 2.6b). The lowest mean phenolic concentration was measured for the lake, at 0.84 ± 0.42 mg/L. Both the stream and ditches had significantly higher concentrations than the lake, at 2.35 ± 1.33 mg/L and 2.27 ± 0.19 mg/L respectively ($p<0.001$), but the mean stream and ditch concentrations did not demonstrate a significant difference ($p>0.05$).

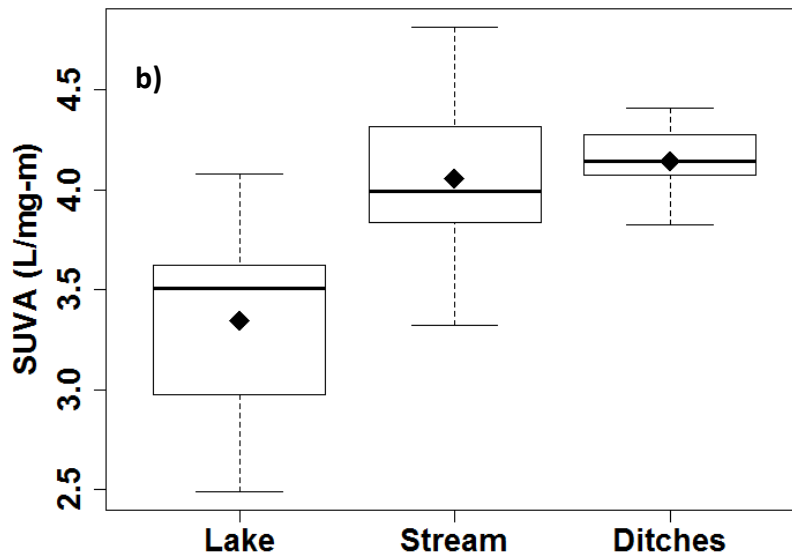
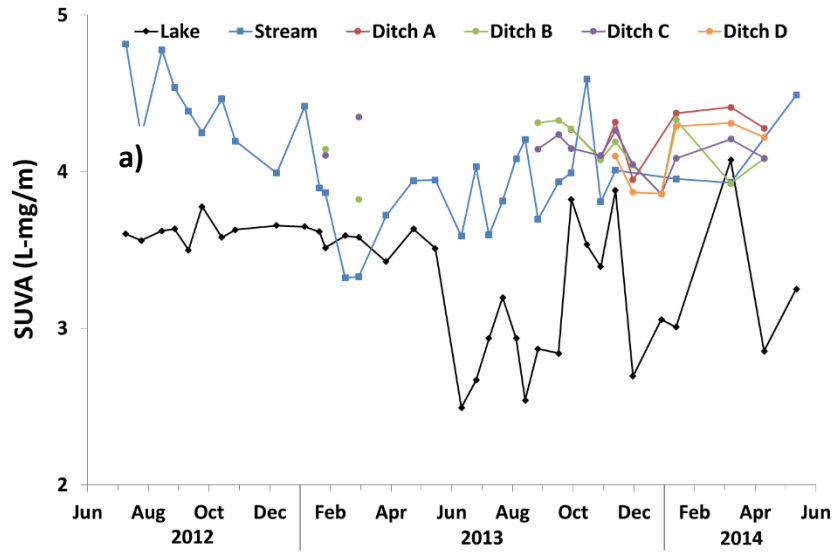


Figure 2.5a) time series and b) mean SUVA L-mg/mg all study sites for the period July 2012 to May 2014. Diamond symbols are mean values, bold horizontal lines are median values, the box represents the interquartile range and whiskers the 5th and 95th percentiles.

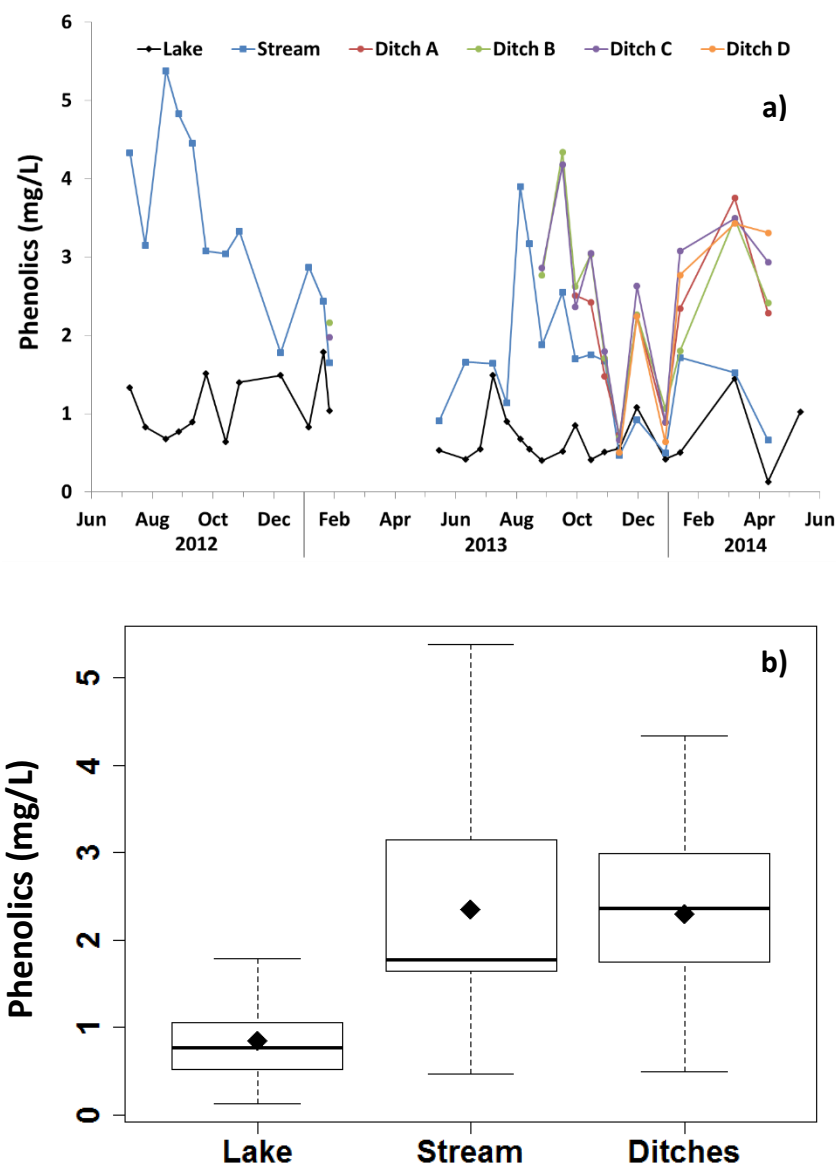


Figure 2.6a) time series and b) mean phenolics concentration mg/L all study sites for the period July 2012 to May 2014. Diamond symbols are mean values, bold horizontal lines are median values, the box represents the interquartile range and whiskers the 5th and 95th percentiles.

Chloroform was the dominant trihalomethane species detected (over 90%), so we have not presented data for the brominated THM species. There was no significant effect of site on the chloroform formation potential ($F=1.180$, $p>0.05$; Figure 2.7). DOC correlated significantly with both Phenolics ($r=0.77$, $p<0.001$; Figure 2.8) and SUVA ($r=0.78$, $p<0.001$; Figure 2.9) (Table 2.4).

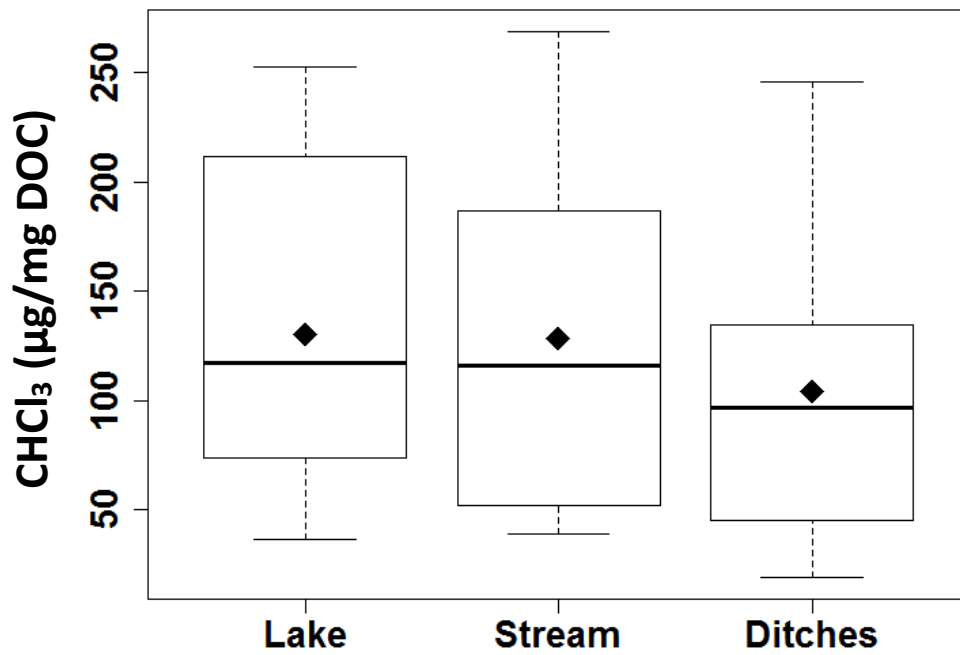


Figure 2.7. Mean Trihalomethane Formation Potential for the three contrasting site types for the entire study period.

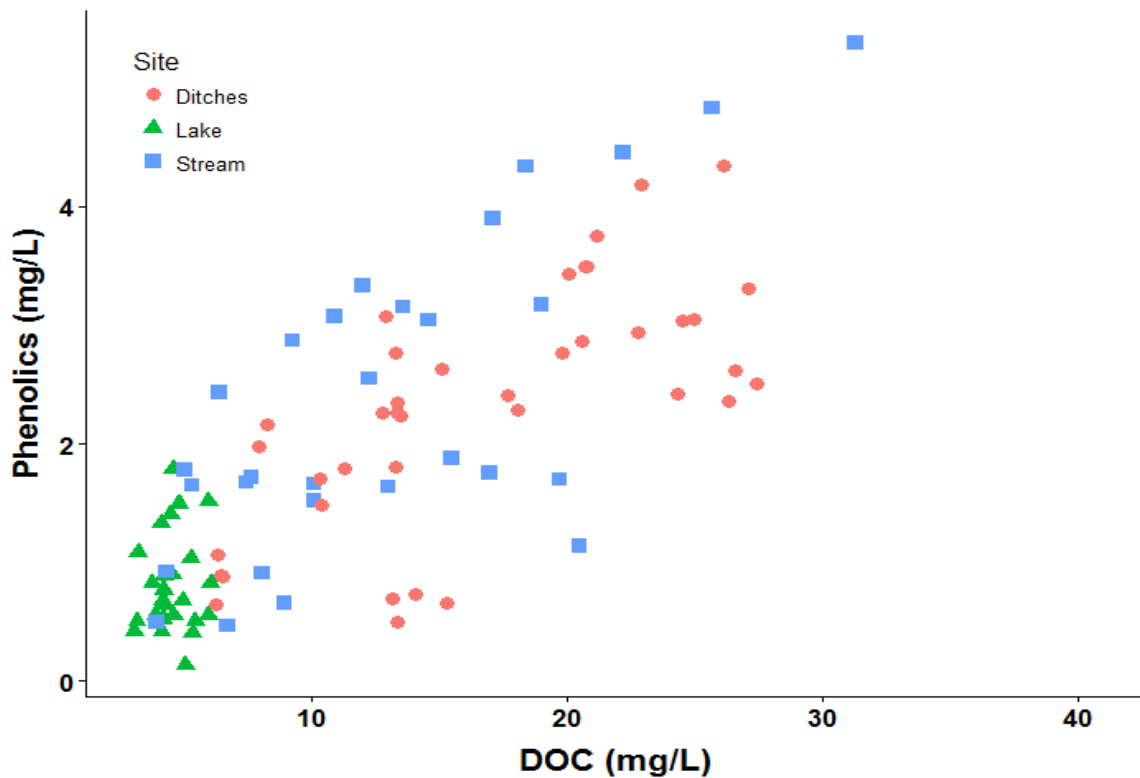


Figure 2.8. DOC v Phenolics for all sampled time points for all six sampling sites.

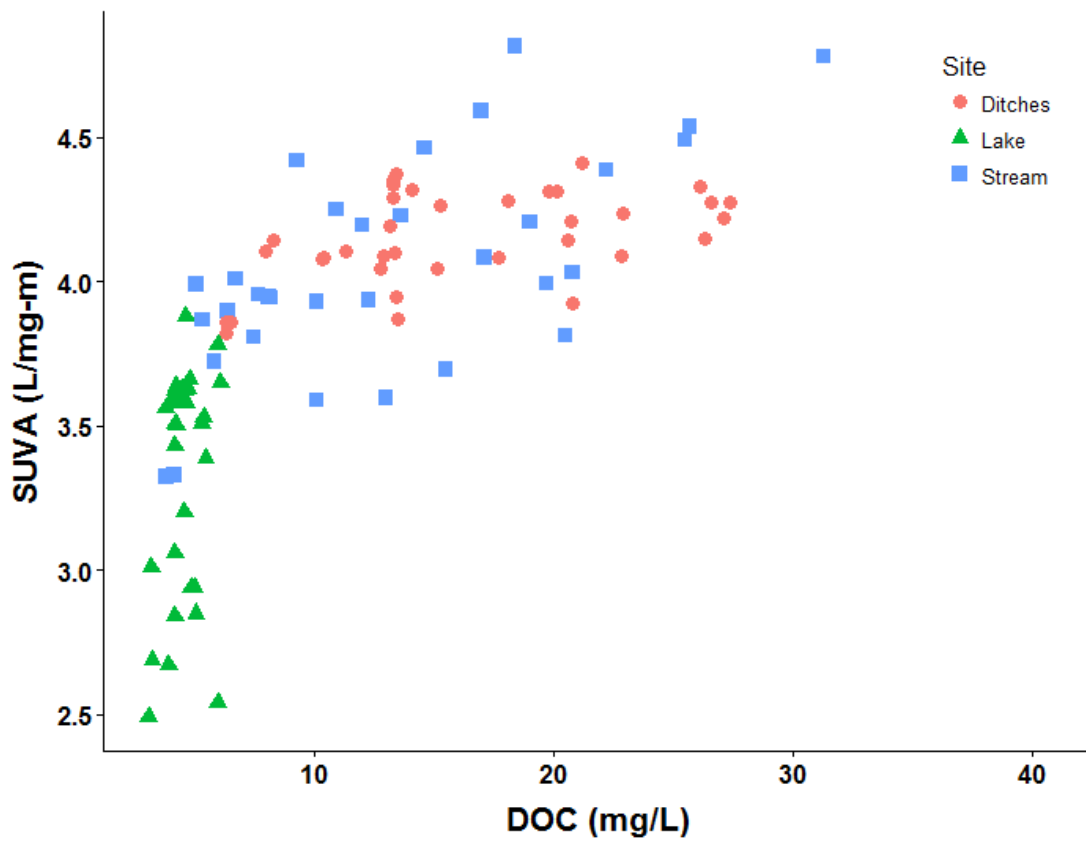


Figure 2.9. DOC v SUVA for all sampled time points for all six sampling sites.

		Conductivity	DOC	SUVA	Phenolics	THMs
pH	Pearson Correlation	-0.603	-0.387	-0.370	-0.188	0.207
	Sig. (2-tailed)	<0.001	<0.001	<0.001		
Conductivity	Pearson Correlation		.036	0.236	-0.058	-0.413
	Sig. (2-tailed)			0.023		<0.001
DOC	Pearson Correlation			0.708	0.779	0.270
	Sig. (2-tailed)			<0.001	<0.001	.024
SUVA	Pearson Correlation				0.696	-0.152
	Sig. (2-tailed)				<0.001	
Phenolics	Pearson Correlation					.149
	Sig. (2-tailed)					

Table 2.4. Results of Pearson correlation analysis run on all dependent variables.

2.5. Discussion

The results shown in Figure 2.4b the higher mean concentration of DOC in the ditches relative to the lake and stream is most likely to be due to greater decomposition processes occurring in the peat soils around the drainage ditches. The soils within the catchment of Llyn Conwy are mostly peat, which is several metres deep and contains large stores of organic carbon. Streams that drain the soils in this region (such as the Nant-y-Brwyn sampled in this study) are high in DOC due to the high organic matter content of the soils. The DOC concentration is higher in the man-made drainage ditches than the natural stream because they were created only a few decades ago and are still releasing DOC due to the continued oxidation of the peat soils they are draining. They were excavated to a depth of approximately 1 metre, exposing a large proportion of the soil profile to oxygen. According to the 'enzymic latch theory' this will have released the main constraint on the enzyme phenol oxidase and enhanced the overall decomposition of the peat (Pourmoghaddas and Stevens, 1995). This in turn will have resulted in enhanced DOC release into the water draining the soil (Freeman *et al.*, 2004) explaining our observation. The natural stream sampled in this study and others in the vicinity are not draining soils that have undergone a disturbance such as this and therefore have a lower mean DOC concentration.

The significantly lower mean DOC concentration was observed for Llyn Conwy compared to those for the ditches and stream is as would be expected from earlier studies (Evans *et al.*, 2012). Lakes are generally sinks for DOC due to long residence times and processes such as photochemical oxidation breaking down organic carbon into CO₂ (Evans *et al.*, 2012). Furthermore, the high rainfall received in the region and the large volume of the lake compared to the size of its catchment will quickly dilute any DOC inputs to the Lake.

A consistent seasonal trend was not observed in the DOC data for Llyn Conwy, although the lowest DOC concentration was recorded in July 2013 and the highest in January 2014. This contrasts to the pattern observed for the stream and ditches, where the DOC peaked in the summer/autumn and was lowest in the winter. This is due to the effects of seasonal weather cycles on DOC cycling. During the summer growing seasonal plants take up CO₂ from the atmosphere; a significant part of this carbon is transferred to the soil during the late summer/autumn period to form soil organic matter. This increases the amount of carbon able to be transferred to freshwater streams and rivers as DOC, hence DOC concentrations in these water bodies (particularly in upland environments where peat is found) are greatest at this time.

Results for phenolics (Figure 2.6 a and b) were similar to DOC and a strong positive relationship was recorded between DOC and the phenolic concentration across the three sample types (Table 2.3). Thus, indicating phenolic compounds are likely to be key component of DOC. The high phenolic concentrations are due to the peat soils from which the waters are draining, with phenolic structures persisting in peat due to the anaerobic conditions in the soil (Hättenschwiler and Vitousek, 2007; Peacock *et al.*, 2013). In previous studies, it has been noted that there are correlations between DOC and phenolic concentrations (Hagedorn *et al.*, 2007).

2.6. Conclusions

The overall finding of this study is that the drainage ditches have the potential to contribute more carbon per unit volume of water to the oligotrophic Llyn Conwy than the natural stream feeding into Llyn Conwy (equivalent to NYB).

This study measured DOC in man-made drainage ditches and discovered that the concentration was consistently higher than in a natural stream, indicating that the water draining artificially drained peat generally has a much higher DOC concentration than water draining natural peat. Drainage ditches lower the water table in the peat soil, thus exposing the peat to oxygen and increasing the decomposition of soil organic matter in the soils surrounding the ditches. Despite the elevated DOC concentrations in the drainage ditches, this study found no significant difference in the propensity to form THMs; a favourable outcome from a drinking water perspective.

Table 2.S1 – Field sampling dates vs locations for this study – Part 1: July 2012 – Feb 2013, Part 2 - May 2013 to May 2014 (• indicate samples collected)

Date	Lake	Stream	Ditches (A-D)
<u>PART 1</u>			
10/07/2012	•	•	B,C
26/07/2012	•	•	
16/08/2012	•	•	
29/08/2012	•	•	
12/09/2012	•	•	
26/09/2012	•	•	
16/10/2012	•	•	
30/10/2012	•	•	
11/12/2012	•	•	
09/01/2013	•	•	
24/01/2013	•	•	
30/01/2013	•	•	B,C
19/02/2013	•	•	
05/03/2013	•	•	B,C
02/04/2013	•	•	
30/04/2013	•	•	
<u>PART 2</u>			
22/05/2013	•	•	
18/06/2013	•	•	
03/07/2013	•	•	
16/07/2013	•	•	
30/07/2013	•	•	
13/08/2013	•	•	
22/08/2013	•	•	
04/09/2013	•	•	A-D
25/09/2013	•	•	A-D
08/10/2013	•	•	A-D

Table 2.S1 - Continued

Date	Lake	Stream	Ditches (A-D)
24/10/2013	•	•	A-D
07/11/2013	•	•	A-D
22/11/2013	•	•	A-D
10/12/2013	•	•	A-D
08/01/2014	•	•	A-D
23/01/2014	•	•	A-D
20/03/2014	•	•	A-D
23/04/2014	•	•	A-D
26/05/2014	•	•	A-D

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Chapter 3: Field experiment investigating effects of peatland drought on dissolved organic carbon (DOC) release and implications for trihalomethane (THM) formation

3.1. Abstract

Peatland ecosystems sequester vast quantities of carbon below ground due to persistent waterlogged conditions and suppressed decomposition. Anthropogenic drainage has resulted in the loss of vast quantities of carbon on a global scale and drought resulting from climate change is a serious long-term risk to the ability of a peatland to continue to act as a carbon sink. The lowering of the water table can also affect the export of dissolved organic carbon (DOC), which has implications for freshwater biota and the provision of drinking water supplies.

In this study, the effect of long-term drought on peatland DOC has been examined, observing reduced porewater concentrations and a shift in quality to lower molecular weight and less aromatic DOC. Despite this, a significant change in standardised trihalomethane formation potential (THMFP) values was not observed. This indicates that DOC removal efficiency during conventional water treatment processes may change following drought, although the decreased loading of DOC to the works would significantly reduce coagulant demand.

3.2. Introduction

Peatlands are one of the planet's most important terrestrial reservoirs of organic matter, occupying approximately 6 million km² of the terrestrial surface (Bridgham *et al.*, 1996). They are found in regions with a positive net water balance, without sustained dry periods (Moore *et al.*, 1998) and accumulate organic matter as peat (Clymo, 1984). Despite only occupying 3% of the Earth's terrestrial surface (Rydin and Jeglum, 2006), peatlands are estimated to have sequestered 480-610 Gt of carbon as peat (Page *et al.* 2011). The carbon sequestration ability of peatlands and their function as carbon sinks, retaining the carbon they have accrued over thousands of years, depends primarily on the position of the water table and maintenance of waterlogged conditions (Fenner and Freeman, 2011). Water saturation reduces oxygen concentrations in the peat, resulting in anoxic conditions that inhibit decomposition and allow organic matter to build up. In the UK, significant peat deposits occur in upland blanket bogs, which form under the warmest and wettest conditions (Wieder and Vitt, 2006), where the rainfall is approximately 3 times more than potential evaporation (Clark, 2010; Pearsall, 1965). Although peatlands have had a net cooling effect on the planet, they do export large quantities of carbon to freshwaters as Particulate Organic Carbon (POC) and Dissolved Organic Carbon (DOC). DOC is generally the largest aquatic carbon flux from a peatland (Billett *et al.*, 2006; Dinsmore *et al.*, 2013) and it is important to include DOC when calculating the carbon budget of a peatland (Billett *et al.*, 2004; Chen *et al.*, 2008; Freeman *et al.*, 2001a; Limpens *et al.*, 2008). DOC is a key component of the global carbon cycle (Evans *et al.*, 2006), as it connects the terrestrial and aquatic biogeochemical cycles. Globally, 0.25–0.45 Gt C/year of DOC is transported from rivers to the world's oceans (Cole *et al.*, 2007; Hedges *et al.*, 1997). The downstream export of DOC from a peatland plays a key role in the redistribution and balance of carbon (Arnosti *et al.*, 2003). In aquatic ecosystems, the transfer of carbon from terrestrial peatlands to downstream locations has a significant influence on water quality (Lennon *et al.*, 2013; Wallage *et al.*, 2010). DOC influences visible and ultraviolet light penetration into freshwater systems due to colouration of the water (Evans *et al.*, 2005), which affects the biological functioning of the water body.

The discharge of surface water from a peatland is mostly dependent on the rate of water flow, seepage through upper peat layers and hydrological properties of peat (Holden *et al.*, 2003; Worrall *et al.*, 2002). The production of DOC is greatest from decomposing vegetation at the peat surface and the majority of DOC exported from an undisturbed peatland is relatively 'young' (Billett, *et al.*, 2007; Blodau *et al.*, 2004; Schiff *et al.*, 1998). Export of DOC from a catchment depends on concentration and discharge (Hope *et al.*, 1997). High runoff results in high DOC export and vice versa (Holden *et al.*, 2005; Scott *et al.*, 1998). When a peatland experiences drought conditions, which occur naturally due

to seasonal rainfall patterns and are expected to increase in frequency due to climate change (Roulet *et al.*, 1992), decomposition of the peat matrix is stimulated to the extent that the peatland can become a net source of carbon (Freeman *et al.*, 1998). When the water table falls, the peat is exposed to oxygen, opening the enzymic latch and stimulating decomposition of the peat matrix to carbon dioxide (Freeman *et al.*, 2001b). Drought reduces surface runoff and therefore the export of DOC (Moore *et al.*, 1998), but DOC production can increase in the aerated zone due to the oxidation of the peat matrix and can be removed by leaching during a wetter period (Blodau *et al.*, 2004). With climate change, water table drawdown is expected to have a greater impact on DOC export than warming (Lou *et al.*, 2014).

In North America and Europe, long term observations show that DOC concentrations have increased in freshwaters in recent decades (Driscoll *et al.*, 2003; Hejzlar *et al.*, 2003; Monteith *et al.*, 2007; Stoddard *et al.*, 2003). Freeman *et al.* (2001a) estimated that in the United Kingdom from 1988-2000, DOC in stream and lake catchments increased by 65%. Increased DOC concentrations in rivers could be indicative of a translocation of terrestrial carbon in peat soils (Limpens *et al.*, 2008) due to climate change. In peatlands the water table has been lowered by human activities such as drainage for agriculture and the effects of global climate change (Aertsde *et al.*, 1999; Moore and Dalva *et al.*, 1993; Price, *et al.*, 2003). The lowering of the water table impairs carbon storage and ecosystem stability in peatlands (Pastor *et al.*, 2003). In the UK, climate projections for the 21st century indicate an overall increase in temperatures and change in the distribution of rainfall with wetter winters and warmer, drier summers (Hulme, 2002; Jenkins, 2009). This may affect the ability of UK peatlands to retain peat and affect the extent to which they export aquatic DOC (Hossell *et al.*, 2000).

Increasing DOC concentrations are a concern for the water industry for a number of reasons. Firstly, the cost to produce clean drinking water will increase, as DOC removal is a key component of water treatment processes; secondly, disinfection by-products, principally trihalomethanes (THMs), are produced due to reactions between chlorine and DOC (Rook *et al.*, 1974) and thirdly, the presence of extra DOC in the distribution system increases the demand for residual chlorine to protect the finished water from biological contamination (Hsu *et al.*, 2001). Therefore, understanding how peatlands may react due to drought in terms of DOC export is currently an important area of research. This study measured the effect of prolonged drought in a peatland catchment on porewater DOC concentrations and quality and the propensity of the DOC to form THMs following chlorination.

We hypothesise that the experimental field-scale peatland drought will a) lower the concentration of DOC and b) change the characteristics of DOC to make it more likely to form THMs compared to a control site with a natural water table.

3.3. Methods

3.3.1. Study site and experimental treatment

Sampling was performed at a long-term experimental site in mid-Wales, UK, that has been the location for several studies on the impacts of drought on peatland carbon cycling (Freeman *et al.*, 1998). The site is located within the Cerrig-yr-Wyn catchment, Plynlimon (UK Nat. Grid Ref. SN 82008 86571). The characteristics of the site are a discontinuous series of peat-accumulating wetlands (length 30 m, wide 5 m) which are dominated by *Sphagnum* and *Juncus* species. In 1995, a large area of the catchment (30 m x 5 m) was droughted by diverting surface water flow elsewhere. An area roughly the same size with similar characteristics was left undisturbed to act as a control.

Ten rhizon (10 cm) porewater samplers (Rhizosphere Research Products, Wageningen, the Netherlands) were installed at both the control and droughted sites, in a 10 m transect at approximately 1 m intervals. The first samples were taken in March 2014 and sampling continued monthly until September 2014. Twenty ml of pore water was extracted using syringes, capped and transported carefully back to the laboratory.

pH and conductivity were measured in the laboratory on an unfiltered aliquot of the original sample on the day of sampling. pH was measured using a SevenEasy pH meter (Mettler Toledo, Leicester, UK). The device was calibrated using buffers of pH 4 and 7 at 25°C (Sigma). Conductivity was measured with a Primo 5 conductivity meter (Hanna Instruments, Bedfordshire, UK). All samples were then filtered through 0.45 µm cellulose acetate filters and refrigerated at 4°C until further analysis was undertaken.

3.3.2. Dissolved organic carbon (DOC)

Concentrations of DOC were determined using a Thermalox TC/TN analyser (Analytical Sciences Ltd, Cambridge, UK), which is a combustion and infrared detection based technique. For DOC, samples were acidified to pH <3 prior to analysis to remove inorganic carbon using 10 M HCl. The instrument was calibrated with potassium hydrogen phthalate standards. The injection volume was 15 µL and oven temperature 680°C. Results were corrected if standard solutions deviated by 10% or more from the correct concentration.

3.3.3. Phenolic compounds

The phenolic component of DOC was determined using a method modified from Box (1983), adapted for 300 µL microplate wells. A calibration curve was generated using a series of phenol

standard solutions ranging in concentration from 0.5 - 10 mg/L. The assay product was detected by measuring the absorbance at 750 nm on a Spectramax M2e spectrophotometer (Molecular Devices, Wokingham, UK).

3.3.4. UV/visible absorption

SUVA is used as an indicator of overall DOC quality i.e. molecular weight and aromaticity. SUVA was calculated by dividing the absorbance value at 254 nm (measured using a 1 cm quartz cuvette on the Spectramax M2e spectrophotometer) by the DOC concentration (mg/L) (Potter, 2003). High SUVA (more than 4 L/mg/m) indicates high humic content with hydrophobic character (aromatic) and low SUVA (less than 4 L/mg/m) indicates more hydrophilic content with reduced aromatic character (Goslan *et al.*, 2002).

3.3.5. Trihalomethane formation potential (THMFP)

The standard trihalomethane formation potential (THMFP) analysis was used to determine the concentration of THMs that can be formed per unit DOC under ideal conditions, to give an indication of the reactivity of DOC with chlorine and its propensity to form THMs. A modified version of the THMFP test detailed in Nieuwenhuijsen *et al.* (2000) was used. In this study, samples were diluted to 1 mg/L DOC in order to derive a standardised THMFP_{7d} value (STHMFP_{7d}) which provides a measure of DOC reactivity. A THMFP_{7d} value was then calculated by multiplying STHMFP_{7d} by DOC concentration. 97.5 mL of diluted sample was dosed with 2.0 mL of 0.5 M KH₂PO₄ to buffer the solution to pH 6.8. Samples were then dosed with 0.5 mL of NaOCl to provide 5 mg of free Cl per mg of DOC. The solution was transferred to a 100 mL amber glass bottle and incubated in the dark at 25°C for 7 days to allow the reaction between organic carbon and chlorine to occur. After 7 days incubation, the reaction was quenched by adding 0.4 mL of 0.8 M Na₂SO₃ to 14.6 mL of incubated sample. This solution was then transferred to a 22 mL amber headspace vial and analysed by the following method. The concentration of THMs was measured using a solid phase micro-extraction (SPME) technique similar to that described by Sarrión *et al.* (2000). The technique uses a Varian GC 450, a Restek MX 1 column (diphenyl/dimethyl polysiloxane phase, 30m length, 0.53 mm i.d.), N₂ carrier gas at a flow rate of 10 mL/min and a ⁶³Ni Electron Capture Detector. The oven temperature was held at 35°C for 9 minutes and then increased at a rate of 10°C/minute to 140°C and held for 2 minutes and finally increased to 180°C and held for 3 minutes. The injector temperature was 290°C and the detector temperature 300°C. THM calibration solutions were made from a stock solution containing 2000 µg/mL of each of the 4 THMs; chloroform (CHCl₃), bromodichloromethane (CHBrCl₂), dibromochloromethane

(CHBr₂Cl), and bromoform (CHBr₃) (Sigma). A series of standard solutions were then prepared ranging from 1-500 µg/L.

3.3.6. Statistical analyses

The effect of one factor; Treatment (2 levels; Control and Drought) was determined on the time averaged data for each measured parameter using one-way ANOVA. If a significant effect was found for a given parameter, significant effects at each time point were tested for using t tests. Most data met the homogeneity and normality assumptions, which were tested using the Bartlett and Shapiro Wilk tests, but those that did not were log-transformed. All statistical analyses were run in R v3.3.1.

3.4. Results

All data are presented as a series of graphs (Figures 3.1-3.8), with a boxplot displaying the average, median and quartile data for both treatments over the entire course of the experiment and, if a significant treatment effect was found, a line graph showing the data as a time series.

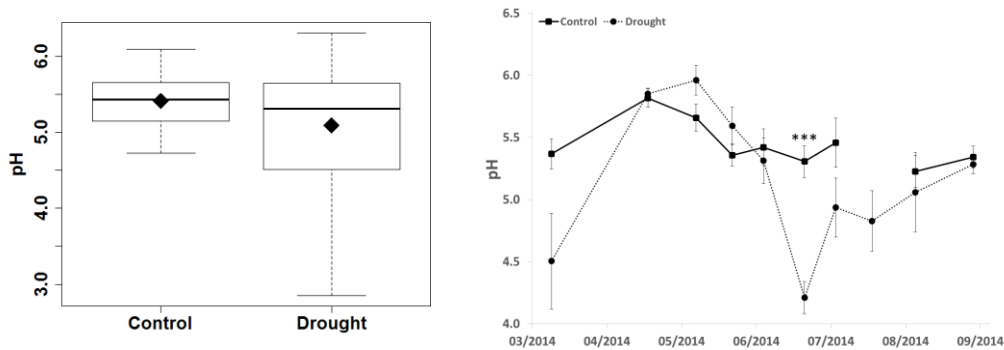


Figure 3.1. (left) – Boxplot for pH (Diamond symbols indicate mean values) and (right) – Time series graph for pH (Error bars are presented as ±SEM). A significant difference between the Control and Drought treatments is indicated by a * ($p < 0.05$).

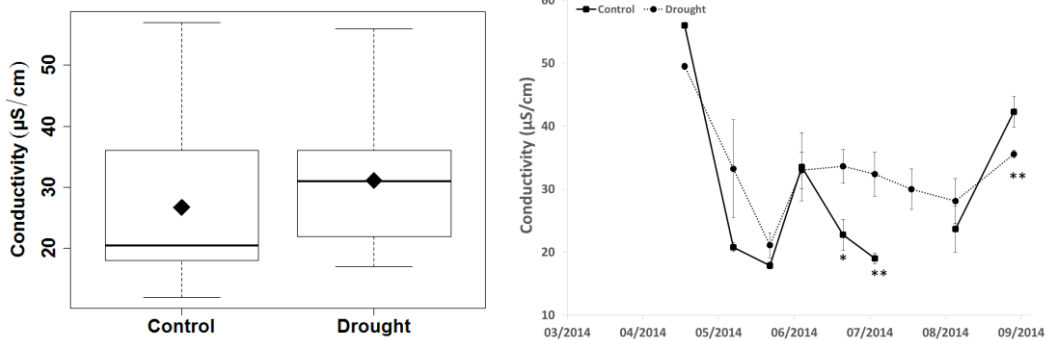


Figure 3.2. (left) – Boxplot for conductivity (Diamond symbols indicate mean values) and (right)– Time series graph for conductivity (Error bars are presented as \pm SEM). A significant difference between the Control and Drought treatments is indicated by a * ($p < 0.05$).

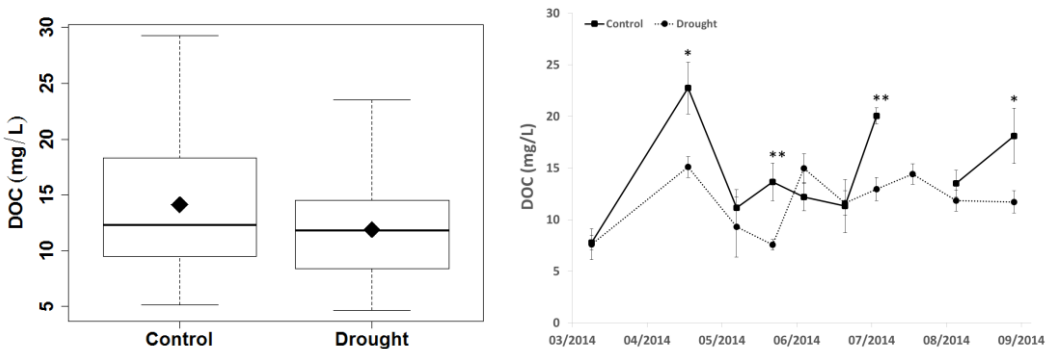


Figure 3.3. (left) – Boxplot for DOC (Diamond symbols indicate mean values) and (right) – Time series graph for DOC (Error bars are presented as \pm SEM). A significant difference between the Control and Drought treatments is indicated by a * ($p < 0.05$).

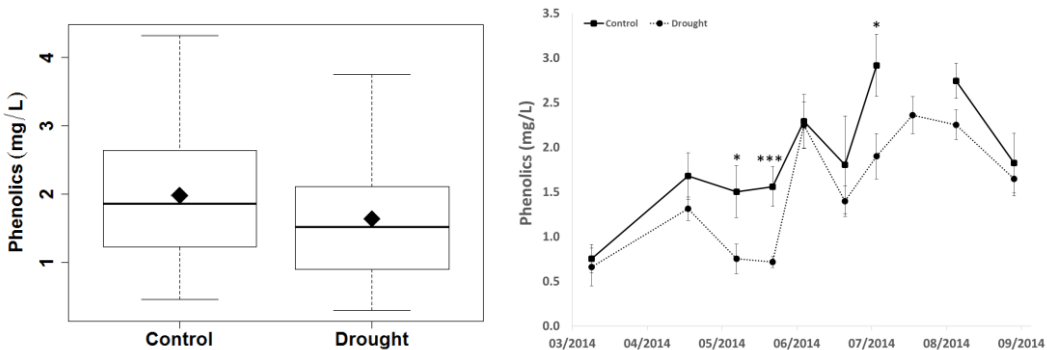


Figure 3.4. (left) – Boxplot for phenolics (Diamond symbols indicate mean values) and (right) –Time series graph for phenolics (Error bars are presented as \pm SEM). A significant difference between the Control and Drought treatments is indicated by a * ($p < 0.05$).

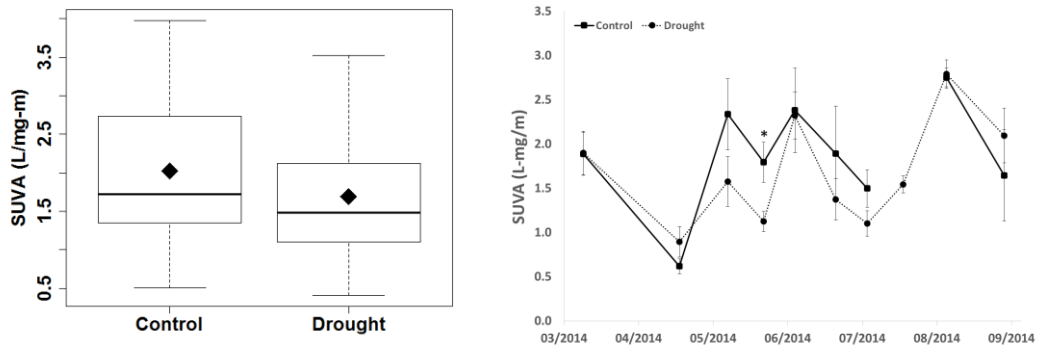


Figure 3.5. (left) – Boxplot for SUVA (Diamond symbols indicate mean values) and (right) – Time series graph for SUVA (Error bars are presented as \pm SEM). A significant difference between the Control and Drought treatments is indicated by a * ($p < 0.05$).

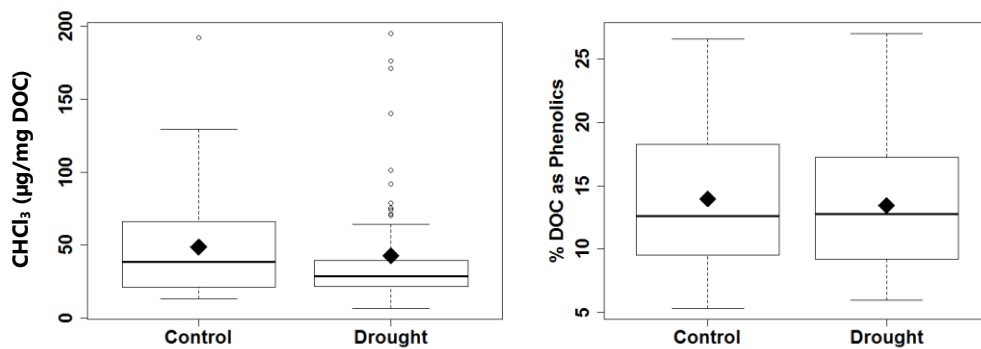


Figure 3.6. (left) – Boxplot for CHCl_3 (Diamond symbols indicate mean values)
 Figure 3.7. (right) – Boxplot for % DOC as Phenolics (Diamond symbols indicate mean values)

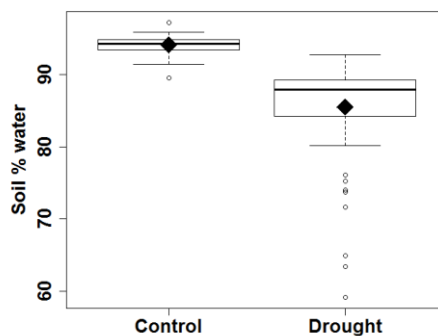


Figure 3.8. Boxplot for Soil % water (Diamond symbols indicate mean values)

All samples were acidic throughout the sampling period. There was a significant main effect on pH ($F=8.29$, $p < 0.01$; Figure 3.1a), with the mean pH of the control being 5.41 ± 0.04 and the drought being 5.10 ± 0.08 . The only individual month when the treatments differed significantly was the 3rd of July, when the drought pH was 1.1 units lower than the control ($p < 0.001$; Figure 3.1b). pH values were generally higher in the spring period before declining in summer and autumn. There was a significant main effect on conductivity ($F=8.42$, $p < 0.01$; Figure 3.2a), with the mean of the control being $26.72 \pm$

1.78 $\mu\text{S}/\text{cm}$ and the drought being $31.1 \pm 1.19 \mu\text{S}/\text{cm}$. The conductivity of the drought treatment was significantly higher than the control on two sampling occasions; 3rd and 16th of July ($p < 0.05$), and significantly lower on the 11th of September ($p < 0.01$; Figure 3.2b). There wasn't a clear seasonal variation in the conductivity for either treatment. There was a significant main effect on DOC ($F = 4.93$, $p < 0.05$; Figure 3.3a), with the mean DOC concentration of the control being $14.1 \pm 0.77 \text{ mg}/\text{L}$ and the drought being $11.9 \pm 0.45 \text{ mg}/\text{L}$. The DOC concentration of the control treatment was significantly higher than the drought on four sampling occasions; 19th of April, 3rd of June, 16th of July and 11th of September ($p < 0.05$; Figure 3.3b). There wasn't a clear seasonal variation in the DOC concentration for either treatment. There was a significant main effect on phenolics ($F = 5.16$, $p < 0.05$; Figure 3.4a), with the mean concentration of the control being $2.00 \pm 0.12 \text{ mg}/\text{L}$ and the drought being $1.64 \pm 0.09 \text{ mg}/\text{L}$. The phenolic concentration of the drought treatment was significantly lower than the control on three sampling occasions; 19th of May, 3rd of June and 16th of July ($p < 0.05$; Figure 3.4b). Unlike DOC, there was a clear seasonal variation in phenolics; concentrations increased throughout the spring, peaked in late summer and declined again in September. There was a significant main effect on SUVA ($F = 4.69$, $p < 0.05$; Figure 3.5a), with the mean SUVA of the control being $2.02 \pm 0.13 \text{ L}/\text{mg}/\text{m}$ and the drought being $1.69 \pm 0.09 \text{ L}/\text{mg}/\text{m}$. The SUVA of the drought treatment was significantly lower than the control on one sampling occasion; 3rd of June ($p < 0.05$; Figure 3.5b). There wasn't a clear seasonal variation in the SUVA for either treatment.

Chloroform was the dominant THM species produced during the laboratory THMFP test, therefore we have focused on this species only. There was no significant main effect on CHCl_3 -FP (chloroform formation potential) ($p > 0.05$; Figure 3.6). The control treatment averaged $48.6 \pm 5.57 \mu\text{g}/\text{mg DOC}$ and the drought $42.7 \pm 4.89 \mu\text{g}/\text{mg DOC}$. There was also no significant effect on the 'percentage of DOC as phenolics' ($p > 0.05$; Figure 3.7), with the control treatment averaging $14.0 \pm 0.72 \%$ and the drought $13.5 \pm 0.52 \%$. There was a significant main effect on soil water content ($F = 195.65$, $p < 0.001$; Figure 3.8). The Control treatment averaged $94.1 \pm 0.12 \%$ and the Drought $85.5 \pm 0.51 \%$.

3.5. Discussion

The location for this study was a long-term experimental site in the UK, where several studies have addressed the impact of drought conditions on peatland carbon cycling (Freeman *et al.*, 1998; Freeman *et al.*, 2004). Part of the site was droughted in the mid-1990s and the soil moisture data collected during 2014 for this study shows that the drought treatment is still functioning effectively (Figure 3.8). Soil water content averaged 94.1% for the control site and 84.1% for the drought site, a difference of exactly 10%. Such a difference would have important implications for biogeochemical and carbon cycling in the peat soil and the maintenance of drought conditions

for over twenty years enables the study of long-term drought of a peatland catchment. In this study, significant differences in porewater DOC concentration and quality were observed between the control and drought treatments.

The concentration of DOC was significantly lower in the drought compared to the control treatment. This finding agrees with the study of (Freeman *et al.*, 2004; Clark *et al.*, 2012), that long-term drought of a peatland decreases porewater DOC. However it is important to acknowledge that some studies have recorded increased DOC in drought (Fenner *et al.*, 2009; Lou *et al.*, 2014; Strack *et al.*, 2008; Worrall *et al.*, 2004). The decrease in DOC and phenolics due to drought in this study can be attributed to oxygenation and improved conditions for decomposition in the soil. The reduced concentration of phenolic compounds in the pore water of the droughted peat will presumably be due to an increase in the activity of phenol oxidase (Lin *et al.*, 2012). Oxygenated conditions in the drought treatment will have resulted in the complete breakdown of soil organic matter to CO₂, rather than incomplete breakdown to DOC, due to the opening of the enzymic latch (Fenner and Freeman, 2011; Freeman *et al.*, 2001b), explaining the reduction in DOC we observed. Another reason why we observed a decrease in DOC in the drought treatment may be related to temperature. A slightly higher soil temperature (data not shown) was recorded in the drought treatment because the soil was drier. This may be allowing microbial and enzymic decomposition processes to be happening at a faster rate, leading to accelerated breakdown of DOC. However, it is also important to consider that higher temperatures can contribute to increased DOC production by increasing microbial and invertebrate activity, and therefore the rate of organic matter decomposition in soils (Cole *et al.* 2002; Fenner *et al.* 2001; Fenner, *et al.* 2005; Freeman *et al.* 2001a; Scanlon and Moor, 2000; Worrall *et al.*, 2008). The phenolics trend demonstrated an autumn flush for both treatments. Increasing phenolics concentrations during the autumn may be due to a combination of vegetation death within the catchment and increased rainfall (Dawson *et al.*, 2008; Scott *et al.*, 2001). Specific Ultra Violet Absorbance (SUVA), which indicates the relative degree of aromaticity (Leenheer and Croue 2003), was used as the main indicator of DOC quality in this study.

SUVA values were significantly lower in the drought treatment. This again provides evidence that under drought conditions there is enhanced microbial breakdown of organic matter and DOC, explaining the reduced SUVA values measured in this study.

The pH of the pore water was significantly lower in the drought treatment compared to the control, particularly in the summer/autumn half of the study. This could be due to greater oxidation of sulphides, which tend to dominate waterlogged soils (Ponnamperuma *et al.*, 1972). Conductivity in the pore water was significantly higher in the drought treatment compared to the

control. This may be due to organic matter mineralisation under oxygenated conditions (Fierer *et al.*, 2007; Philipp and Schink 2012; Winsborough, 2010).

3.6. Conclusion

Long-term drought of a peatland has had a strong impact on porewater DOC, with reduced concentration and a shift in quality to lower molecular weight and aromaticity. Despite this, we did not observe a significant change in THMFP. Therefore, part a) of our hypothesis was correct but part b) was not. The observed differences are likely to be due to oxygenated conditions in the drought treatment. This will be leading to enhanced decomposition of both soil organic matter and the existing pool of DOC.

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Chapter 4: Laboratory experiment investigating the effects of Peatland Drought on Dissolved Organic Carbon (DOC) and Trihalomethane (THM) Formation

4.1. Abstract

Dissolved organic carbon (DOC) present in freshwaters that are used as sources of drinking water acts as a precursor to potentially harmful carcinogenic compounds following disinfection treatment. The rising DOC trend observed in many surface waters therefore poses a threat to human health as it may lead to increased disinfection by-product (DBP) production if treatment processes are not improved. Drought conditions are known to affect the ability of peatlands to retain carbon and previous studies have demonstrated that drought can affect the export of DOC from peatlands to freshwaters. In this study, we examine the effect of drought on the porewater DOC concentrations and characteristics of two contrasting types of peatland, fen and bog. We observe lower DOC concentrations in the peat cores subject to a drought treatment, but greater trihalomethane formation potential (THMFP) values, indicating that the drought changes the characteristics of the DOC to make it more likely to form DBPs.

4.2. Introduction

Carbon is the key constituent of natural organic matter (NOM), therefore NOM is often referred to as either dissolved or particulate organic carbon (DOC/POC). The analytical techniques used to quantify and characterise organic matter in freshwaters are usually based on measuring the carbon in NOM. DOC is the dominant form of organic carbon in freshwaters, usually comprising 90% of total organic carbon (Jones and Mulholland, 1998). It is derived from the cycling of carbon between the terrestrial environment and the atmosphere. Organic carbon present in soil can be transferred to freshwaters during rainfall and this is generally the dominant source of DOC in rivers, streams and lakes globally (allochthonous DOC). It is common to observe strong correlations between lake DOC concentrations and the size of the lake's catchment. DOC is also produced within freshwaters by algae and microorganisms (autochthonous DOC). Freshwaters with a high concentration of DOC will usually be coloured brown due to the presence of humic substances which strongly absorb ultraviolet and visible light. The contribution from each source varies on a seasonal basis; allochthonous DOC inputs are greatest during the late summer/autumn period whilst autochthonous DOC production peaks in the summer months, when temperatures and light levels are greatest. Therefore freshwater DOC concentrations tend to peak in September-October when the contribution from both sources is high. On average globally, allochthonous sources tend to dominate DOC inputs over autochthonous sources (Manny and Wetzel, 1973).

One of the most important factors controlling the DOC concentration of a freshwater stream, river or lake is the soil type within its catchment. Peatlands play a key role in influencing the DOC concentration of freshwaters, particularly in temperate regions of the northern hemisphere where most peatlands exist. Peat soils export high concentrations of DOC, because the soil is carbon rich and precipitation is high in areas where peat is located, so there is a continual flush of DOC rich waters.

There are two main types of peatland; bog and fen, which are differentiated according to the way they receive water. Bogs receive all their water from precipitation and are therefore nutrient poor and acidic. Fens receive water from precipitation and other more nutrient rich sources, such as groundwater and drainage from surrounding mineral soils and therefore have a more neutral pH (Mitsch and Gosselink, 2000).

There is significant evidence that the concentration of DOC in many freshwater environments in the northern hemisphere is increasing. Freeman et al. (2001a) reported that from 1988 to 2000 DOC increased by 65% on average in streams and lakes in the UK. There is also evidence of this increasing trend in other parts of Europe and North America (Evans et al., 2006; Monteith et al.,

2007). The rising DOC trend has been attributed to increasing temperature (Freeman et al., 2001b), atmospheric CO₂ concentrations (Freeman et al., 2004) and more frequent droughts and reduced acid deposition (Fenner and Freeman, 2011). These changes can lead to peatlands changing from carbon sinks to carbon sources (Evans et al., 2012).

As peatlands export large amounts of DOC to freshwaters they have an important influence on the production of drinking water. In the UK, many catchments used to source drinking water contain peat and are therefore high in DOC. The presence of DOC is not harmful to humans, but when water containing DOC is treated with disinfectant, usually chlorine, potentially harmful disinfection by-products (DBPs) may be produced, which can lead to cancer (Sketchell et al., 1995). The most common DBP's are the trihalomethanes (THMs), which are simple derivatives of methane formed when 3 of the hydrogen atoms are replaced by a halogen (Peterson et al., 1993). Final DBP concentrations depend on concentration of DOC, chlorine concentration, water temperature and pH (Rodrigues et al., 2007).

Regulatory bodies have set limits for final water DBP concentrations because of the potential effects on human health. In the UK, the limit for the sum of the four trihalomethane compounds is 100 µg/L due to the carcinogenic effect of these compounds.

Peatlands may be affected by climate change through the lowering of the water table (Roulet et al., 2007). A number of processes affect the export of DOC from peatlands including lowering of the water table which is known to destabilise peat and lead to increased release of DOC to freshwaters (Strack et al., 2008). However, there has been little work undertaken to investigate how a lowering of the water table changes the characteristics of DOC, and what the implications of this are for DBP production which has prompted the study described herein.

4.3. Methods

4.3.1. Experimental design and study sites

This experiment used mesocosms of peat to investigate the impacts of a controlled drought on DOC and how readily the DOC forms THMs. Twenty peat cores were collected from two locations in North Wales in June 2013; 10 each from Migneint bog and Cors Erddreiniog fen. The Migneint (grid ref SH7795942829) is the largest blanket bog in North Wales and Cors Erddreiniog (grid ref SH4715882581) is the largest area of fen on the island of Anglesey.

Each core was 20 cm wide and 50 cm deep. The cores were collected by inserting a cylindrical PVC drainpipe carefully into the soil to minimise disturbance of the vegetation and extracting once the

vegetation was just above the top surface of the core. The cores were kept in a controlled temperature room (9-10°C), each in separate buckets, until the experiment began. The water table was maintained at the same height as it was when the cores were collected. Holes were installed in the side of the cores for water sampling at depths 5 cm and 20 cm below the surface. 10 cm rhizon porewater samplers (Rhizosphere Research Products, Wageningen, The Netherlands) were inserted into these holes for sampling soil porewaters.

4.3.2. Treatment regime

A roughing treatment was applied to half of the cores from each soil type by removing water from the external bucket to lower the water table in the cores. This was initiated on 23rd September 2013 (day 0). The water table steadily declined until it reached 20 cm below the surface on 25th of November 2013 (day 63), where it remained stable until 30th December 2013. It was then increased steadily back to the surface, reaching this position on 12th February 2014. The water table was maintained at the original height for the other half of the cores (the control treatment). There were 5 replicate cores for each of the 4 treatments. Soil pore water samples were extracted from the cores on 6 dates; 10th November 2013 and 5th December 2013 and 10th January, 21st January, 13th February and 22nd February 2014. Some of the 5 cm samples for the drought cores during the peak of the drought could not be collected as the soil was too dry.

4.3.3. Analytical techniques

All samples were analysed for pH and conductivity on the same day as collection, 20 ml of sample was filtered through 0.45 µm syringe filters (Triple Red, Buckinghamshire, UK) into acid-washed 20 ml plastic vials and refrigerated at 4°C until further analysis was undertaken. Samples were analysed for DOC, phenolics and ion concentrations, UV-Vis absorbance and trihalomethanes formation potential (THMFP).

pH was measured using a Metler Toledo Seven Easy pH meter. The device calibrated using buffers of pH 4 and 7 at 25°C.

4.3.4. Laboratory Analyses

4.3.4.1. Dissolved organic carbon (DOC)

Concentrations of DOC were determined using a Thermalox TC/TN analyser (Analytical Sciences Ltd, Cambridge, UK), which is a combustion and infrared detection based technique. For DOC, samples were acidified to pH <3 prior to analysis to remove inorganic carbon using 10 M HCl. The instrument was calibrated with potassium hydrogen phthalate standards. The injection volume

was 15 μL and oven temperature 680°C. Results were corrected if standard solutions deviated by 10% or more from the correct concentration.

4.3.4.2. Phenolic compounds

The phenolic component of DOC was determined using a method modified from Box (1983), adapted for 300 μL microplate wells. A calibration curve was generated using a series of phenol standard solutions ranging in concentration from 0.5 - 10 mg/L. The assay product was detected by measuring the absorbance at 750 nm on a Spectramax M2e spectrophotometer (Molecular Devices, Wokingham, UK).

4.3.4.3. UV/visible absorption

Specific ultra violet absorbance (SUVA) is used as an indicator of overall DOC quality i.e. molecular weight and aromaticity, with most freshwaters having values in the range 2-4. SUVA was calculated by dividing the absorbance value at 254 nm (measured on the Spectramax M2e spectrophotometer) by the DOC concentration (mg/L).

4.3.4.4. Trihalomethane formation potential (THMFP)

The standard trihalomethane formation potential (THMFP) analysis was used to determine the concentration of trihalomethanes that can be formed per unit DOC under ideal conditions, to give an indication of the reactivity of DOC with chlorine and its propensity to form THMs. A modified version of the THMFP test detailed in (Nieuwenhuijsen *et al.*, 2000) was used. In this study samples were diluted to 1 mg/L DOC in order to derive a standardised THMFP_{7d} value (STHMFP_{7d}) which provides a measure of DOC reactivity. A THMFP_{7d} value was then calculated by multiplying STHMFP_{7d} by DOC concentration. 97.5 mL of diluted sample was dosed with 2.0 mL of 0.5 M KH_2PO_4 to buffer the solution to pH 6.8. Samples were then dosed with 0.5 mL of NaOCl to provide 5 mg of free Cl per mg of DOC. The solution was transferred to a 100 mL amber glass bottle and incubated in the dark at 25°C for 7 days to allow the reaction between organic carbon and chlorine to occur. After 7 days incubation, the reaction was quenched by adding 0.4 mL of 0.8 M Na_2SO_3 to 14.6 mL of incubated sample. This solution was then transferred to a 22 mL amber headspace vial and analysed by the following method. The concentration of THMs was measured using a solid phase micro-extraction (SPME) technique similar to that described by Sarrión *et al.* (2000). The technique uses a Varian GC 450, a Restek MX 1 column (diphenyl/dimethyl polysiloxane phase, 30 m length, 0.53 mm i.d.), N_2 carrier gas at a flow rate of 10 mL/min and a ^{63}Ni Electron Capture Detector. The oven temperature was held at 35°C for 9 minutes and then

increased at a rate of 10 °C /minute to 140°C and held for 2 minutes and finally increased to 180°C and held for 3 minutes. The injector temperature was 290°C and the detector temperature 300°C.

Trihalomethane calibration solutions were made from a stock solution containing 2000 µg/mL of each of the 4 THMs; chloroform (CHCl₃), bromodichloromethane (CHBrCl₂), dibromochloromethane (CHBr₂Cl), and bromoform (CHBr₃) (Sigma). A series of standard solutions were then prepared ranging from 1-500 µg/L.

4.3.4.5. Statistical analysis

Most of data met the homogeneity and normality assumptions, which were tested using the Bartlett and Shapiro Wilk tests, but those that did not were log-transformed. Data points were removed if they were outliers. A four-way ANOVA was used to test effects of the factors Soil Type (two levels; bog and fen), Treatment (two levels; Control and Drought); Depth (two levels; 5 and 20 cm), and Date (six levels for each sampling date) for each measured parameter. Following this, t tests was performed to test the effect of Treatment on each date. All tests were run in R v3.3.1. A p value of 0.05 was used to determine significance.

4.4. Results

Data is presented in Figures 4.1 to 4.6 as a series of graphs showing the variation in measured parameters over time, alongside the mean water table height measured within each mesocosm throughout the experiment.

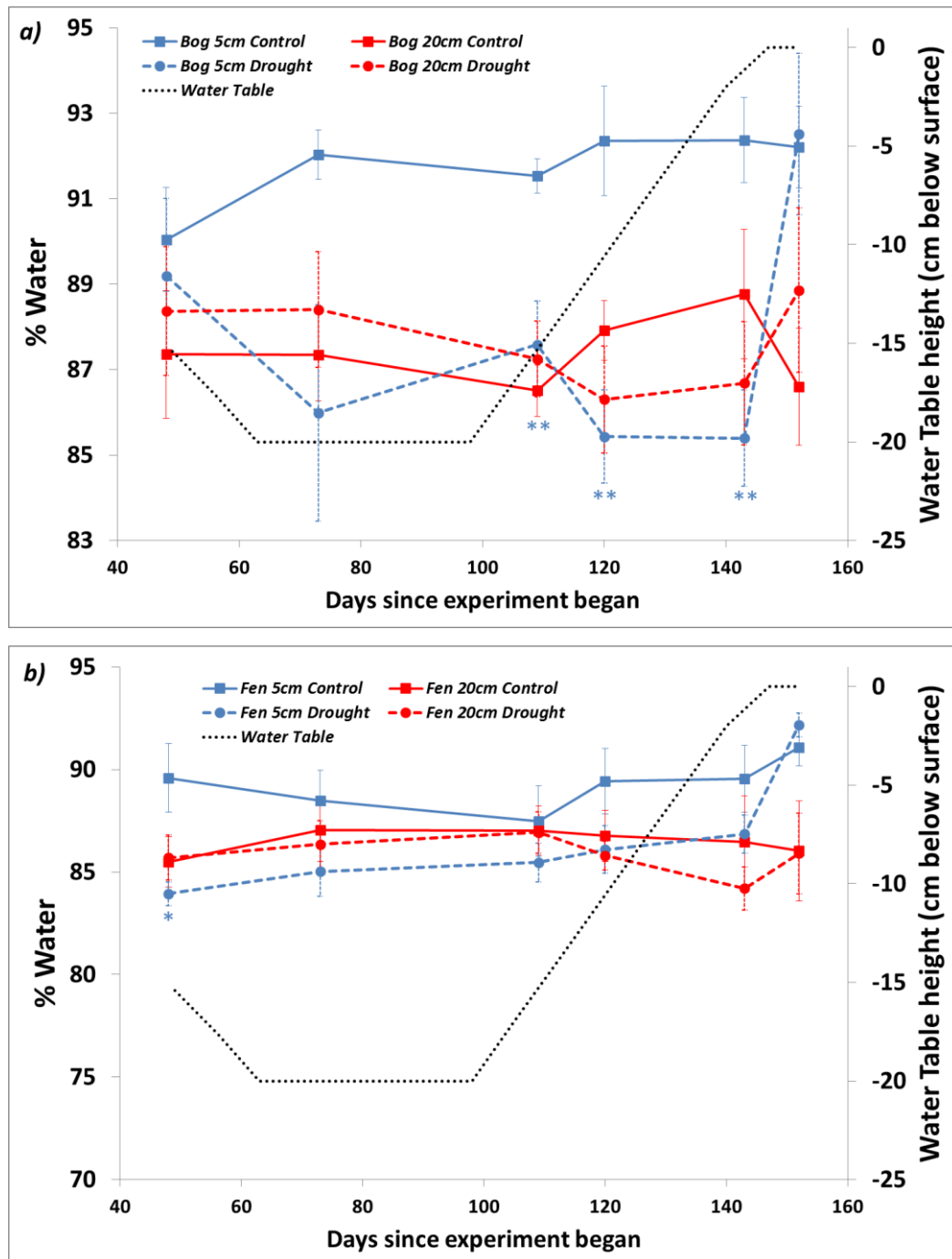


Figure 4.1. – Soil water content (%) at 5 and 20 cm and water table height for a) bog and b) fen mesocosms, with the water table height either maintained at the surface (Control) or reduced and restored (Drought). Asterisks indicate significant differences between Control and Drought treatments at individual time and depths (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$)

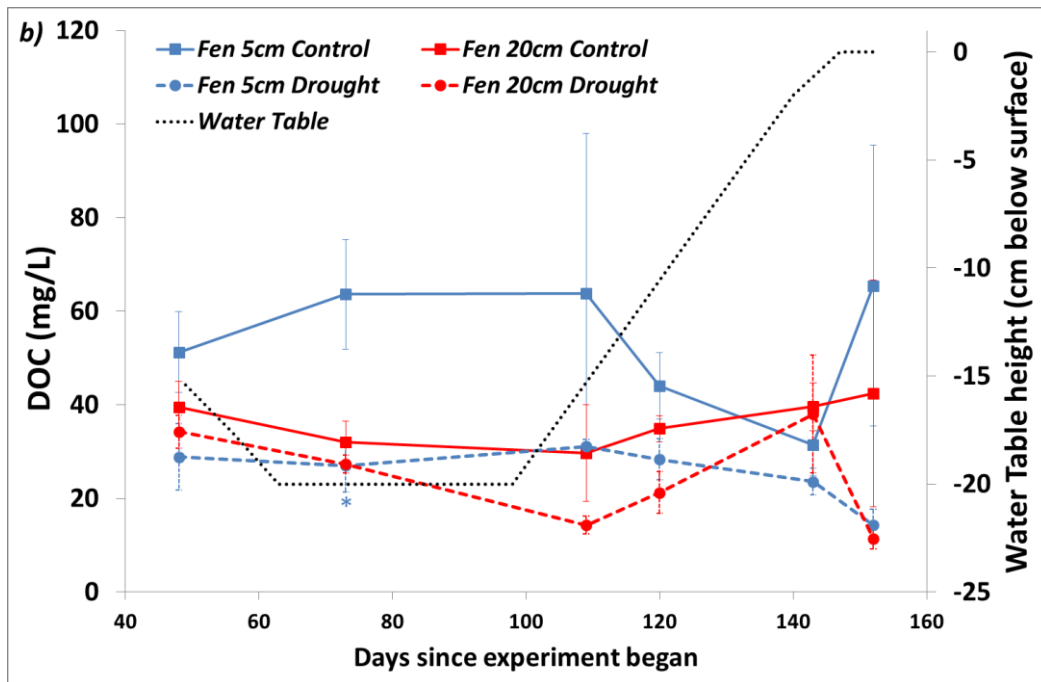
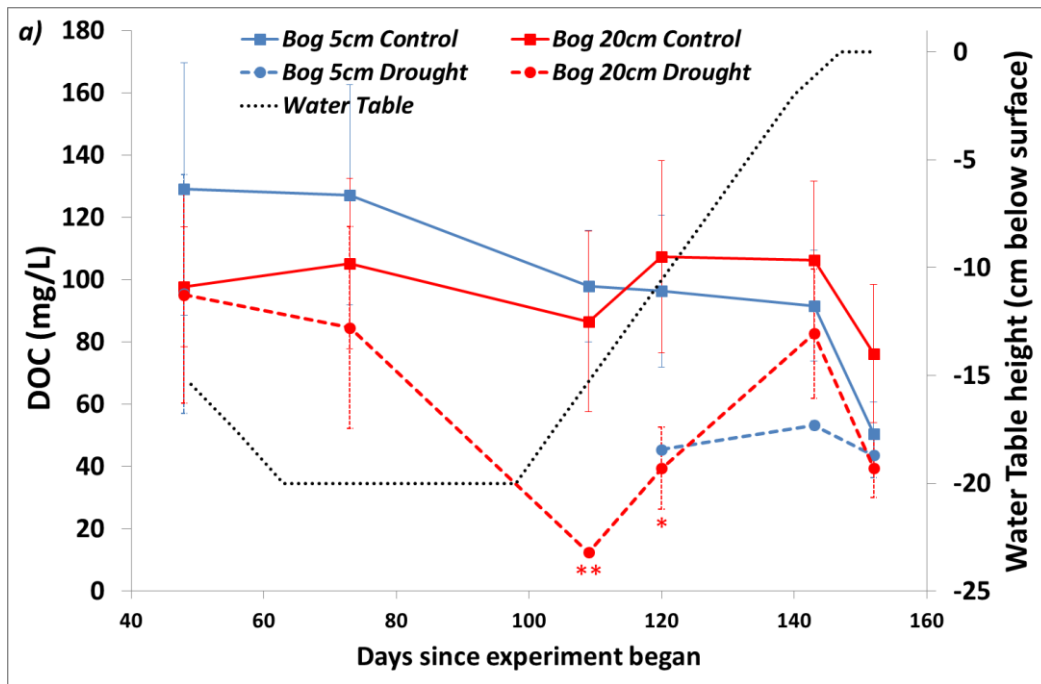


Figure 4.2. – Soil porewater Dissolved Organic Carbon (DOC) concentrations at 5 and 20 cm and water table height for a) bog and b) fen mesocosms, with the water table height either maintained at the surface (Control) or reduced and restored (Drought). Asterisks indicate significant differences between Control and Drought treatments at individual time and depths (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

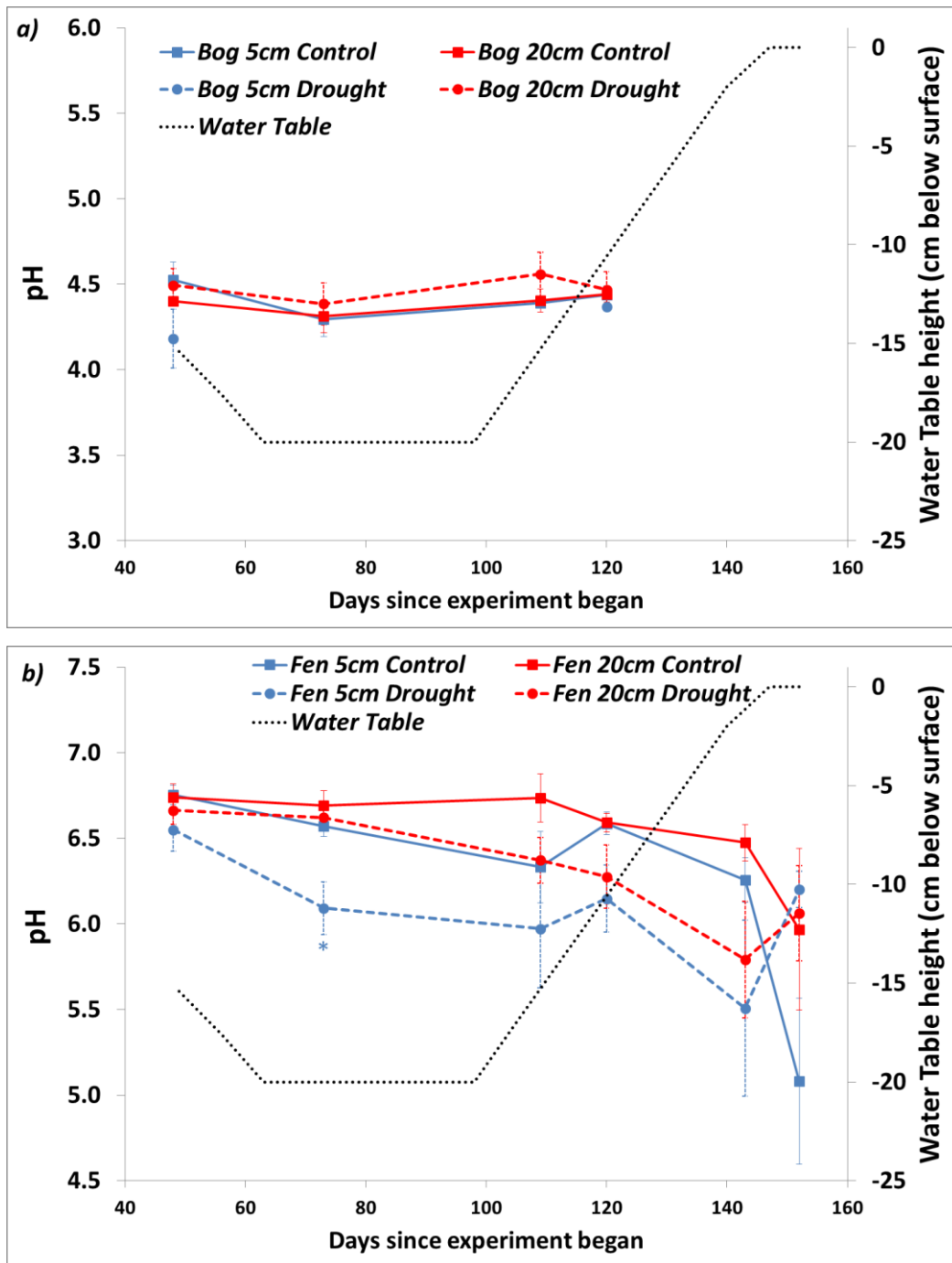


Figure 4.3. – Soil porewater pH at 5 and 20 cm and water table height for a) bog and b) fen mesocosms, with the water table height either maintained at the surface (Control) or reduced and restored (Drought). Asterisks indicate significant differences between Control and Drought treatments at individual time and depths (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

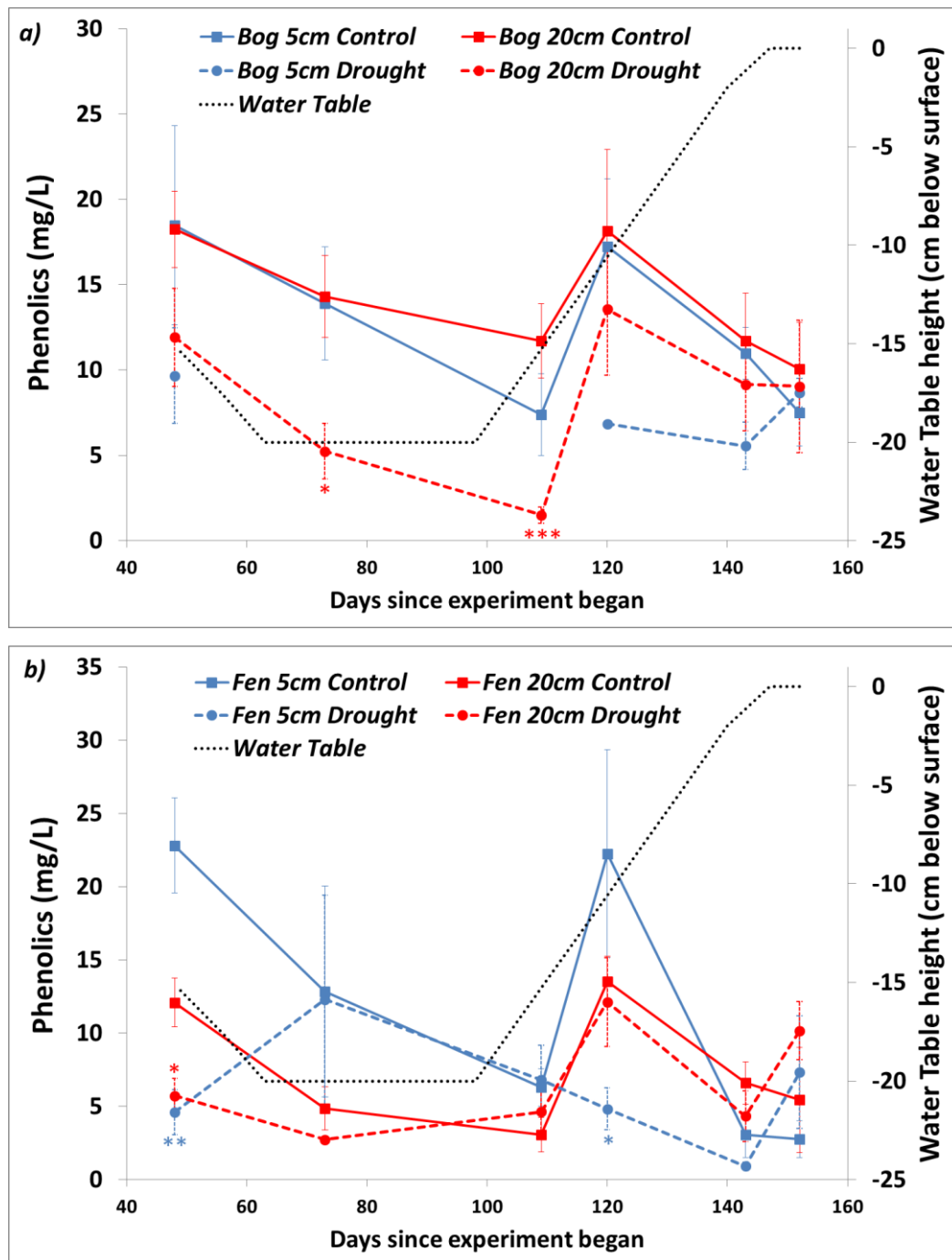


Figure 4.4. – Soil porewater phenolics concentration at 5 and 20 cm and water table height for a) bog and b) fen mesocosms, with the water table height either maintained at the surface (Control) or reduced and restored (Drought). Asterisks indicate significant differences between Control and Drought treatments at individual time and depths (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

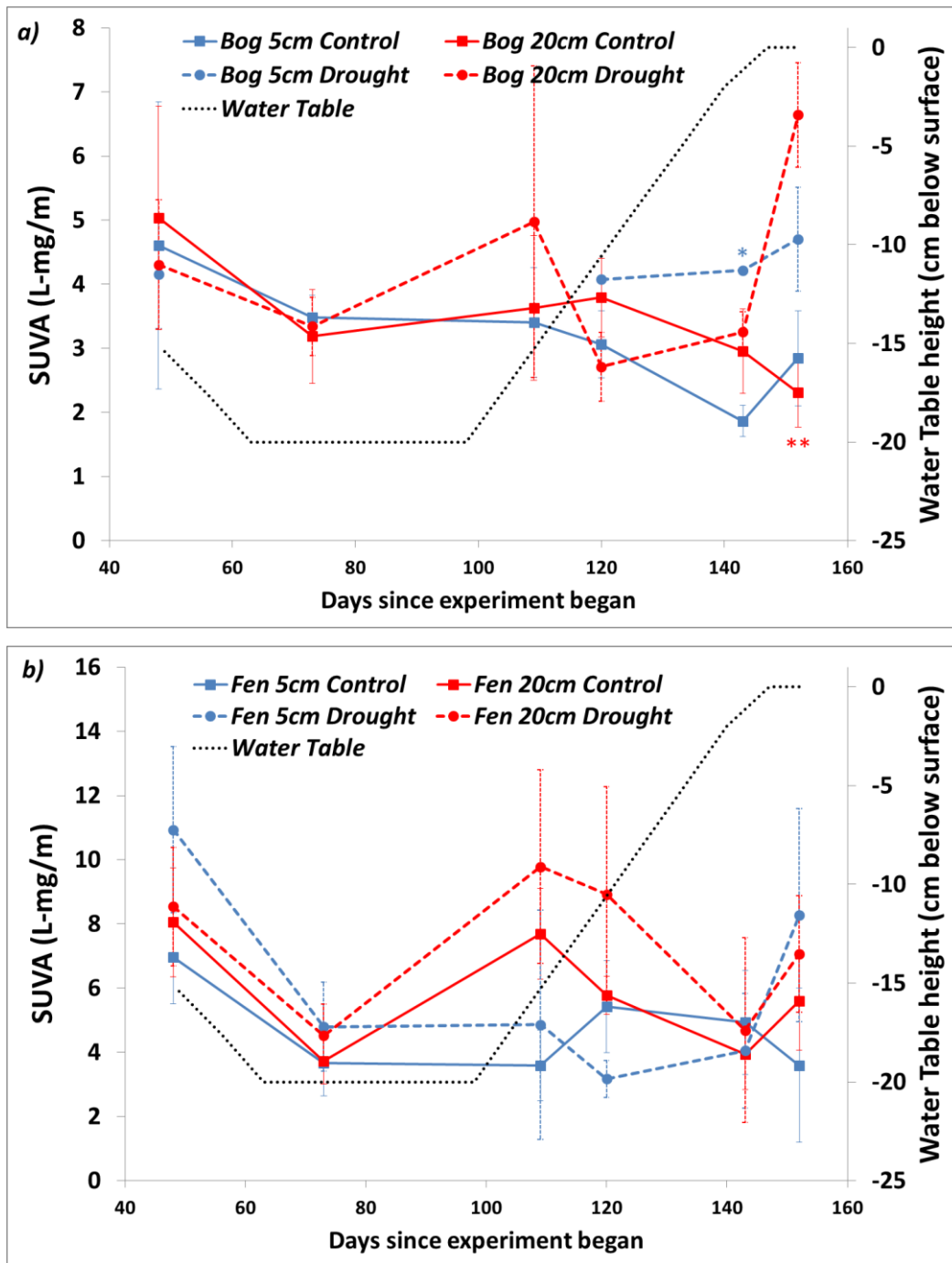


Figure 4.5. – Soil porewater SUVA at 5 and 20 cm and water table height for a) bog and b) fen mesocosms, with the water table height either maintained at the surface (Control) or reduced and restored (Drought). Asterisks indicate significant differences between Control and Drought treatments at individual time and depths (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

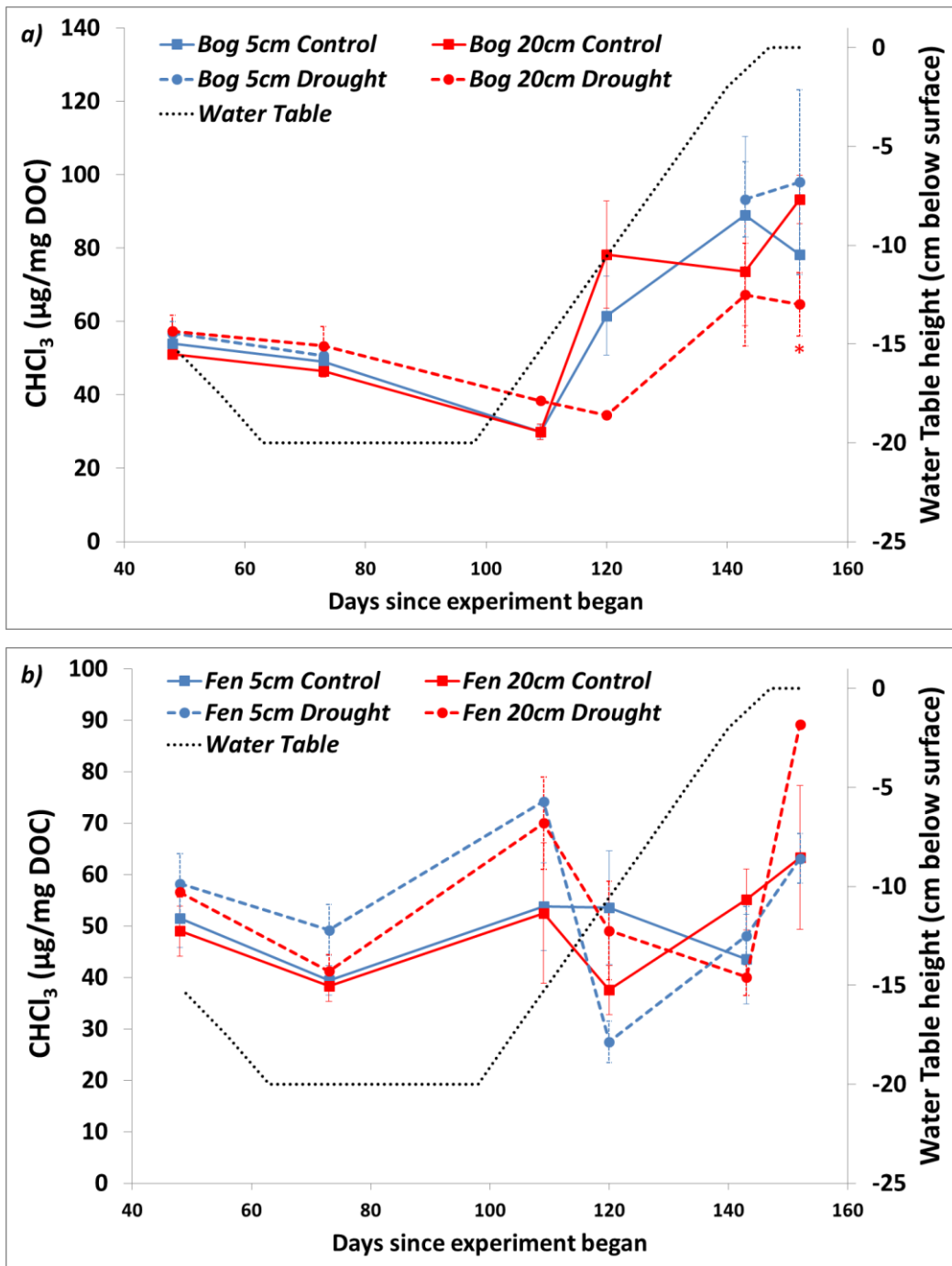


Figure 4.6. – Soil porewater CHCl_3 formation at 5 and 20 cm and water table height for a) bog and b) fen mesocosms, with the water table height either maintained at the surface (Control) or reduced and restored (Drought). Asterisks indicate significant differences between Control and Drought treatments at individual time and depths (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

Parameter	Factor	F value	P value
Water content	Soil type	15.86	<0.001
	Treatment	20.40	<0.001
	Depth	26.57	<0.001
	Date	2.58	0.028
DOC	Soil type	104.59	<0.001
	Treatment	44.89	<0.001
	Depth		
	Date	6.13	<0.001
pH	Soil type	512.88	<0.001
	Treatment		
	Depth	4.51	0.035
	Date	5.22	<0.001
Phenolics	Soil type	31.78	<0.001
	Treatment	18.89	<0.001
	Depth		
	Date	11.06	<0.001
SUVA	Soil type	15.89	<0.001
	Treatment	6.62	0.011
	Depth		
	Date	4.24	<0.01
THMFP	Soil type	12.51	<0.001
	Treatment	5.18	0.024
	Depth		
	Date	10.20	<0.001

Table 4.1. Result of 4-way ANOVA to determine the effect of Soil type, Treatment, Depth and Date on several measured parameters.

The water table manipulation successfully lowered the soil water content of the bog mesocosms but not the fen (Figure 4.1, Table 4.1). The overall results of the four-way ANOVA showed there was a significant effect of soil type, treatment, date and depth on soil water content (Table 4.1). Overall, soil water content was higher for the bog (88.62 ± 3.56 %) compared to the fen (87.07 ± 4.43 %), for the control (88.74 ± 3.52 %) over the drought (86.96 ± 3.42 %) treatment and for the 5 cm (88.88 ± 3.80 %) over the 20 cm (86.83 ± 3.03 %) depth.

For the bog, the soil water content was relatively stable for the 5 cm control throughout the sampling period, the drought treatment was lower % water than the control for all but the final time point (day 152, restored water table). The soil water content at 5cm was significantly lower for the drought compared to the control on days 109, 120 and 143 ($p < 0.01$), and during the phase of the experiment when the water table was beginning to recover. For the bog at 20 cm, there was no time point when the soil water content of the drought treatment was significantly lower than the control treatment.

For the Fen, the soil water content of the drought treatment was generally lower than the control treatment, but only significantly so for one time point and one depth; day 48 and 5 cm. Therefore, the water table manipulation did not induce consistent effects on soil water content for the fen.

There was a significant effect of soil type, treatment and date, but not depth, on DOC concentrations (Table 4.1). Mean DOC was higher for the bog (83.77 ± 57.67 mg/L) compared to the fen (34.76 ± 26.00 mg/L) and for the control (71.68 ± 53.3 mg/L) over the drought (39.69 ± 38.66 mg/L) treatment. For the bog at 20 cm, the control treatment was relatively stable throughout the sampling period but the drought treatment DOC decreased during the drought period and was significantly lower than the control treatment at days 109 ($p < 0.01$) and 120 ($p < 0.05$), when the water table was beginning to recover. The concentrations on these dates were 63-86% lower than the 20 cm control treatment. At 5 cm, DOC declined from 129.12 ± 40.52 mg/L on day 48 to 50.44 ± 10.16 mg/L on day 152 in the control treatment (-60.9%). For the drought, on the dates that where a sample could be collected, all drought treatment data points were lower than the control treatment, but not significantly so. The differences at days 120 and 143 (recovering water table) were similar to day 48 (declining water table).

For the fen, the 5 cm drought DOC concentrations were always lower than the control, but only significantly so at day 73 (57% lower, $p < 0.05$). DOC concentrations at 20 cm were relatively stable for both treatments and always lower in the drought treatment but there were no significant differences at any time point.

There was a significant effect of soil type, depth and date, but not treatment, on pH (Table 4.1). Mean pH was higher for the fen (6.30 ± 0.62) compared to the bog (4.41 ± 0.66) mesocosms and at 5 cm (5.49 ± 1.09) compared to 20 cm (5.40 ± 1.18). For the bog, the pH was relatively stable throughout the sampling period for both control and drought treatments and at both depths. Due to the stable values recorded for the first four dates, which included the greatest change in water table height, pH was not measured for the bog mesocosms on days 143 and day 153.

For the fen, the control treatment decreased from 6.75 at day 48 to 5.08 at day 153 at 5cm, and from 6.74 at day 48 to 5.97 at day 153 at 20 cm. The drought treatment displayed a similar declining trend at both depths but the pH was generally lower than the control, however the only significant treatment effect was at day 73, when the drought was lower than the control treatment by 0.53 of a pH unit ($p < 0.05$).

There was a significant effect in mean phenolic concentrations of soil type, treatment and date, but not depth, on phenolic concentrations (Table 4.1). Mean phenolic concentrations were higher

for the bog (11.46 ± 7.50 mg/L) compared to the fen (7.80 ± 8.00 mg/L) and for the control (11.39 ± 8.67 mg/L) over the drought (7.19 ± 6.29 mg/L) treatment.

For the bog, the phenolics concentration of the control treatment was very similar at both depths, displaying a declining trend over time, apart from a spike at day 120. The drought treatment phenolic concentration was almost always lower than the control at both depths, especially during the period when the water table was lowest, but only significantly so at days 73 and 109 ($p < 0.05$ and $p < 0.001$) and for 20 cm depth. There were no time points when there was a significant treatment effect at 5 cm. For the fen, there was a similar trend for the control treatment to the bog control treatment, with a generally declining trend but a spike at day 109. However, for the fen, the phenolics concentrations at 20 cm were generally much lower than at 5 cm. As for the bog, phenolics in the drought treatment were generally lower than the control, significantly so at day 48 for both 5 cm ($p < 0.01$) and 20 cm ($p < 0.05$) and day 120 at 5 cm ($p < 0.05$). The phenolics concentration of the drought treatment was higher than the control treatment at both depths on day 152, when the water table was fully recovered.

There was a significant effect of soil type, treatment and date, but not depth, on SUVA (Table 4.1). Mean SUVA was higher for the fen (6.06 ± 4.19 L/mg/m) compared to the bog (3.68 ± 2.10 L/mg/m) and for the drought (5.80 ± 4.32 L/mg/m) over the control (4.32 ± 2.73 L/mg/m) treatment.

For the bog, SUVA showed a similar trend of declining slowly at both 5 cm and 20 cm for the control treatment. The drought treatment had a higher SUVA than the control at most time points, significantly so at day 143 at 5 cm ($p < 0.05$) and day 152 at 20 cm ($p < 0.01$).

For the fen, there were no time points when there was a significant treatment effect, although the SUVA was generally higher for the drought compared to the control at both depths.

There was a significant effect of soil type, treatment and date, but not depth, on THMFP (Table 4.1). Mean THMFP was higher for the bog (61.87 ± 26.60 $\mu\text{g CHCl}_3/\text{mg DOC}$) compared to the fen (50.76 ± 15.91 $\mu\text{g CHCl}_3/\text{mg DOC}$) and for the drought (57.75 ± 20.33 $\mu\text{g CHCl}_3/\text{mg DOC}$) over the control (54.59 ± 23.63 $\mu\text{g CHCl}_3/\text{mg DOC}$) treatment. For the bog, the THMFP displayed a similar trend at both 5 and 20 cm, declining from day 48 to 109 but then increasing up to the final days of sampling (day 143 and 152) to finish with a value approximately 50% greater than the day 48 value. The Drought treatment had a similar trend but at 5 cm (on the dates when it was possible to collect a sample) the THMFP was higher than the control and at 20 cm the THMFP was lower than the control. The only significant difference was at 20 cm on day 152, when the water table

had fully recovered, when the drought treatment THMFP was approximately 30% lower than the control. For the fen, the control THMFP was relatively stable at both depths, but there was more variation within the drought treatment data. The drought treatment THMFP was generally higher than the control treatment THMFP but there were no significant differences at any of the six time points.

4.5. Discussion

Overall, the results of this study indicate that applying a drought treatment to cores of peat dramatically reduced the concentration of DOC in the porewater. It would therefore be expected that if such conditions were recreated in the field then DOC export to freshwaters would be reduced by drought conditions in catchments where peat soils dominate. The effect was especially pronounced in the bog soil, where the DOC concentration was approximately 4 times lower during the peak of drought conditions. For the fen, the DOC concentration reduced by 30-50% during the peak of drought conditions. During the re-wet period the DOC concentrations recovered so they were comparable to pre-drought values. The trend in phenolics concentrations followed a similar pattern to the DOC, indicating that phenolics were affected by the drought treatment. The loss of DOC and phenolics due to drought can be attributed to oxygenation and improved conditions for decomposition in the soil. This will have resulted in the complete breakdown of soil organic matter to CO₂, rather than incomplete breakdown to DOC, due to the opening of the enzymic latch (Freeman *et al.*, 2001b; Fenner and Freeman, 2011).

Drought generally lowered pH and conductivity (data not presented), especially for the fen. This is because the fen receives its nutrients from groundwater so if this supply is cut off, as it was during the drought treatment, the effect on the peat will be dramatic.

Phenolic concentration in pore water was lower during drought treatments in the bog and fen due to the much larger pool of phenolic compounds in the soil itself which changes in drought, which is not that easy to observe. There is previous evidence of the decline of phenolic compound concentration, shown in the literature (e.g. Fenner and Freeman, 2011). The mean concentration of phenolic compounds in pore water was lower in the fen than in the bog; this can be due to the greater activities of phenol oxidase in the fen (Jassey *et al.*, 2011). However, a previous study mentioned that the carbon in the bog degrades less than carbon in the fen (Tfaily *et al.*, 2013). Moreover, bacterial genes in the fens are higher than bogs (Ausec *et al.*, 2011), so perhaps the bog conditions are not suitable for phenolic microbes, which are responsible for the breakdown of phenolic compounds. Some previous studies show that precipitation was one of the changes in the effect on fungal communities (Toberman *et al.*, 2008). In addition, other previous studies

show that fungal communities control the low pH in soils. (Rousk et al., 2010) and that the anaerobic conditions in peatlands might prevent fungal growth (Golovchenko et al., 2013).

Although the treatment differences are not significant, the THMFP values were higher for the drought treatment compared to the control during the peak of the drought and initial rise in water table back to the surface. This suggests that drought conditions lead to the release of proportionally more THM precursors from peat than if the water table was close to the surface. This is a finding that has not previously been published in scientific literature.

4.6. Conclusions

The main overall conclusion from this study is that artificially exposing peat cores to a drought treatment reduced DOC concentrations compared to control cores but increased THMFP values, although none of the effects were significant. From a water treatment perspective, the increase in THMFP during peatland drought suggests that for source water from reservoirs whose catchments are dominated by peat, during drought conditions, the DOC exported from the peatland to the reservoir will be proportionally more likely to form THMs, however, this effect is more than offset by the lower DOC concentrations.

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Chapter 5: Impact of constructed wetlands on DOC characteristics and THMFP

5.1. Abstract

In England and Wales, two-thirds of drinking water comes from surface water such as reservoirs, lakes and rivers, and the main problem is the potential eutrophication of those reservoirs. In lakes and reservoirs, DOC originate from both internal (allochthonous) is DOC produced within the lake and external (autochthonous) is DOC that intend to the lake from the catchment biochemical inputs. Eutrophication is one of the biggest issues for drinking water production, that characterized by excessive plant and algal growth due to the increased availability of one or more of growth factors needed for photosynthesis such as sunlight, carbon dioxide, and nutrient fertilizers. Eutrophication also increases dissolved organic carbon (DOC) concentrations and the removal of DOC is usually the costliest stage of water treatment process that is because the reaction of DOC with chlorine during disinfection can lead to formation of carcinogenic disinfection by-products (DBPs) such as THMFP. The presence of DOC in water is not harmful however, when water containing DOC that reacts with chlorine as a disinfectant in water treatment plant, it can produce THMs that lead to cancerous diseases and the most common THMs is chloroform. Constructed wetlands are one solution to the eutrophication issue, because wetlands sequester nutrients. The objective of this study was determining whether a constructed wetland used for to remove nutrients from a stream, flowing into a eutrophic reservoir affects the quantity and quality of DOC in the stream. Inflow and outflow of the Free Water Surface was observed twice a week over a period of nine months. Physicochemical parameters (pH, conductivity), nutrients, dissolved organic carbon (DOC), Phenolics, trihalomethans (THMs) and greenhouse gases (CH_4 , CO_2 , N_2O) concentration were measured.

5.2. Introduction

In England and Wales, two-thirds of drinking water is derived from surface waters such as man-made reservoirs, natural lakes and rivers. Such raw water supplies can contain naturally high concentrations of natural organic matter (NOM). Aquatic NOM is a heterogeneous mixture of complex organic compounds, is derived from allochthonous and autochthonous sources and plays important roles in several biotic and abiotic processes (Wetzel, 2001). Carbon (C) is the key constituent of NOM, therefore NOM in the dissolved form is often referred to and measured as dissolved organic carbon (DOC). Allochthonous DOC originates outside of the waterbody, from within its catchment, and is mainly derived from decaying plant material and the leaching of organic detritus from soils that is transported to the aquatic system by run off, streams and shallow groundwater flow (Thurman, 1985; Aiken and Cotlaris, 1995). Autochthonous DOC is that which is produced within the water body itself, from macrophytes and phytoplankton. The main factors controlling the DOC concentration of surface waters are catchment properties (land use/soil type), hydrological characteristics and climatic conditions (Pacheco *et al.*, 2013).

Eutrophication is the enrichment of a waterbody with nutrients that leads to rapid growth of algae (Nixon 1995; Carpenter *et al.* 1998). Sunlight, carbon dioxide and nutrients are the factors that increase the magnitude of the eutrophication (Schindler 2006). In addition, eutrophication can lead to blooms of algae that are directly harmful to human health (Smith and Schindler, 2009). Eutrophication can also increase the DOC concentration of water, which is particularly an issue for lowland reservoirs in agricultural catchments (Gough *et al.*, 2015; Pierson-Wickmann *et al.*, 2011). During algal growth algogenic organic matter is leached from algal cells by diffusion (extracellular organic matter) or released from dying algal cells during cell lysis (intracellular organic matter), which can lead to taste and odour problems in potable water supplies (Gough *et al.*, 2015). Some algal species produce toxic metabolites which can pose a serious acute public health risk (Zegura *et al.*, 2011; Ritson *et al.*, 2014). The increase in dissolved organic carbon concentrations during eutrophication events will also increase coagulant and chlorine demand and lead to greater formation of potentially-harmful disinfection by-products (DBPs) such as trihalomethanes (THMs) (Nguyen *et al.*, 2005; Li *et al.*, 2012).

Allochthonous and autochthonous DOC differs in characteristics and quality. Allochthonous DOC is generally comprised of high-molecular weight, aromatic humic and fulvic acids whilst autochthonous DOC is generally much lower-molecular weight, less aromatic and comprised of much smaller compounds (Chupakov *et al.*, 2017). The contribution from each source changes seasonally;

autochthonous production peaks in late spring, summer and early autumn due to greater sunlight and warmer temperatures that allow photosynthesis and primary production by algae and vegetation. The contribution from allochthonous sources generally peaks during late summer/autumn, when the weather turns cooler and wetter and the vegetation within the catchment starts to die back and decompose (Chupakov et al., 2017; Tremblay and Benner, 2006).

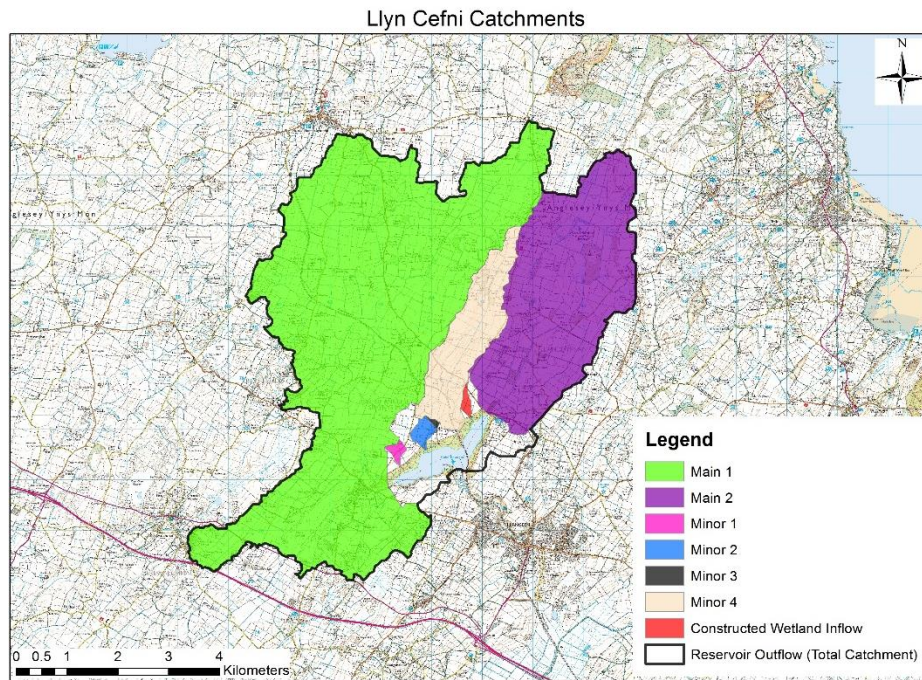
DOC quality is a key influence on water treatability and likelihood to form DBPs. High molecular weight, aromatic, hydrophobic allochthonous DOC, which generally has a high Specific Ultraviolet Absorbance (SUVA), generally reacts more readily with chlorine to form THMs (Fang et al., 2010; Chow et al., 2005). SUVA used to determine the disinfection by-product formation potential and calculated by dividing the absorbance at 254nm by DOC concentration mg/L of a water, High SUVA indicates that there is a large portion of organics present in the water is aromatic, high SUVA due to high aromatic organics compounds that easily react with disinfectants to create DBPs, so high SUVA point to a high potential for the formation of DBP's. However, many studies shows that reducing the SUVA value by removing the NOM by coagulation (Archer and Singer, 2006).

Constructed wetlands are increasingly being used as a natural solution for cleaning freshwaters impacted by pollution and suffering from poor water quality (Vymazal, 2007), sometimes with the goal of ensuring compliance with environmental legislation standards (Gouriveau, 2009). As water flows through a constructed wetland, much of the suspended sediment will settle out, whilst nutrients in inflow waters are either taken up by plants or processed by microbes in the soil. Constructed wetlands are particularly well suited to removing nitrate and phosphate from agricultural run-off, with several biogeochemical processes within wetlands allowing for the sequestration of nutrients and pollutants. This study builds on the publication of Scholz et al. (2016), which reports findings on the success of a newly built constructed wetland for removing nitrate and phosphate from a stream flowing into a lowland eutrophic lake. The paper reported net removal of nutrients but increased the DOC concentration of the stream, which may impact negatively in the downstream water treatment works at this site. The aim of this study was to assess whether the constructed wetland remained a net sink of nutrients but a net source of DOC in the autumn and winter time and to determine whether the constructed wetland changes the characteristics of the DOC flowing into the reservoir and how readily it reacts with chlorine.

5.3. Methods

5.3.1. Study site

The study site was Llyn Cefni (UK Grid Ref: SH443774), a man-made eutrophic reservoir on the Island of Anglesey, north Wales, UK. The reservoir was created in 1940 by damming the Afon Cefni river to produce a lake 2.3 km in length and 0.86 km² in surface area, which is now used as a source of drinking water. There are two main water sources feeding into Llyn Cefni reservoir; the Afon Frogwy on the south-west side and Afon Erddreiniog on the north-east side, the latter originating within Cors Erddreiniog, a National Nature Reserve and Site of Special Scientific Interest that is a rich fen and contains significant deposits of peat. The DOC concentration of the water can be high, partly due to intensive farming practices within the catchment leading to excess nutrients entering the lake and fuelling algal growth (Scholz *et al.* 2016). Management practices have been implemented aimed at minimising the autochthonous production of DOC, such as the installation of straw bales within the lake to suppress algal growth and the construction of a treatment wetland within the catchment to reduce nutrient inputs. This study followed on from the work presented by Scholz *et al.* (2016) at the same site and was undertaken to a) assess the ability of the constructed wetland to sequester nutrients outside of the growing season and b) to determine the impact of the constructed wetland on DOC characteristics and therefore propensity to form DBPs following chlorination. In total, nine sampling sites were identified and were mostly similar to those monitored during the Scholz *et al.* (2016) study. The two main inputs were sampled and identified as Main 1 and 2 respectively. Five other smaller stream inputs were also sampled, including the inflow to the constructed wetland (labelled as Minor 1-4 and Constructed Wetland inflow). The outflow from the same constructed wetland was also sampled in addition to the reservoir at the point where the water discharges from the reservoir to a downstream river (Afon Cefni). Figure 5.1 is a map showing the reservoir, its watershed boundary and the catchments of the seven sampled inflowing rivers/streams. The constructed wetland was built in March 2014 on a stream previously identified as supplying high concentrations of nitrate and phosphate, which are the key nutrients for the algal growth. Table 5.1 presents the catchment sizes of the seven streams and reservoir as a whole, demonstrating the larger sizes of the two main inflows.



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Figure 5.1. Watershed area of sampled inflowing streams to Llyn Cefni Reservoir, and the reservoir itself. Map created using ArcGIS 10.4.1 software (Environmental Systems Research Institute (ESRI), California, USA).

Stream name	Catchment area (km ²)
Main 1	27.63094
Main 1	10.27868659
Minor 1	0.0941424
Minor 2	0.184136973
Minor 3	0.026807824
Minor 4	2.993129
Constructed Wetland Inflow	0.080882
Reservoir	44.1606002

Table 5.1: Catchment area (km²) of sampled streams at the Cefni Reservoir

5.3.2. Land use analysis

The land use of the catchment of each stream, and the reservoir as a whole, was analysed using ArcGIS 10.4.1 software (Environmental Systems Research Institute (ESRI), California, USA), utilising digital map data acquired from Digimap (University of Edinburgh, UK), Catchments were delineated using the ‘Hydrology’ function located in the ‘Spatial Analyst’ add-on, as described by Hopes et al (submitted for publication).

5.3.3. Sampling regime

Sampling was undertaken approximately twice a month from September 2014 to March 2015 at all nine sampling sites. Three replicates were collected at each site in 100 ml amber glass bottles. pH and conductivity were measured in the laboratory on unfiltered samples on the day of sampling. The pH was measured using a Seven Easy pH meter (Mettler Toledo, Leicester, UK). The probe was calibrated using buffers of pH 4 and 7 at 25°C (Sigma, Dorset, UK). Conductivity was measured with a Primo 5 conductivity meter (Hanna Instruments, Bedfordshire, UK). All samples were filtered through 0.45 µm syringe filters (Triple Red, Buckinghamshire, UK), and refrigerated at 4°C until further analysis was undertaken.

5.3.4. Laboratory Analyses

5.3.4.1. Dissolved organic carbon (DOC)

Concentrations of DOC were determined using a Thermalox TC/TN analyser (Analytical Sciences Ltd, Cambridge, UK), which is a combustion and infrared detection based technique. For DOC, samples were acidified to pH <3 prior to analysis to remove inorganic carbon using 10 M HCl. The instrument was calibrated with potassium hydrogen phthalate standards. The injection volume was 15 µL and oven temperature 680°C. Results were corrected if standard solutions deviated by 10% or more from the correct concentration.

5.3.1.2. Phenolic compounds

The phenolic component of DOC was determined using a method modified from Box (1983) and adapted for 300 µL microplate wells. A calibration curve was generated using a series of phenol standard solutions ranging in concentration from 0.5 - 10 mg/L. The assay product was detected by measuring the absorbance at 750 nm on a Spectramax M2e spectrophotometer (Molecular Devices, Wokingham, UK).

5.3.1.3. Trihalomethane formation potential (THMFP)

The standard trihalomethane formation potential (THMFP) analysis was used to determine the concentration of trihalomethanes that can be formed per unit DOC under ideal conditions, to give an indication of the reactivity of DOC with chlorine and its propensity to form THMs. A modified version of the THMFP test detailed in Nieuwenhuijsen, *et al.* (2000) was used. THMFP analysis was performed on only five sites; Main 1, Main 2, Constructed Wetland Inflow and Outflow, and the reservoir, and at each sampling site and time point the three replicates were combined into one and the DOC concentration re-measured. All samples were then diluted to 1 mg/L DOC in order to derive

a standardised THMFP_{7d} value (STHMFP_{7d}) which provides a measure of DOC reactivity. To a 100 mL amber glass bottle the following solutions were added; 97.5 mL of diluted sample, 2.0 mL of 0.5 M KH₂PO₄ buffer (to adjust the solution to pH 6.8) and 0.5 mL of NaOCl (to provide 5 mg of free Cl per mg of DOC). The bottles were incubated in the dark at 25°C for 7 days to allow the reaction between organic carbon and chlorine to occur. After 7 days, 14.6 mL of sample was transferred to a 22 ml amber headspace vial and the reaction was quenched by adding 0.4 mL of 0.8 M Na₂SO₃. The samples were then analysed using a solid phase micro-extraction (SPME) technique similar to that described by Sarrión, et al., (2000). The analysis used a Varian 450 GC, a Restek MX 1 column (diphenyl/dimethyl polysiloxane phase, 30m length, 0.53 mm i.d.), N₂ carrier gas at a flow rate of 10mL/min and a ⁶³Ni Electron Capture Detector. The oven temperature was held at 35°C for 9 minutes and then increased at a rate of 10°C /minute to 140°C and held for 2 minutes and finally increased to 180°C and held for 3 minutes. The injector temperature was 290°C and the detector temperature 300°C. Trihalomethane calibration solutions were made from a stock solution containing 2000 µg/mL of each of the 4 THMs; chloroform (CHCl₃), bromodichloromethane (CHBrCl₂), dibromochloromethane (CHBr₂Cl), and bromoform (CHBr₃) (Sigma). A series of standard solutions were then prepared ranging from 1-500 µg/L.

5.3.5. Statistical analyses

The effect of Site (nine levels) and Date (15 levels) on each measured parameter were determined using two-way ANOVA and, for the Site factor only, Tukey HSD post-hoc analyses. Further statistical analysis was undertaken using Pearson correlation to test for significant relationships between all dependent variables. A p value of <0.05 was used to determine significance throughout and analysis was undertaken in IBM SPSS Statistics v22.

5.4. Results

5.4.1. Land use

The catchment areas for the seven sampled streams and reservoir are presented in Table 5.1, showing the much larger catchment sizes of the Main 1 and Main 2 streams and the much smaller catchment sizes of streams Minor 1-4 and the Constructed Wetland inflow. The land use of each of the eight catchments is shown in Figure 5.2. All are dominated by “Improved grassland”, from 61.1% for Main 2 to 99.5% for Minor 1. The next most common land use types are generally “Rough grassland” and “Arable Horticulture”. The catchment of Main 2 is the most unique, with 13.7% of the land comprised of “Fen/Marsh/Swamp”, a category not found in any of the other stream’s catchments. The represents

a large area of deep fen peat, Cors Erddreiniog SSSI, which may have an important influence on the water quality of Llyn Cefni.

Treatments	Site		Date	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
pH	126.72	<0.001	209.76	<0.001
Conductivity	589.864	<0.001	411.574	<0.001
Phenolic	216.673	<0.001	189.899	<0.001
DOC	92.548	<0.001	92.423	<0.001
Nitrate	2070.080	<0.001	165.395	<0.001
Nitrite	8.803	<0.001	56.879	<0.001
Phosphate	99.890	<0.001	207.384	<0.001
Bromide	64.570	<0.001	16.485	<0.001
CHCl ₃	0.480	0.751	11.901	<0.001
CHCl ₂ Br	3.711	0.006	16.382	<0.001
CHClBr ₂	2.735	0.030	12.534	<0.001

Table 5.2. Results of two-way ANOVA analysis two test effect of sites on measured parameters.

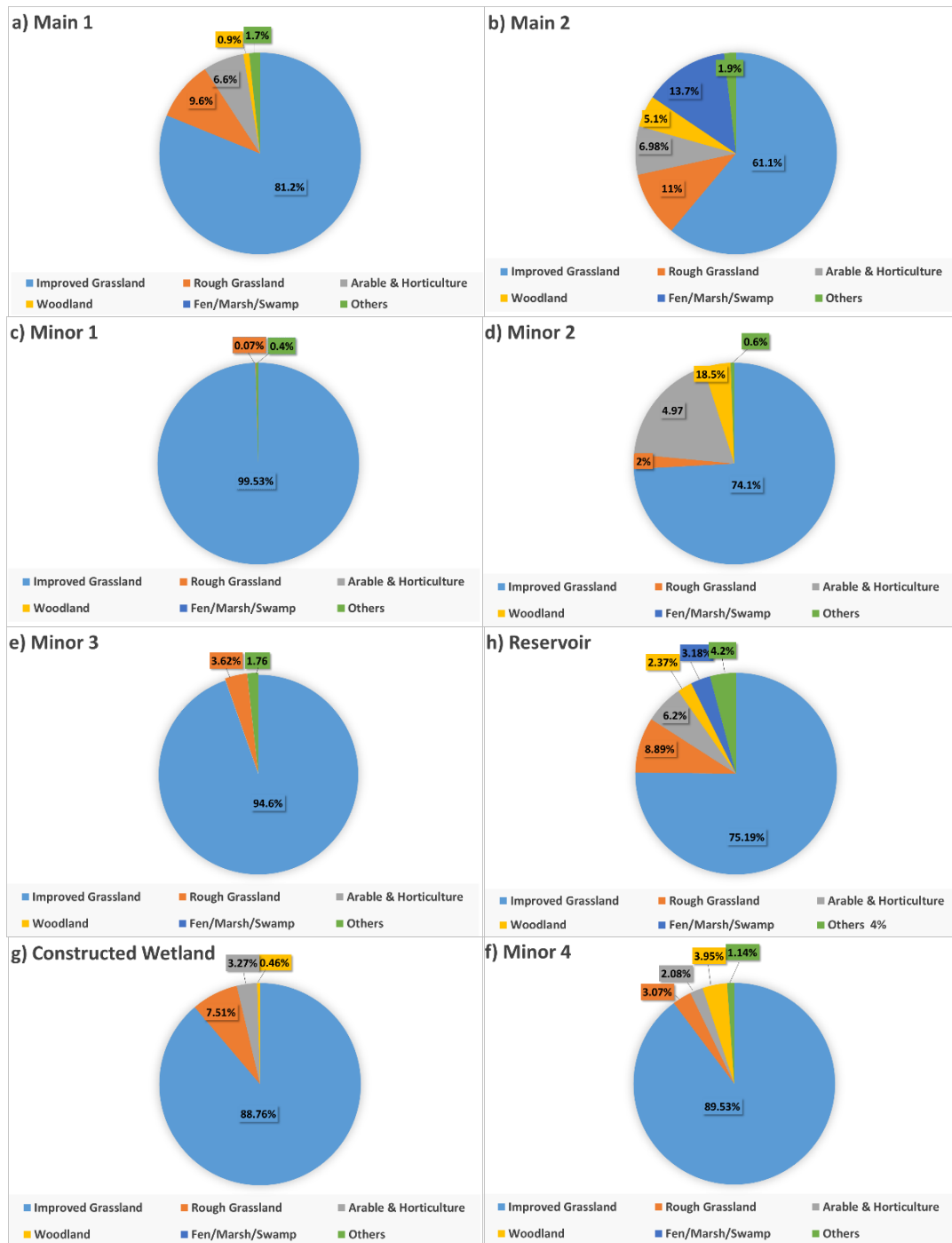


Figure 5.2. Shows the percent land use within the Cefni Catchment a) Main 1, b) Main 2, c) Minor 1, d) Minor 2, e) Minor 3, f) Minor 4, g) Constructed Wetland inflow, h) Reservoir

5.4.2. Water quality

The pH of all samples was almost always slightly alkaline (Figure 5.3a). The reservoir had the highest mean pH of all sites during the sampling period, at 7.82 ± 0.04 , and Minor 3 had the lowest at 7.31 ± 0.04 (Figure 5.3b). There is evidence of some seasonal variation in pH; with the values

declining by approximately half a unit from autumn 2014 to spring 2015 across most sites. When comparing the influence of the constructed wetland, the outflow (7.41 ± 0.03) was slightly lower than the inflow (7.45 ± 0.04), but the difference was not significant ($p > 0.05$.) Main 1 had a significantly lower pH than Main 2 and both had a significantly lower pH than the Reservoir (Table 5.3). The highest mean conductivity was recorded for Main 2, at $358 \pm 9.41 \mu\text{S}/\text{cm}$ and the lowest was for Main 1, at $219 \pm 7.56 \mu\text{S}/\text{cm}$ (Figure 5.4b). The conductivity was generally stable throughout the sampling period apart from some very low values on 24th October 2014 for all sites (Figure 5.4a). For the constructed wetland, the outflow was slightly lower than the inflow, at $215.7 \pm 7.04 \mu\text{S}/\text{cm}$ and $219.5 \pm 8.92 \mu\text{S}/\text{cm}$ respectively, a difference that was significant ($p < 0.01$; Table 5.3). The large difference between the mean conductivity of Main 1 and Main 2 were significantly different, whilst the conductivity of the reservoir was midway between both.

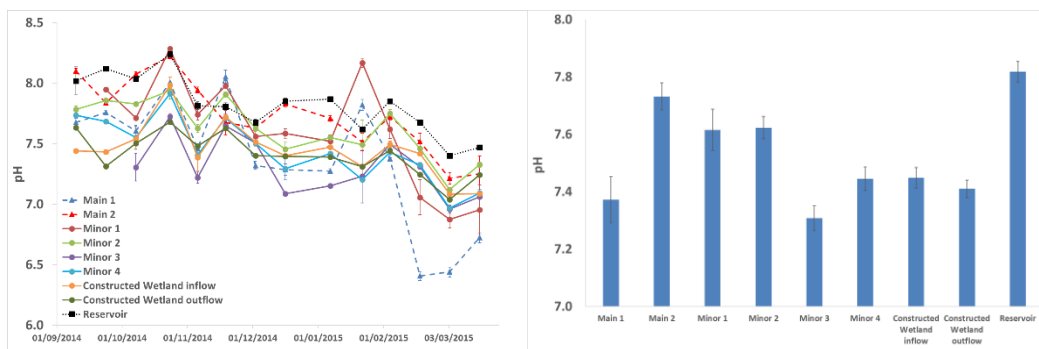


Figure 5.3 a) time series for pH ($\pm\text{SEM}$) and b) mean pH ($\pm\text{SEM}$) of all study sites for the period September 2014 to March 2015.

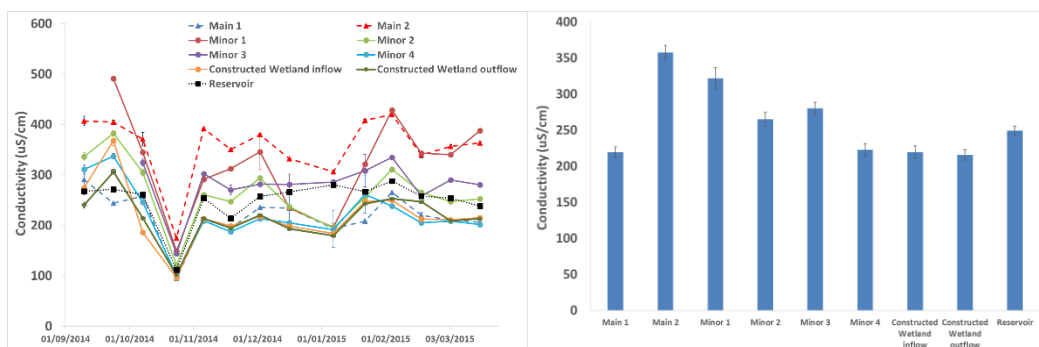


Figure 5.4 a) time series for Conductivity ($\pm\text{SEM}$) and b) mean Conductivity ($\pm\text{SEM}$) of all study sites for the period September 2014 to March 2015

	Main 1 v Main 2	Constructed Wetland Inflow v Outflow	Main 1 v Reservoir	Main 2 v Reservoir
pH	<0.001		<0.001	0.001
Conductivity	<0.001	0.006	<0.001	<0.001
Phenolic	<0.001		0.017	0.048
DOC		<0.001	<0.001	<0.001
Nitrate	<0.001		<0.001	<0.001
Phosphate				
Bromide	0.002			
CHCl₃				
CHCl₂Br			0.026	
CHClBr₂				

Table 5.3. Results of Tukey HSD post-hoc tests for each level of the Site factor

Minor 1 had the highest mean DOC concentration of all sites, at 16.60 ± 1.26 mg/L and Minor 3 had the lowest, at 5.31 ± 0.51 mg/L (Figure 5.5b). There is some evidence of a seasonal variation in DOC, with generally higher concentrations in the autumn of 2014 compared to the spring of 2015 and particularly high concentrations on 10th September 2014 and 7th January 2015 (Figure 5.5a). The constructed wetland outflow (12.21 ± 0.73 mg/L) had a significantly lower DOC concentration than the inflow (14.90 ± 1.05 mg/L) ($p < 0.001$). Main 1 and Main 2 had similar mean concentrations that were not significantly different and both were significantly lower than the Reservoir (Table 5.3).

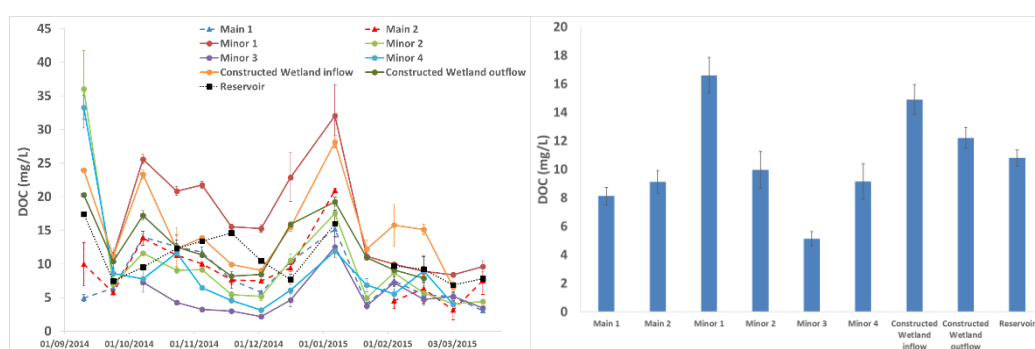


Figure 5.5 a) time series for DOC (\pm SEM) and b) mean DOC (\pm SEM) of all study sites for the period September 2014 to March 2015.

The trend in the phenolics data was generally similar to DOC, indicating that phenolic compounds comprise a significant component of DOC (Figure 5.6a). This is confirmed by a significant positive relationship between DOC and phenolics for the data from all sites combined ($R=0.75$, $p < 0.01$; Table 5.4). Minor 1 had the highest mean phenolics concentration of all sites, at 1.13 ± 0.07 mg/L and Minor 3 had the lowest, at 0.37 ± 0.03 mg/L (Figure 5.6b).

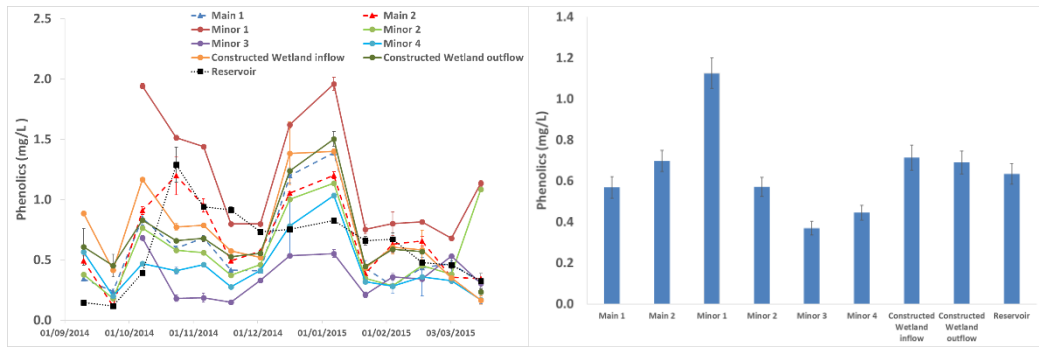


Figure 5.6 a) time series for phenolics (\pm SEM) and b) mean phenolics (\pm SEM) of all study sites for the period September 2014 to March 2015

Correlations																		
		conductivity	DOC	phenolics	Nitrate	phosphate	Bromide	Nitrite	ImprGrass	RoughGrass	AraHor	Wood	Fen	Others	CHCL3	CHCL2Br	CHCLBr2	TTHMs
pH	Pearson Correlation	.437	.326	.408	-.602	-.552	-.486	.433	-.637	.264	.413	.542	.576	.469	-.845	-.960	-.985	-.853
	Sig. (2-tailed)	.279	.430	.316	.115	.156	.222	.284	.090	.527	.309	.165	.135	.241	.155	.040	.015	.147
conductivity	Pearson Correlation		.073	.447	.063	.116	.143	-.351	-.322	-.052	.036	.273	.719	.016	-.079	-.380	-.612	-.094
	Sig. (2-tailed)		.863	.266	.882	.785	.735	.394	.436	.903	.932	.514	.044	.971	.921	.620	.388	.906
DOC	Pearson Correlation			.868**	-.704	.192	-.046	.041	.284	-.247	-.140	-.256	-.142	-.418	.052	.215	.013	.061
	Sig. (2-tailed)			.005	.051	.649	.915	.923	.496	.555	.740	.540	.738	.302	.948	.785	.987	.939
phenolics	Pearson Correlation				-.606	.248	.077	.015	.183	-.212	-.176	-.256	.100	-.277	.056	-.033	-.425	.052
	Sig. (2-tailed)				.112	.554	.856	.972	.664	.614	.677	.541	.814	.507	.944	.967	.575	.948
Nitrate	Pearson Correlation					.499	.505	-.571	.267	-.115	-.386	-.246	-.048	.109	.427	.124	-.113	.412
	Sig. (2-tailed)					.208	.202	.139	.522	.786	.345	.558	.910	.798	.573	.876	.887	.588
phosphate	Pearson Correlation						.780	-.731	.759	-.479	-.588	-.819	-.403	-.391	.533	.701	.537	.543
	Sig. (2-tailed)						.023	.039	.029	.230	.125	.013	.323	.339	.467	.299	.463	.457
Bromide	Pearson Correlation							-.554	.708	-.819	-.279	-.511	-.532	-.339	.349	.600	.833	.362
	Sig. (2-tailed)							.154	.049	.013	.504	.196	.175	.412	.651	.400	.167	.638
Nitrite	Pearson Correlation								-.262	.306	.132	.296	.003	.524	-.614	-.524	-.128	-.611
	Sig. (2-tailed)								.531	.460	.755	.477	.994	.183	.386	.476	.872	.389
ImprGrass	Pearson Correlation									-.676	-.685	-.770	-.767	-.424	.287	.569	.666	.302
	Sig. (2-tailed)									.066	.061	.025	.026	.295	.713	.431	.334	.698
RoughGrass	Pearson Correlation										.128	.197	.578	.519	-.029	-.290	-.255	-.043
	Sig. (2-tailed)										.763	.639	.133	.188	.971	.710	.745	.957
AraHor	Pearson Correlation											.638	.160	.008	-.229	-.462	-.371	-.241
	Sig. (2-tailed)											.089	.705	.986	.771	.538	.629	.759
Wood	Pearson Correlation												.580	.167	-.272	-.557	-.718	-.287
	Sig. (2-tailed)												.132	.692	.728	.443	.282	.713
Fen	Pearson Correlation													.339	-.121	-.419	-.642	-.136
	Sig. (2-tailed)													.411	.879	.581	.358	.864
Others	Pearson Correlation														-.898	-.904	-.654	-.901
	Sig. (2-tailed)														.102	.096	.346	.099

Table 5.4. Results of Pearson correlation analysis run on all dependent variables.

For the constructed wetland, the mean phenolics concentration of the outflow (0.69 ± 0.06 mg/L) was slightly lower than the inflow (0.71 ± 0.06 mg/L), but the difference was not significant ($p > 0.05$). Main 2 had a significantly higher concentration than Main 1 (Table 5.3).

Minor 3 had by far the highest nitrate concentration, averaging 29.51 ± 0.53 mg/L and Minor 1 had the lowest, at 3.01 ± 0.28 mg/L (Figure 5.7b). There is a clear seasonal trend in the data, with lower concentrations in autumn 2014 (when the concentration in the reservoir was below the limit of detection) and rising throughout winter and spring (Figure 5.7a). The outflow of the constructed wetland had a mean nitrate concentration of 5.06 ± 0.55 mg/L, slightly lower than the inflow concentration of 5.95 ± 0.70 mg/L but this was not significant (Table 5.3). Main 2 had a significantly higher nitrate concentration than Main 1 and both had a significantly higher concentration than the reservoir (Table 5.3).

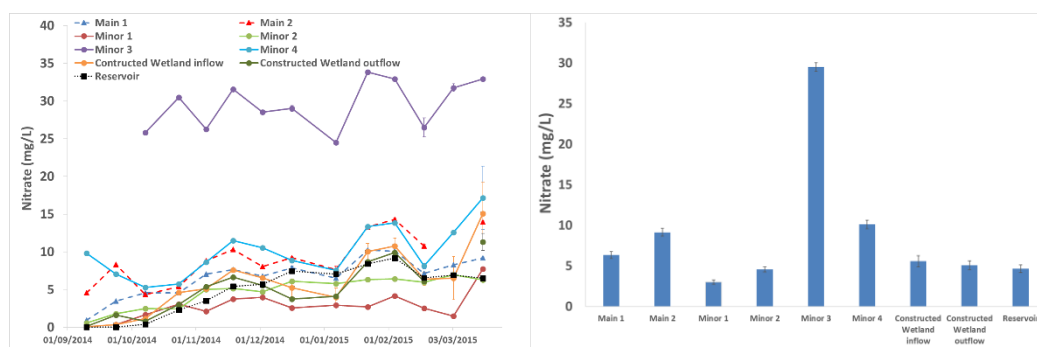


Figure 5.7 a) time series for nitrate (\pm SEM) and b) mean nitrate (\pm SEM) of all study sites for the period September 2014 to March 2015.

Minor 3 also had the highest mean phosphate concentration of all sites, at 0.83 ± 0.29 mg/L, with Minor 4 having the lowest at 0.06 ± 0.02 mg/L (Figure 5.8b). There was no consistent seasonal variation in phosphate concentrations, with high concentrations occurring sporadically for most streams (Figure 5.8a). For the constructed wetland, the outflow (0.41 ± 0.21 mg/L) had a slightly lower concentration than the inflow (0.54 ± 0.27), but the difference was not significant. There were also no significant differences between Main 1, Main 2 and the Reservoir (Table 5.3).

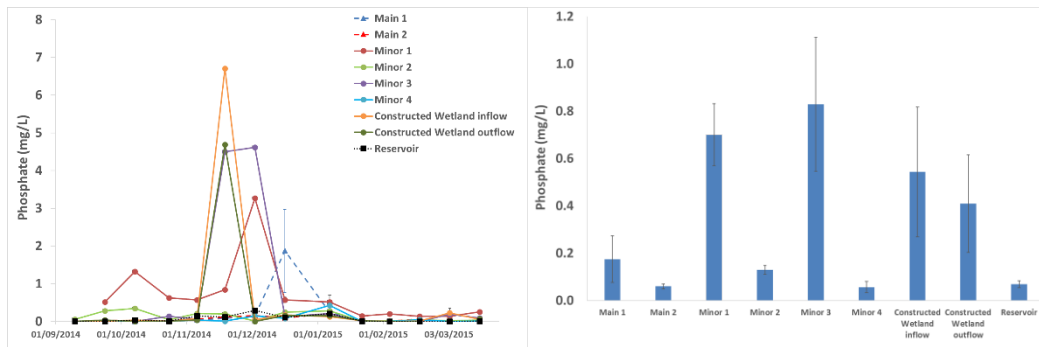


Figure 5.8 a) time series for phosphate ($\pm SEM$) and b) mean phosphate ($\pm SEM$) of all study sites for the period September 2014 to March 2015.

Minor 3 also had the highest mean bromide concentration, at 0.16 ± 0.00 mg/L and Main 2 had the lowest, at 0.09 ± 0.01 mg/L (Figure 5.9b). There was no clear seasonal variation in bromide concentration across all sites (Figure 5.9a). For the constructed wetland there was a small but significant increase in bromide concentration from 0.10 ± 0.01 mg/L in the inflow to 0.11 ± 0.01 in the outflow, but this was not significant ($p > 0.05$; Table 5.3).

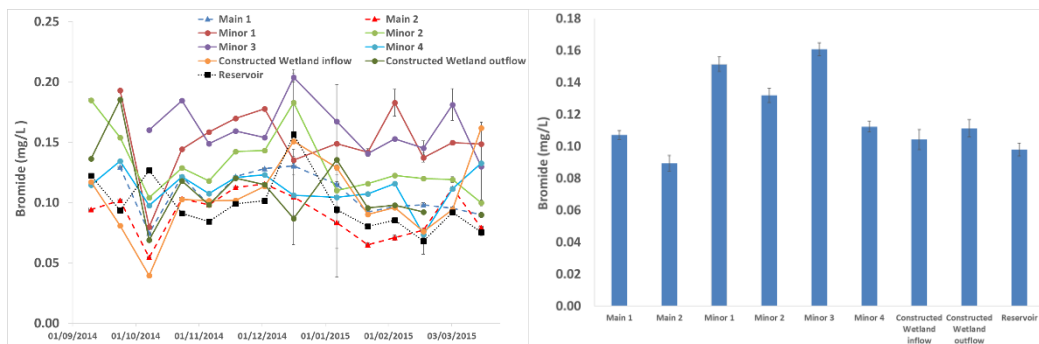


Figure 5.9a) time series for bromide ($\pm SEM$) and b) mean bromide ($\pm SEM$) of all study sites for the period September 2014 to March 2015.

The THMFP analysis generated chloroform as the dominant trihalomethane species; with much lower concentrations of dichlorobromomethane and dibromochloromethane (bromoform was not detected). There were only minor differences between the five sampling sites for all three THM species and no evidence of seasonality. For chloroform, there were no significant differences between any of the sites (Figure 5.10a). For the constructed wetland, the mean chloroform concentration of the inflow was 577.2 ± 37.0 $\mu\text{g/L}$ and the outflow was $580.5 \mu\text{g/L} \pm 36.8 \mu\text{g/L}$ (Figure 5.10b). For dichlorobromomethane, Main 1 had the highest mean concentration of all sites at 26.45 ± 2.6 $\mu\text{g/L}$ (Figure 5.11b) and the reservoir had the lowest at 19.53 ± 3.0 $\mu\text{g/L}$. The constructed wetland inflow, at 19.9 ± 2.02 $\mu\text{g/L}$, was lower than the outflow, at 25.25 ± 2.8 , but the difference was not significant ($p > 0.05$) (Figure 5.11a). The trend for dibromochloromethane was very similar. Main 1 had the highest mean concentration, at 0.33 ± 0.04 $\mu\text{g/L}$, and the reservoir had the lowest, at $0.24 \pm$

0.04 $\mu\text{g/L}$ (Figure 5.12b). For the constructed wetland, the outflow was higher than the inflow, at $0.29 \pm 0.04 \mu\text{g/L}$ and $0.23 \pm 0.02 \mu\text{g/L}$ respectively, but this difference was not significant ($p > 0.05$).

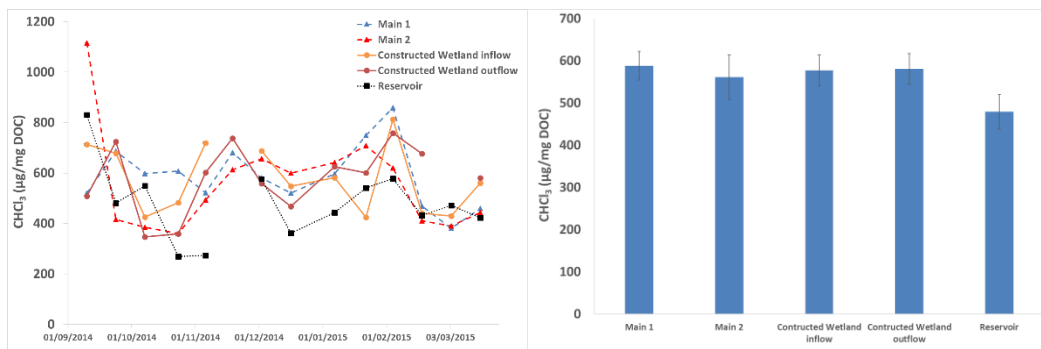


Figure 5.10a) time series for Chloroform ($\pm\text{SEM}$) and b) mean Chloroform ($\pm\text{SEM}$) of all study sites for the period September 2014 to March 2015

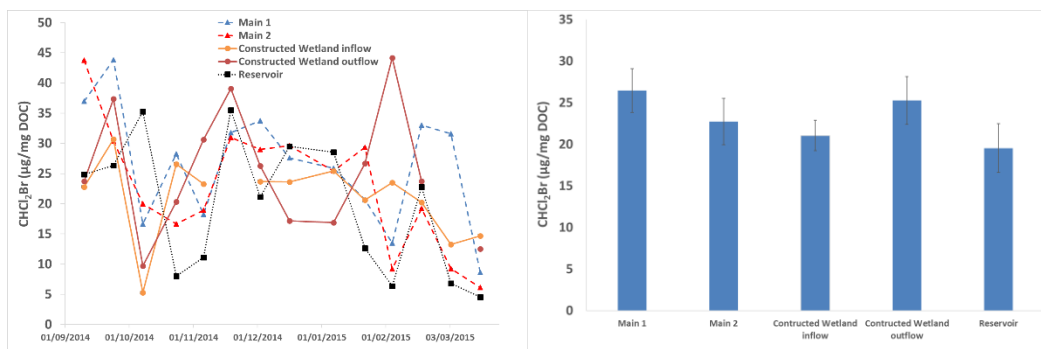


Figure 5.11a) time series for dichlorobromomethane ($\pm\text{SEM}$) and b) mean dichlorobromomethane ($\pm\text{SEM}$) of all study sites for the period September 2014 to March 2015

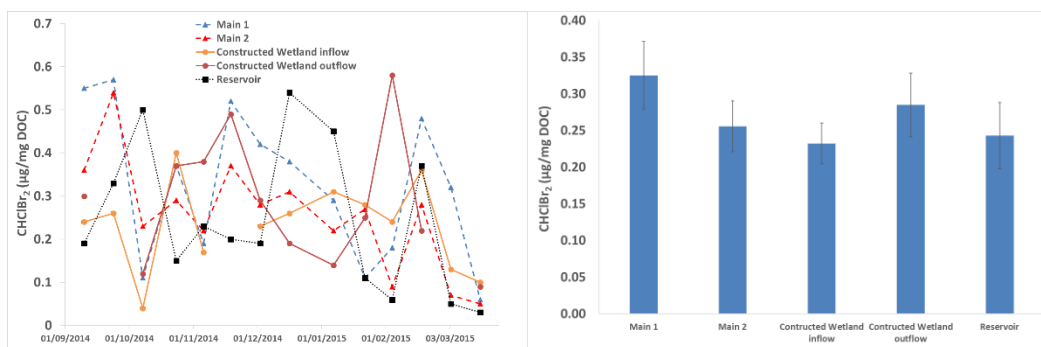


Figure 5.12a) time series for dibromochloromethane ($\pm\text{SEM}$) and b) mean dibromochloromethane ($\pm\text{SEM}$) of all study sites for the period September 2014 to March 2015.

5.5. Discussion

The pH of all streams was relatively constant, ranging from approximately 7.3 to 7.8, and are slightly basic because the sites are lowland and influenced by groundwater. The reservoir outlet had the highest pH; this is because of the eutrophic conditions leading to extensive algae which generate alkalinity and increase the pH of water (Scholz et al., 2016). DOC concentrations in the two main inflow streams were similar but the concentration in the reservoir was higher, therefore, DOC is being produced within the lake by phytoplankton and algal growth and senescence (Wetzel 2001). Rapid autochthonous production of DOC can occur within freshwaters as a result of eutrophication (Pierson-Wickmann et al., 2011).

For the constructed wetland, in contrast to the 12% increase in DOC reported at this site by Scholz et al. (2016), DOC decreased significantly from inlet to outlet by 18%. Furthermore, nitrate and phosphate only reduced by 9% and 21% respectively. These decreases were not significant and much lower than the 72% and 53% reductions in the concentration of these two key nutrients recorded by Scholz et al. (2016). The different removal rates can be attributed to the contrasting times of year in which the two studies were undertaken. This study mainly involved sampling in the late autumn/winter/early spring period, when primary production and vegetation biomass is much lower than during the summer. Nitrate and phosphate reductions within a constructed wetland system are partly due to plant uptake and this mechanism will be much reduced outside of the growing season. Reduced activity of plants would also explain why DOC did not increase through the constructed wetland in this study, as Scholz et al. (2016) linked the increase they recorded with greater inputs from plants.

Although DOC concentration decreased, all five studied sites had very similar THMFP yields, with no significant differences recorded. This suggests that there were only minor changes in DOC quality or that any variability in DOC quality did not impact on how the DOC reacts with chlorine.

5.6. Conclusion

A eutrophic reservoir used for drinking water, its inflowing streams and a constructed wetland within the catchment were monitored for parameters including DOC, nutrients and THMFP. The main conclusion from the study is that there was no difference in the THMFP of the streams and no impact on THMFP of the constructed wetland. This indicates that there were no differences in DOC characteristic between streams in terms of reactivity with chlorine. However, there were large differences in DOC concentration between the streams and the constructed wetland, which reduced DOC concentration by 18% on average. The constructed wetland was less efficient at

sequestering nutrients than in the summer, highlighting the role of plants in a constructed wetland. It can be concluded that in the wintertime the constructed wetland is having a positive impact on water quality of the reservoir because it is still sequestering some nitrate and phosphate, but also reducing the DOC concentration of the treated inflow. Reducing the amount of nutrients going to the lake will lessen the algal growth in the lake and therefore decrease the amount of DOC being formed within the lake.

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Chapter 6: Simultaneous analysis of water quality and temperature on the stability of trihalomethanes in water samples during prolonged storage

6.1. Abstract

The presence of natural dissolved organic carbon (DOC) in drinking water supplies can cause a number of issues during water treatment due to its effect on aesthetics such as taste and odour and its reaction with chlorine, resulting in the formation of disinfection by-products (DBPs). These compounds include haloacetic acids (HAAs) and the four trihalomethanes (THMs), trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane. THM compounds have carcinogenic properties and can potentially cause damage to the reproductive system. DBP formation is a complex process and depends on a number of factors such as DOC concentration, chlorine dose, water temperature, reaction time and pH. The two DOC reactants (humic and fulvic acid) are the principal THM precursors. In the UK DOC concentrations have increased by 91% in freshwater rivers and lakes during the last 15 years partly due to the destabilisation of peatland soils resulting from climate change. This rising trend represents a challenge for water companies who need to minimise the formation of harmful DBP compounds. This project investigated the effect of THMs storage at 4°C and room temperature on the formation of THMs for selected surface waters (Nant-y-Brwyn stream and Cefni reservoir) in north Wales. Quantitative analysis of THMs (CHCl_3 , CHCl_2Br , CHClBr_2 and CHBr_3) was carried out using a solid phase micro extraction (SPME) technique.

6.2. Introduction

During conventional water treatment processes, a disinfectant, usually chlorine, is added to reduce microbial contamination and the occurrence of waterborne diseases. Before widespread disinfection of potable water supplies was undertaken outbreaks of diseases such as typhoid and cholera were common throughout the world (USEPA, 1998). In developed areas of the world where water treatment is undertaken at an industrial scale the occurrence of such water-borne diseases has virtually ceased (Richardson, 2003), but contaminated water is still a big issue in the developing world. In chlorinated drinking water supplies, other compounds do exist which are a threat to human health. When water containing natural organic matter (NOM) comes into contact with chlorine a suite of halogenated disinfection by-products (DBPs) are formed, some of which are known to be carcinogenic (Jimenez *et al.*, 1993; Sketchell *et al.*, 1995; Palacios *et al.*, 2000). DBPs are organo-halide compounds and the most common are the trihalomethanes (THMs), which are simple derivatives of methane formed when three of the hydrogen atoms are replaced by a halogen (Peterson *et al.*, 1993). DBP concentrations in final, treated water depend on several factors, such as the concentration and characteristics of NOM, chlorine dosing amount, water temperature and pH (Rodrigues *et al.*, 2007). The humic and fulvic acids component of NOM is considered to be the principal type of precursor to DBP formation (Bond *et al.*, 2009). DBPs form either by the oxidation of carbon-carbon double bonds or by substitution, where a functional group is replaced by a halogen (Westerhoff *et al.*, 2004). Higher temperatures lead to higher DBP yields, so the concentration of DBPs formed at water treatment works in summer are usually higher than in winter (Williams *et al.*, 1997). Derivatives of chloro-bromo DBPs are formed when the bromide ion is present e.g. bromoform (Pourmoghaddas and Stevens, 1995). The four main THM species are trichloromethane/chloroform (CHCl_3), bromodichloromethane (CHCl_2Br), dibromochloromethane (CHClBr_2) and tribromomethane/bromoform (CHBr_3), with chloroform generally being the dominant THM in most chlorinated water supplies (Sketchell *et al.*, 1995). In addition, pH influences the species of THMs formed; at pH 4–6 CHCl_3 tends to dominate, but when the pH increases then relatively more brominated THMs are produced, assuming a sufficient supply of bromide (Italia and Uden, 1992). Regulatory bodies have set limits for final water THM concentrations because of the potential effects on human health. In the UK, the safe limit for total THM concentration is 100 $\mu\text{g/L}$, whilst the Environmental Protection Agency in the United States regulates the sum of the four THMs to a limit of 80 $\mu\text{g/L}$ and also regulates another class of DBPs, haloacetic acids (HAAs) (Goslan *et al.*, 2009). THM formation is minimised in chlorinated water supplies through water companies removing as much DOC as possible prior to disinfection, using techniques such as coagulation and filtration. DOC removal is the costliest

stage of water treatment, but it is impossible to remove all DOC, therefore some THM formation will always occur. The standard method for THM analysis is the US EPA, Method 524.2 (Measurement of purgeable organic compounds in water by capillary column gas chromatography/mass spectrometry) and states that samples for VOC analysis, including THMs, must be stored in the dark at 4°C and analyzed within 14 days of collection (Eichelberger *et al.*, 1998), although the reference does not state why. However, Pepich *et al.* (2004) found that HAAs, which are similar to THMs, will remain stable for at least 28 days. However, we are not aware of any studies that have assessed the effects of temperature and water quality on THM stability.

6.3. Methods

6.3.1. Study site and sampling

Water samples were taken from two contrasting sites in north Wales on 4th February 2016 (Table 6.1). They were an oligotrophic, upland stream that drains predominantly blanket bog (the Migneint-Arenig-Dduallt Special Area of Conservation); the Nant-y-Brwyn stream (UK grid ref SH 79147 45267) and the outflow of a shallow, lowland, eutrophic lake, Llyn Cefni, which receives water that drains agricultural land (UK grid ref SH 44611 77150). One litre of sample was collected in a glass amber bottle at both sites.

6.3.2. Laboratory analyses

pH and conductivity were measured in the laboratory on unfiltered samples on the day of sample collection. pH was measured using a SevenEasy pH meter (Mettler Toledo, Leicester, UK), which was calibrated using buffers of pH 4 and 7 at 25°C (Sigma). Conductivity was measured with a Primo 5 conductivity meter (Hanna Instruments, Bedfordshire, UK). Both samples were then filtered through 0.45 µm cellulose acetate filters, acidified to pH 2.5 and refrigerated at 4°C until further analysis was undertaken.

6.3.3. Trihalomethane formation potential (THMFP)

The samples were chlorinated using a modification of the standard THM formation potential (THMFP) test. Samples were diluted so they contained final concentrations of 1 mg/L DOC after adding the appropriate volumes of chlorine dosing solution (5 mL/L of 0.28 mM sodium hypochlorite) and buffer (0.5 M monopotassium phosphate) added to generate THMs under standard conditions. The dose of sodium hypochlorite was sufficient to provide 5 mg of free Cl per mg of DOC. The samples were then incubated at 25°C in the dark for 7 days and quenched with 0.4 mL of 0.8 M sodium

sulphite to prevent continued THM formation. Each of the 1 L samples were then divided into 66 separate aliquots by pouring 15 mL of the sample into a 22 mL amber headspace vial. Half of the samples for each site were then stored in a refrigerator at 4°C and the other half in an oven at 25°C. At time zero, three vials were removed for each site and temperature conditions (12 samples in total) and analysed for their THM concentration. This process was repeated every week for four weeks and thereafter every two weeks until 11 weeks had passed. The concentration of THMs was measured using a solid phase micro-extraction (SPME) technique similar to that described by (Sarrión *et al.*, 2000). The technique uses a Varian GC 450, a Restek MX-1 column (diphenyl/dimethyl polysiloxane phase, 30 m length, 0.53 mm i.d.), N₂ carrier gas at a flow rate of 10 mL/min and a ⁶³Ni Electron Capture Detector. The oven temperature was held at 35 °C for 9 minutes and then increased at a rate of 10 °C /minute to 140 °C and held for 2 minutes and finally increased to 180 °C and held for 3 minutes. The injector temperature was 290 °C and the detector temperature 300 °C. Trihalomethane calibration solutions were made from a stock solution containing 2000 µg/mL of each of the 4 THM species; chloroform, bromodichloromethane, dibromochloromethane and bromoform (Sigma). A series of standard solutions were then prepared ranging from 1-500 µg/L.

6.3.4. Statistical Analysis

The effect of water type (two levels; oligotrophic and eutrophic) and temperature (two levels; 4 °C and 25 °C) on each measured parameter were determined using two-way ANOVA and Tukey HSD post-hoc tests in SPSS v22 (IBM Statistics). Most data met the homogeneity and normality assumptions, which tested using the Bartlett and Shapiro Wilk tests, but those that did not were log-transformed. A p value of 0.05 used to determine significance.

6.4. Results

The oligotrophic water had a lower pH, conductivity and DOC concentration compared to the eutrophic water (Table 6.1). The greater SUVA value of the oligotrophic water indicates that the DOC is comprised of proportionally more recalcitrant, aromatic and higher molecular weight moieties than the eutrophic water. Mean concentrations of chloroform and dichlorobromomethane for each site and temperature following the THMFP treatment procedure on each sample and 11 weeks of storage are presented in Table 6.2 and statistical analysis in Table 6.3.

	Oligotrophic	Eutrophic
pH	4.57	7.01
Conductivity ($\mu\text{S}/\text{cm}$)	39	253
DOC (mg/L)	4.55	19.3
SUVA (L-mg/m)	4.06	3.17

Table 6.1. Water quality of sampling sites.

Trihalomethane species	Temperature ($^{\circ}\text{C}$)	Oligotrophic	Eutrophic
Chloroform ($\mu\text{g}/\text{L}$)	4	467.8 (± 56.93)	212.5 (± 20.39)
	25	474.3 (± 44.63)	204.6 (± 18.63)
Dichlorobromomethane ($\mu\text{g}/\text{L}$)	4	9.15 (± 2.36)	7.32 (± 2.35)
	25	8.89 (± 2.59)	6.34 (± 1.64)

Table 6.2. Average THM concentration over 11 weeks of sample storage for two contrasting water types and at two storage temperatures.

		CHCl_3	CHCl_2Br
Temperature	F value	0.009	1.82
	P value	0.925	0.180
Site	F value	1103	22.4
	P value	0.000	0.000
Temperature x Site	F value	0.820	0.600
	P value	0.368	0.441

Table 6.3. Results of one-way ANOVA analysis two test effect of sites on measured parameters

The mean concentration of chloroform for the oligotrophic site samples was more than twice as high as the eutrophic site samples, with a highly significant difference for the Site factor ($F=1103.6$; $p<0.000$) but there was no significant temperature effect ($F=0.009$, $p>0.05$) and no significant interaction effect ($F=0.820$, $p>0.05$). For dichlorobromoform, the mean concentrations were more similar between the two sites but there was still a highly significant effect of Site ($F=22.4$, $p<0.000$), with the oligotrophic site having approximately 32% higher mean dichlorobromoform formation. There was also no significant temperature effect ($F=1.82$, $p>0.05$) or interaction effect ($F=0.600$, $p>0.05$) for this THM species.

The general trend over time for chloroform for the oligotrophic water was for concentrations to increase during the first three weeks of sample storage but to then decrease to week 0 levels (not statistically different) for the next few sample weeks (Figure 6.1). For the eutrophic water, concentrations showed much less variation over the 11 weeks of storage.

For the oligotrophic water samples at 4°C , the chloroform concentration was $436.4 \pm 17.37 \mu\text{g}/\text{L}$ at week 0 and $419.6 \pm 31.74 \mu\text{g}/\text{L}$ after 11 weeks of storage, a difference of just $16.8 \mu\text{g}/\text{L}$ that was not significantly different ($p>0.05$). The chloroform concentration was significantly higher than the week 0 concentration on three occasions; week 1 ($504.7 \pm 29.45 \mu\text{g}/\text{L}$; $p<0.05$), week 2 ($548.9 \pm 8.9 \mu\text{g}/\text{L}$; $p<0.001$) and week 3 ($532.3 \pm 29.74 \mu\text{g}/\text{L}$; $p<0.01$). Differences between weeks 5, 7 and 9 compared

to week 0 were not significant ($p>0.05$). The trend for the 25 °C treatment was very similar to the 4 °C treatment, but only the week 2 and week 3 samples were significantly different to week 0 ($445.9 \pm 35.8 \mu\text{g/L}$ for week 0 vs. $522.31 \pm 7.17 \mu\text{g/L}$ for week 2 and $533.6 \pm 20.8 \mu\text{g/L}$ for week 3; both $p<0.05$). The week 11 CHCl_3 concentration was $439.5 \pm 18.8 \mu\text{g/L}$, a difference of just $6.4 \mu\text{g/L}$ from week 0.

For the eutrophic water treated samples, there was minimal variation of chloroform concentration over the 11 weeks of storage for either temperature. At 4°C, the week 0 chloroform concentration was $192.9 \pm 7.5 \mu\text{g/L}$ and at week 11 was $207.7 \pm 17.5 \mu\text{g/L}$, a difference of just $14.8 \mu\text{g/L}$, which was not significant ($p<0.05$). There were no significant differences between week 0 and any of the time points beyond week 0 for the 4 °C treatment. For the 25°C treatment, there were three occasions when the chloroform concentration was significantly higher than the week 0 concentration of $173.2 \pm 15.6 \mu\text{g/L}$; $p<0.05$; week 2 ($213.1 \pm 10.7 \mu\text{g/L}$; $p<0.05$), week 3 ($220.4 \pm 11.1 \mu\text{g/L}$; $p<0.01$) and week 7 ($222.2 \pm 19.8 \mu\text{g/L}$; $p<0.01$). At week 11 the concentration was $199.1 \pm 8.5 \mu\text{g/L}$, a difference of just $25.9 \mu\text{g/L}$ which is not significant ($p>0.05$).

For dichlorobromomethane, concentrations generally increased over the 11 week storage period for all four site/temperature combinations, especially over the initial 7 weeks. For the oligotrophic water at 4°C, the concentration was $6.02 \pm 0.97 \mu\text{g/L}$ at week 0 and $10.07 \pm 2.89 \mu\text{g/L}$ after 11 weeks of storage, a difference of $4.05 \mu\text{g/L}$, but this was not significantly different ($p>0.05$). The dichlorobromomethane concentration was significantly higher than the week 0 concentration on two occasions; week 7 ($12.67 \pm 0.62 \mu\text{g/L}$; $p<0.001$), and week 9 ($10.96 \pm 2.89 \mu\text{g/L}$; $p<0.05$). The trend for the 25°C treatment was reasonably similar to the 4 °C treatment, but only the week 7 samples were significantly different to week 0 ($5.99 \pm 1.54 \mu\text{g/L}$ for week 0 vs. $13.0 \pm 3.47 \mu\text{g/L}$ for week 7; $p<0.01$). The week 11 dichlorobromomethane concentration was $8.07 \pm 1.73 \mu\text{g/L}$, a difference of just $2.08 \mu\text{g/L}$ from week 0, which was not significant ($p>0.05$).

For the eutrophic water at 4°C, there were three occasions when the dichlorobromomethane concentration was significantly higher than the week 0 concentration of $5.00 \pm 0.39 \mu\text{g/L}$; week 7 ($10.17 \pm 1.03 \mu\text{g/L}$; $p<0.01$), week 9 ($8.67 \pm 1.24 \mu\text{g/L}$; $p<0.05$) and week 11 ($10.7 \pm 1.79 \mu\text{g/L}$; $p<0.001$). For the 25°C treatment, there were two occasions when the dichlorobromomethane concentration was significantly higher than the week 0 concentration of $4.33 \pm 0.56 \mu\text{g/L}$; week 7 ($9.23 \pm 2.08 \mu\text{g/L}$; $p<0.001$) and week 9 ($7.14 \pm 0.44 \mu\text{g/L}$; $p<0.05$). At week 11 the concentration was $6.98 \pm 0.95 \mu\text{g/L}$, a difference of $5.8 \mu\text{g/L}$ from week 0, but this was not significant ($p>0.05$).

6.5. Discussion

The current study was designed to assess long term stability of THM compounds in water samples and to test for differences in storage temperature (4°C vs. 25°C) and water type (nutrient poor (oligotrophic) vs. nutrient rich (eutrophic)). The preservation of organic compounds in water samples is a complex issue due to the potential for microbiological degradation. For THMs, if samples cannot be analysed for some time following the 7 day incubation period it is important to quench samples to inhibit further THM development and because THMs are reasonably volatile it is important to consider how the samples are stored.

6.5.1. Storage effects

Over an 11 week period the majority of the weekly measurements of the concentrations of key DBPs showed no statistical differences from the week 0 values, regardless of water source types or storage temperatures (Figures 6.1 & 6.2). However, there are statistically significant higher concentrations of both chloroform and dichlorobromomethane for some weeks prior to week 11, especially weeks 1, 2 and 3 for chloroform for the oligotrophic site. This suggests that there are aspects of the chemistry of these samples post quenching that is not well understood. From an analytical perspective these results suggest that to get reliable data either perform the concentration analysis immediately following the standard 7 day incubation period and quenching or store them for between 4 – 11 weeks.

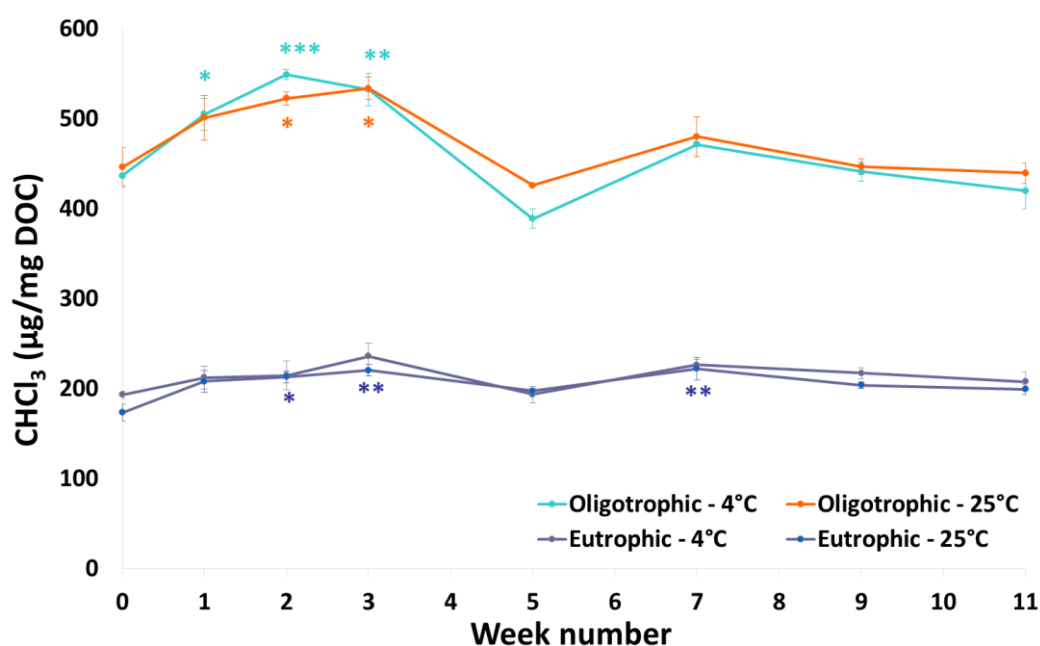


Figure 6.1. Chloroform concentrations following THMFP procedure during sample storage for two contrasting water types stored at two different temperatures for a total period of 11 weeks.

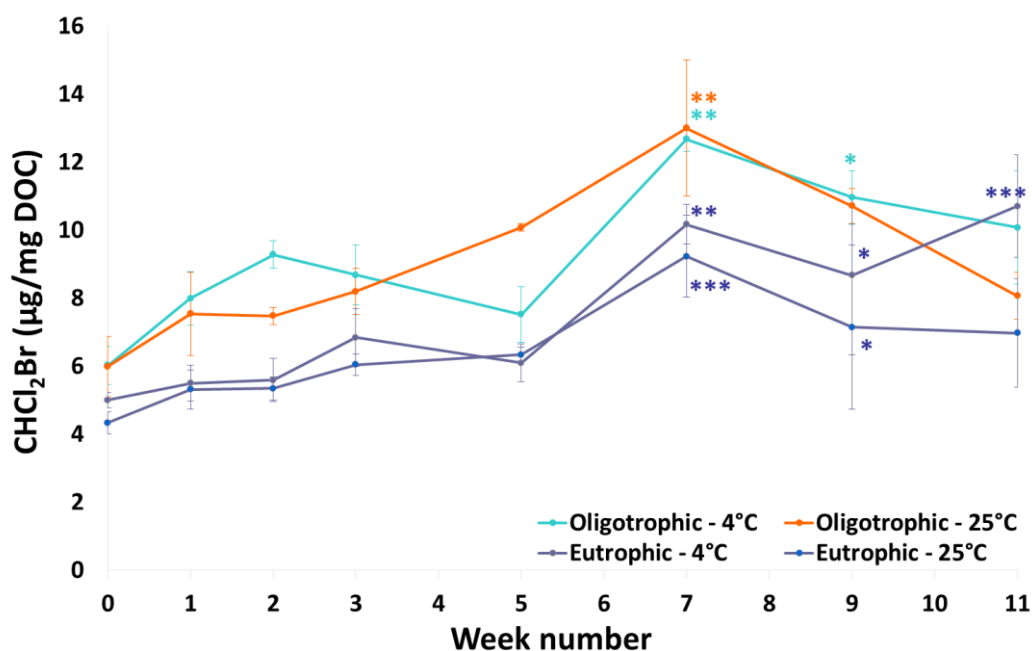


Figure 6.2. Dichlorobromomethane concentrations following THMFP procedure during sample storage for two contrasting water types stored at two different temperatures for a total period of 11 weeks.

6.5.2. Oligotrophic v Eutrophic THMFP

The formation of chloroform and dichlorobromomethane was significantly greater for the oligotrophic water compared to the eutrophic water. This result was also reported by Scholz *et al.* (2016). The catchment of the sampled oligotrophic river is dominated by blanket bog peat, which is rich in organic carbon. Freshwaters that drain these areas tend to be high in DOC and the DOC relatively high molecular weight and high in aromatics. The eutrophic site is high in nutrients due to intensive farming practises within the catchment, occasionally leading to algal blooms within the reservoir and the generation of low molecular weight DOC. The differences in SUVA (an indication of DOC molecular weight/aromaticity) between the two sites can at least partly explain their differences in terms of THMFP. Related studies show that generally SUVA was lower for the eutrophic water (3.13 ± 0.04) (Scholz *et al.*, 2016), suggesting a less aromatic DOC than the oligotrophic water (4.06 ± 0.37) (Chapter 2). The oligotrophic DOC would have a higher proportion of humic compounds, which have a higher surface charge, compared to the more fulvic rich eutrophic water DOC (Sharp *et al.*, 2006). However, studies demonstrate that THMs are also formed by non-aromatic molecular structures (Weishaar *et al.*, 2003; Qunsham *et al.*, 2008; Fram *et al.*, 1999).

High SUVA (more than 4 L/mg/m) indicates the presence of humic matter due to the aromatic and hydrophobic compounds leading to high THMFP, while SUVA between 2 to 4 L/mg/m indicates the presence of a mixture of humic and non-humic matter and a mixture of hydrophobic and hydrophilic material, leading to relatively lower THMFP. By contrast, SUVA less than 2 indicates the presence of a high fraction of non-humic matter of hydrophilic character with low UVA, leading to the lowest THMFP (PEA 2012).

There have been a number of studies that have examined the correlation between SUVA and formation of different types of DBP (Goslan *et al.*, 2002). When studying samples from the same water type there is generally quite a strong correlation between SUVA and the various DBP types (Hua *et al.*, 2015). However a number of studies have provided evidence that this is not the case when a variety of water sample are tested. Different species of DBPs have been found to exhibit different relationships with SUVA (Hua *et al.*, 2015). For instance, a strong positive relationship between SUVA and trihaloacetic acids ($R^2 = 0.80$) was found, and between SUVA and total organic halogens ($R^2 = 0.75$). However, weaker correlations ($R^2 = 0.50$) were found between SUVA and trihalomethanes, and between SUVA and dihaloacetic acid ($R^2 = 0.19$), during chlorination. This work shows that, overall, SUVA is a good indicator for unknown total organic halogens, however, an overall average of 56% for unknown total organic halogens, di- and tri- halo acetic acids and trihalomethanes together is classed as a weak correlation (Hua *et al.*, 2015).

6.6. Conclusions

The hypothesis of his study was that the trihalomethanes concentrations will not be stable after two weeks of storage and the change in concentration will be greater at 25°C compared to 4°C and for the nutrient-rich water compared to the nutrient-poor water. The data we have analysed indicates this hypothesis is true for all of the chloroform samples regardless of site/temperature apart from the eutrophic water samples stored post treatment at 4°C. However, for the dichlorobromomethane there is no significant difference in concentration of the samples from any site/temperature combination for up to 6 weeks.

6.7. References

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Chapter 7: Final Discussion

7.1. Effect of drought conditions on DOC

Chapters 2, 3 and 4 examined the impact of drought conditions on peatland and freshwater dissolved organic carbon (DOC) concentrations, quality and propensity to form trihalomethanes (THMs) following experimental chlorination. Chapter 2 assessed the differences in water quality and DOC between a series of man-made drainage ditches and a natural stream in the peat-dominated Upper Conwy catchment over a 12-month period. Chapter 3 focused on the Plynlimon study site in Mid-Wales, where soil porewater was sampled for 6 months from a control area and an area subject to long-term drought and assessed for DOC concentration and quality. Chapter 4 involved the collection of peat cores from two sites (fen from Cors Eddreniog, Anglesey, and bog from Migneint, Conwy, both UK) and application of a drought and re-wet treatment, again with DOC as the main focus. These studies have addressed the impact of drained/drought conditions on peatland carbon (Freeman *et al.*, 1998; Freeman *et al.*, 2004). This section synthesises some of the key results from these chapters.

7.1.1. General water chemistry

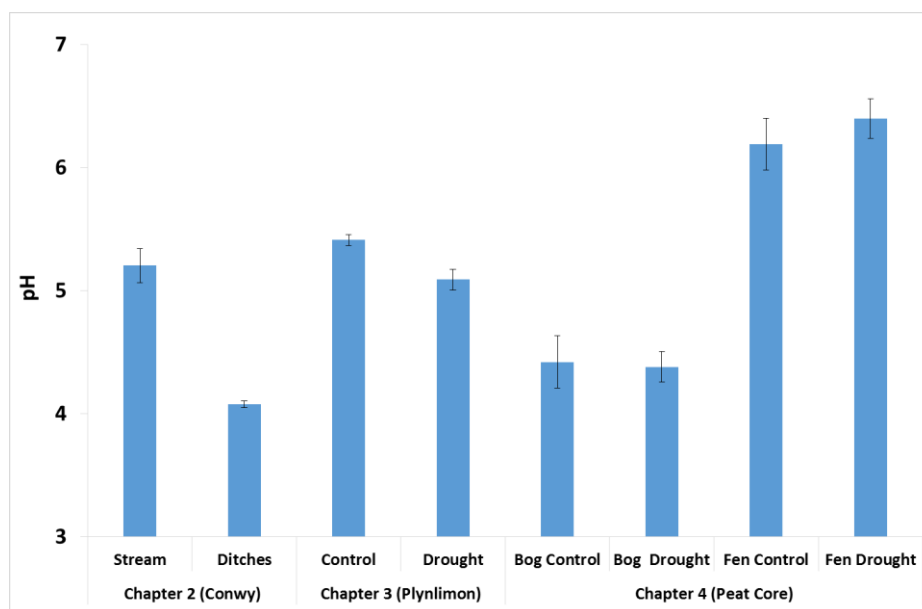


Figure 7.1. mean pH of all drought/drainage experimental sites.

The mean pH value of all the sites, shown in Figure 1, demonstrate that all sites were acidic, ranging from 6.39 ± 0.16 , in the fen drought conditions to 4.07 ± 0.15 in Conwy ditches. The data suggests that the fen has the highest pH, due to the fact that fens mainly receive water from nutrient-rich

groundwater (Leonard *et al.* 2013). Conwy ditches demonstrated the lowest average pH, due to the site deriving its water from nutrient-poor precipitation and due to organic acids leaching from the drained peat soils.

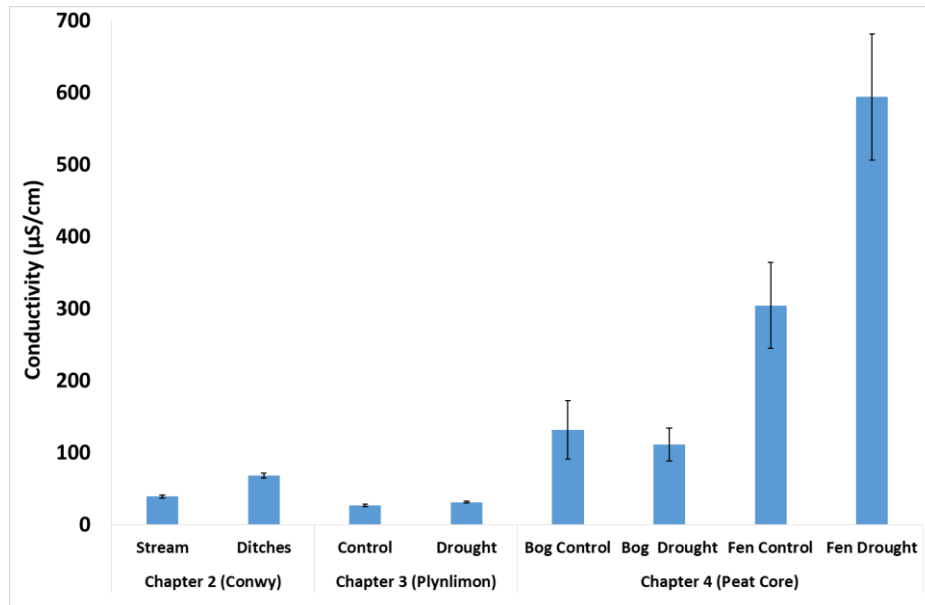


Figure 7.2. Mean specific conductivity of all drought/drainage experimental sites

For conductivity, the greatest mean value of all sites was $594.0 \pm 87.3 \mu\text{S/cm}$ for the fen drought conditions and the lowest was for the Plynlimon control, at $26.7 \pm 1.88 \mu\text{S/cm}$. This again reflects the different sources of water for the different sites.

7.1.2. Dissolved Organic Carbon (DOC) concentrations

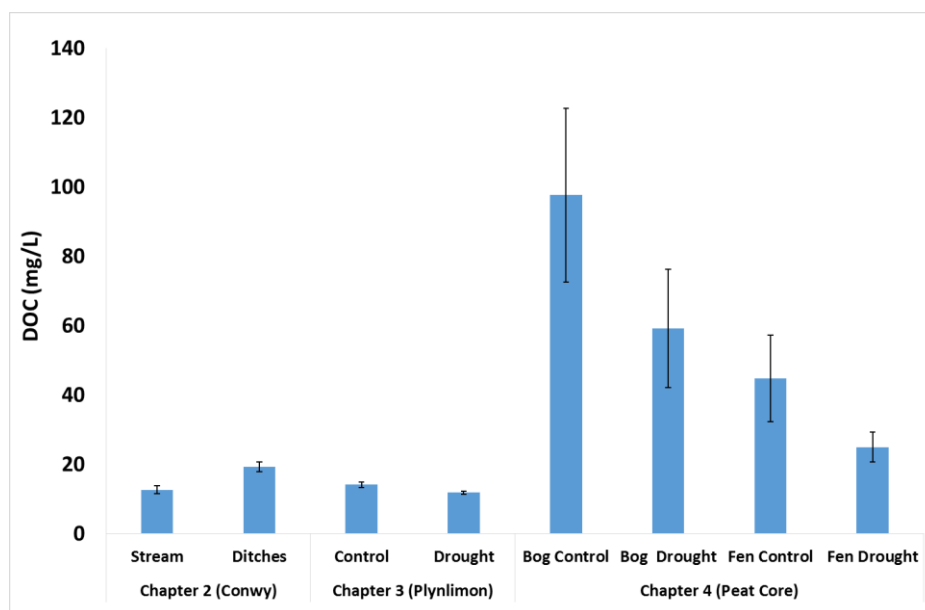


Figure 7.3. Mean DOC concentrations of all drought/drainage experimental sites

The highest mean concentration of DOC of all sampling sites was measured in the bog cores under control conditions (97.6 ± 25.0 mg/l) and the lowest mean concentration was recorded in Plynlimon under drought conditions (11.89 ± 0.45 mg/l). Peatlands represent a major terrestrial store of carbon (Limpens *et al.* 2008), that has formed due to the incomplete decay of plant material in waterlogged conditions (Billett *et al.*, 2010). Climate change is expected to lead to an increase in the frequency of drought and this will not only affect the ability of peatlands to continue to sequester carbon, but will also lead to the release of significant amounts of stored carbon to freshwaters (as DOC) and the atmosphere (as CO₂) (Bates, 2008; Clark *et al.*, 2010; Evans and Warburton, 2010; Fenner and Freeman, 2011; Gallego-Sala and Prentice, 2013).

Mean DOC concentrations across the control sites over the entire period of sampling (from June 2013 – June 2016) were as follows: Bog > Fen > Plynlimon control > Conwy Stream. The drivers of the observed differences between the sites could be attributed to the topography, soil type and extent of anthropogenic influences within the catchments, and as well as external influences such as rainfall and temperature (Fenner *et al.*, 2005; Worrall *et al.* 2003; Wetzel *et al.* 1992). There were seasonal variations in DOC concentration of the sites, most noticeably increasing during the autumn months (Figure 2.4a, Chapter 2). This effect is largely driven by vegetation dieback and senescence within the catchments and the subsequent release of degradation products and DOC during the increasing autumn rainfall (Scott, 2001; Dawson *et al.*, 2008). During drought conditions (for example, at Plynlimon) reduced rainfall and water table drawdown occurs, leads to an increasing influx of oxygen into the upper peat layers. However, rising temperatures throughout spring and summer can lead to increased microbial activity, which can lead to a further increase in degradation products (Freeman *et al.*, 2004). For the peat core experiment, DOC concentrations were higher in the bog than in the fen and higher in the control than in the drought, as was previously found by Lin *et al.* (2012) and Tfaily *et al.* (2013).

We observed slightly greater losses of DOC for the fen cores compared to the bog cores when subject to experimental drought conditions. There was a 39.4% reduction in DOC for the bog and a 44.8% reduction for the fen. This suggests organic matter and DOC from bogs is more resistant to degradation than in fens. This agrees with the findings from Corbett *et al.* (2013), and the reason for this could be due to the difference in the vegetation and therefore substrate quality. *Sphagnum* moss, much more prevalent in bogs than fens, is resistant to decomposition and bogs generally have higher concentrations of phenolic compounds (Lang *et al.*, 2009). In addition, higher porewater sulphate concentrations in the droughted fen peat (due to higher sulphur availability from ground water) may have lowered pH and reduced DOC solubility (Clark *et al.*, 2012), therefore

resulting in lower DOC concentrations. The decline in the concentration of DOC during drought (see Figure 4.2, Chapter 4 and Figure 5.3, Chapter 5), has been reported previously; Freeman *et al.* (2004), Ellis *et al.* (2009), Fenner and Freeman (2011) and Clark *et al.* (2012). However, some other studies have reported an increase in DOC during drought (Fenner *et al.*, 2009; Lou *et al.*, 2014; Strack *et al.*, 2008; Worrall *et al.*, 2004). This could be due to acidification which leads to a decrease in the solubility of DOC.

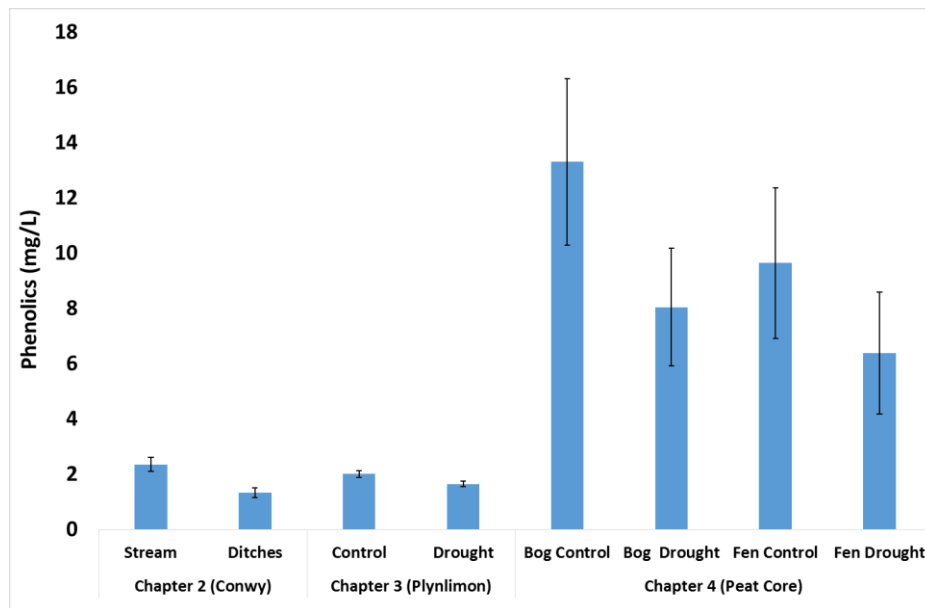


Figure 7.4. Mean phenolic concentrations of all drought/drainage experimental sites

The highest mean phenolic compound concentration of all sites was the bog under control conditions (13.3 ± 3.0 mg/l) and the lowest was Conwy ditches (1.32 ± 0.17 mg/l).

Generally, the concentration of phenolic compounds mirrored that of DOC, indicating that phenolic compounds are a significant fraction of total DOC at these study sites. For all three experiments, the phenolic concentration was lower in the drought/drainage treatment than the control. This finding agrees with the study of Fenner and Freeman (2011) who state that under conditions of lowered water table in peat, oxygen ingress results in the degradation of phenolic compounds and the pool of phenolic DOC can take a long time to recover after rewetting. The data from the peat core experiment (chapter 4) shows that the concentration of phenolic compounds in porewater was higher in the bog than the fen. This could be due to the greater activities of phenol oxidase in the fen (Jassey *et al.*, 2011). Moreover, bacterial activity in fens have been reported to be higher than in bogs (Ausec *et al.*, 2011), suggesting that the bog conditions are less suitable for phenolic-degrading microbes.

Additionally, Figure 5.4 in chapter 5, demonstrates that there was a higher phenolic concentration in control conditions as opposed to drought conditions, presumably due to the increase in the activity of phenol oxidase (e.g. Fenner and Freeman, 2011), although some previous studies have demonstrated a weak relationship between phenol oxidase activity and phenolic concentration (Romanowicz *et al.*, 2015). It is suspected that oxygenated conditions in the drought treatment will have resulted in the complete breakdown of soil organic matter to CO₂, rather than incomplete breakdown to DOC, due to the opening of the enzymic latch (Fenner and Freeman, 2011; Freeman *et al.*, 2001).

Figure 2.6 (a and b) in Chapter 2 shows a higher phenolic concentration in the stream and ditches than Llyn Conwy. This could be due to the peat soils from which the waters are draining providing a higher phenolic concentration, however, it would be reasonable to assume that all water in Llyn Conwy would have been derived from this peat rich catchment, and therefore the phenolic concentration should also be high. The low phenolics concentration is likely a dilution effect taking place here, due to the site's high rainfall and the large volume of the lake, and a larger surface area of the lake compared to the stream would result in a greater exposure to UV radiation from sunlight, which has been shown to degrade phenolic compounds (Jones *et al.* 2016). Figure 2.8 (Chapter 2) shows that the strong positive relationship between DOC and phenolic concentrations applies for the ditch and stream samples but less so for the lake, therefore indicating that the DOC within the lake contains proportionally less phenolics.

7.1.3. Dissolved Organic Carbon (DOC) quality

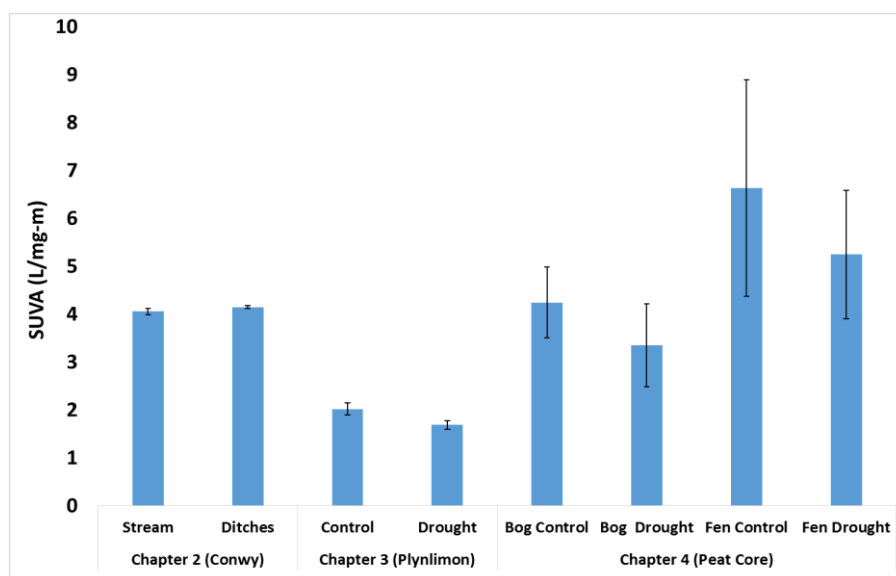


Figure 7.5. Mean Specific Ultraviolet Absorbance (SUVA) concentrations L mg m⁻¹ of all sites during study period. Error bars represent variance in data.

The characteristics of DOC of all sites were analysed by measuring specific UV absorbance (SUVA). The highest mean SUVA value was in the Fen under control condition (6.63 ± 2.25 L/mg-m) and lowest SUVA value was in Plynlimon under drought conditions (1.69 ± 0.09 L/mg-m).

SUVA is an important measurement in drinking water treatment because it indicates higher molecular weight, aromatic compounds which some previous studies have shown have a higher propensity to form some of the regulated DBPs upon chlorination, such as THMs (Bond *et al.*, 2011; Volk *et al.*, 2002; Kitis *et al.*, 2002). The SUVA value is used to describe the aromaticity of DOC (Weishaar *et al.*, 2003) and it is an important predictor for DOC removal during coagulation, and DBP formation in water treatment plants (Matilainen *et al.*, 2011). Ritson *et al.* (2017) shows that high SUVA values were observed in peat soil. In chapter 4, SUVA was higher for the fen compared to the bog and for the control compared to the drought (see Figure 4.5, Chapter 4). SUVA for the drought treatment was significantly lower than the control for the Plynlimon experiment (Figure 5.5, Chapter 5). This could be due to the fact that drought conditions enhance the microbial breakdown of organic matter and DOC.

The data from Chapter 2 figure 2.9 shows that there is a good relationship between DOC and $SUVA_{254}$ ($R^2=0.50$) and this finding agrees with Chow *et al.*, (1999), Bell-Ajy *et al.*, (2000) and Singer and Bilyk, (2002) ($R^2=0.88$).

7.1.4. Trihalomethane Formation Potential (THMFP)

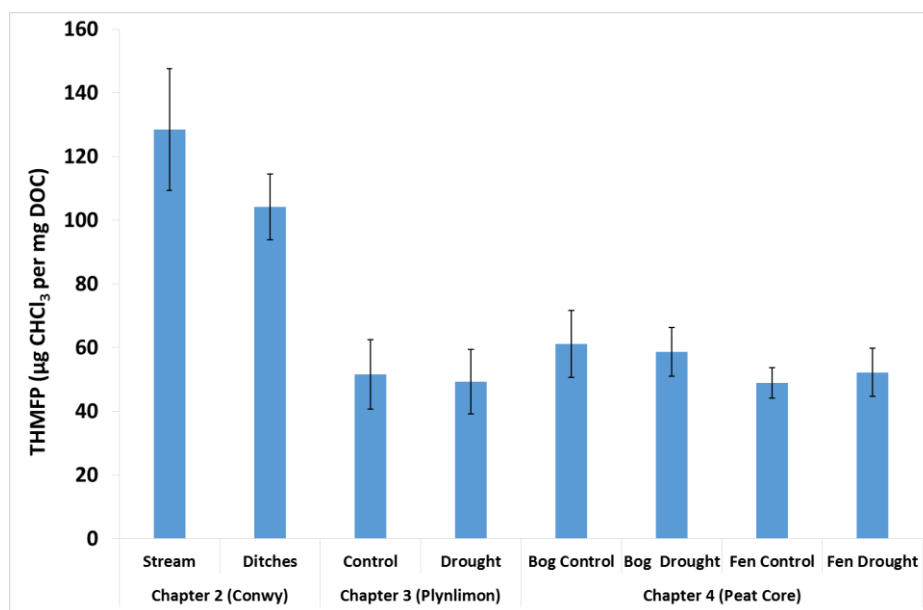


Figure 7.6. Mean total THM formation potential concentrations ($\mu\text{g/L}$) of all sites. Error bars represent variance between data.

The highest mean THMFP value was observed in the Conwy Stream, at $128.51 \pm 76.55 \mu\text{g/L}$ and lowest was in the Fen Control at $48.8 \pm 4.77 \mu\text{g/L}$.

The THMFP for Conwy was higher in the natural stream than the man-made drainage ditches, although the difference was not significant (Chapter 2, figure 2.7). The trihalomethane formation potential (THMFP) in the porewater was generally lower during drought conditions ($42.7 \pm 4.89 \text{ CHCl}_3 \mu\text{g/mg DOC}$) than control ($48.6 \pm 5.57 \text{ CHCl}_3 \mu\text{g/mg DOC}$), but the effect was not significant (see chapter 3, Figure 3.6). The mean THMFP in Figure 4.6 (Chapter 4) was higher for the drought ($57.75 \pm 20.33 \mu\text{g CHCl}_3/\text{mg DOC}$) over the control ($54.59 \pm 23.63 \mu\text{g CHCl}_3/\text{mg DOC}$) treatment, but this effect was not significant. There are no previous studies examining drought effects on THMFP and the lack of significant drainage/drought treatment effects on THMFP was surprising, given the significant changes in DOC quality that were observed in chapters 2, 3 and 4. Our hypotheses all stated that any changes in DOC quality should affect THMFP yields. Our data shows no relationship between SUVA and THMFP for any chapter, a finding only found in one other paper; Weishaar, *et al.* (2003) ($R^2 = 0.4153$). Others studies show significant relationships between the two parameters, including Ma *et al.* (2013), who reported a strong relationship between SUVA_{254} and THMFP ($R^2 = 0.993$). High SUVA should indicate high THMFP because highly hydrophobic and aromatic compounds absorb ultraviolet radiation most strongly and also react readily with chlorine (Ma *et al.*, 2013; Zhao *et al.*, 2006).

7.2. Effect of nutrient status on DOC

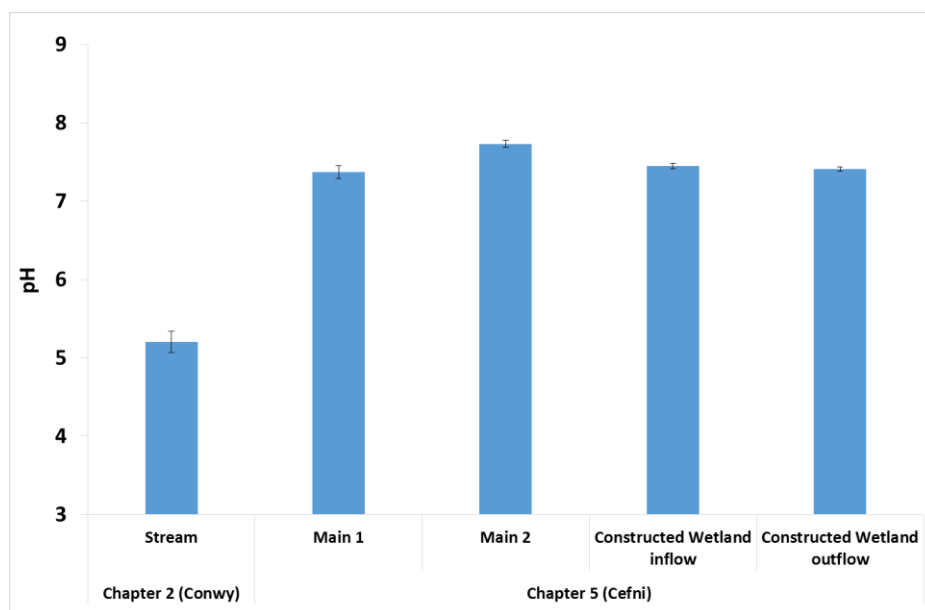


Figure 7.7. mean pH of all Conwy stream and Cefni site.

The mean pH value of all the sites in chapters 2-4 (Figure 7.1) were acidic. By contrast, the eutrophic Cefni was basic with a mean pH of 7.49 ± 0.048 , much higher than the oligotrophic Conwy stream, which was acidic (5.2 ± 0.138). The higher pH in the Cefni is due to the greater influence of groundwater and because algae generate alkalinity (Leonard *et al.* 2013).

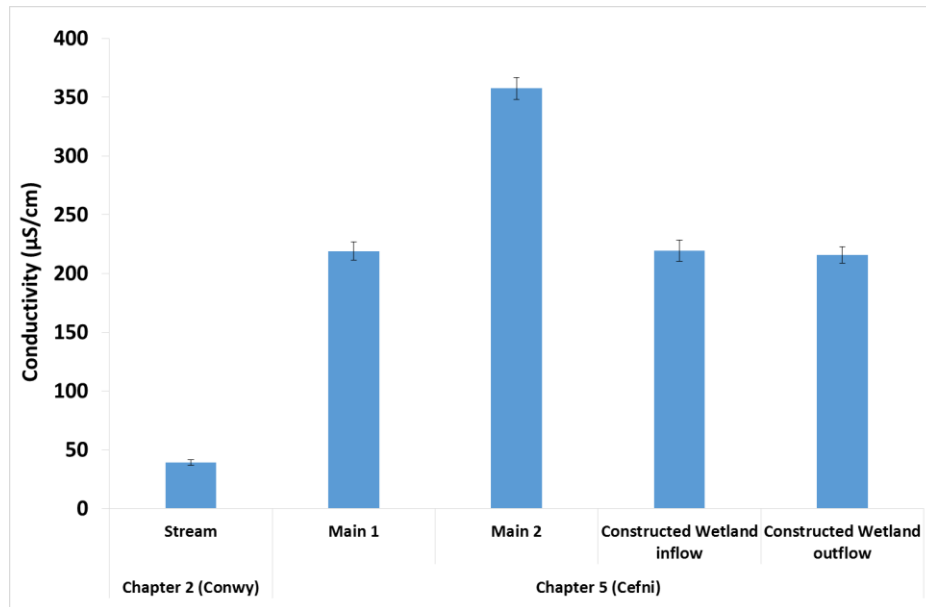


Figure 7.8. Mean specific conductivity for Conwy stream and Cefni site.

The mean conductivity was greater at all Cefni sites ($252.98 \mu\text{S}/\text{cm} \pm 8.230$) than in the Conwy stream ($39.03 \mu\text{S}/\text{cm} \pm 2.416$). Again, this is likely to be due to the influence of groundwater.

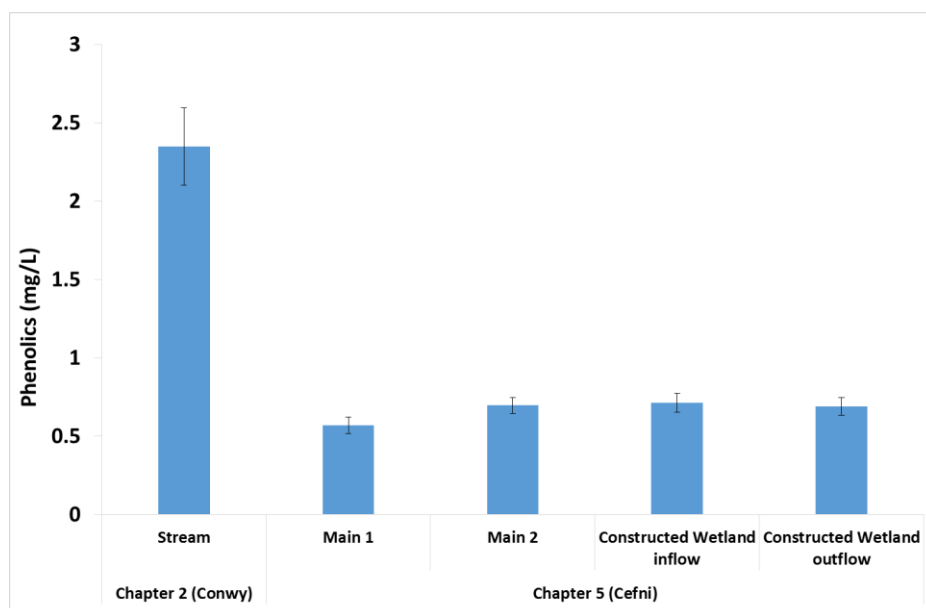


Figure 7.9. Mean phenolics concentrations of stream and Cefni experimental sites.

The highest mean concentration of phenolics was in Conwy stream $2.34 \text{ mg/L} \pm 0.24$ and the lowest was in Cefni $0.67 \text{ mg/L} \pm 0.05$. This is likely to be due to the leaching of phenolic compounds from the peat substrate in the Conwy catchment.

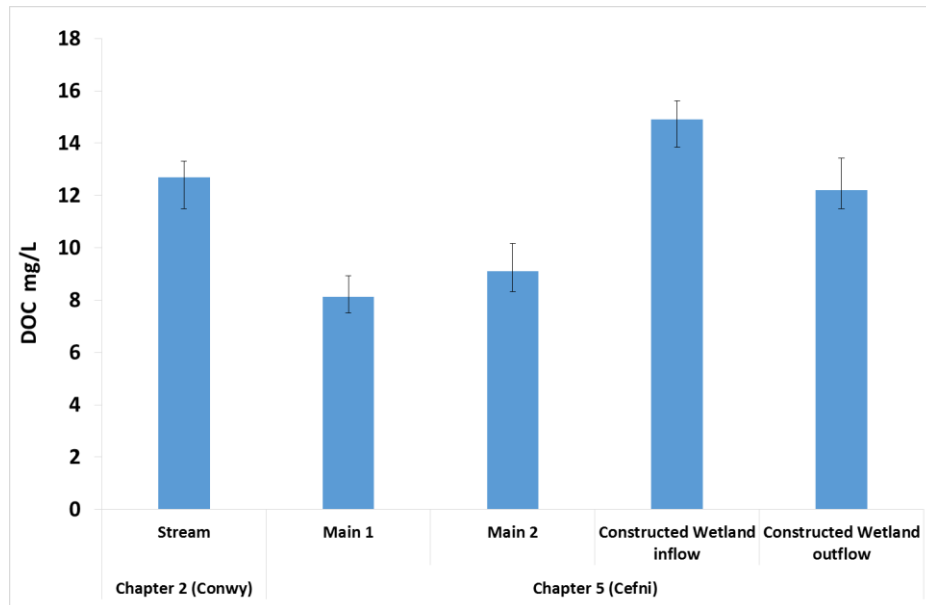


Figure 7.10. Mean DOC concentrations of stream and Cefni experimental sites.

The highest mean concentration of DOC was in Conwy stream $12.69 \text{ mg/L} \pm 1.209$ and the lowest was in the Cefni $11.09 \text{ mg/L} \pm 0.79$. Despite this, the Cefni had the highest THMFP per mg DOC (Figure 7.12), reflecting its higher reactivity.

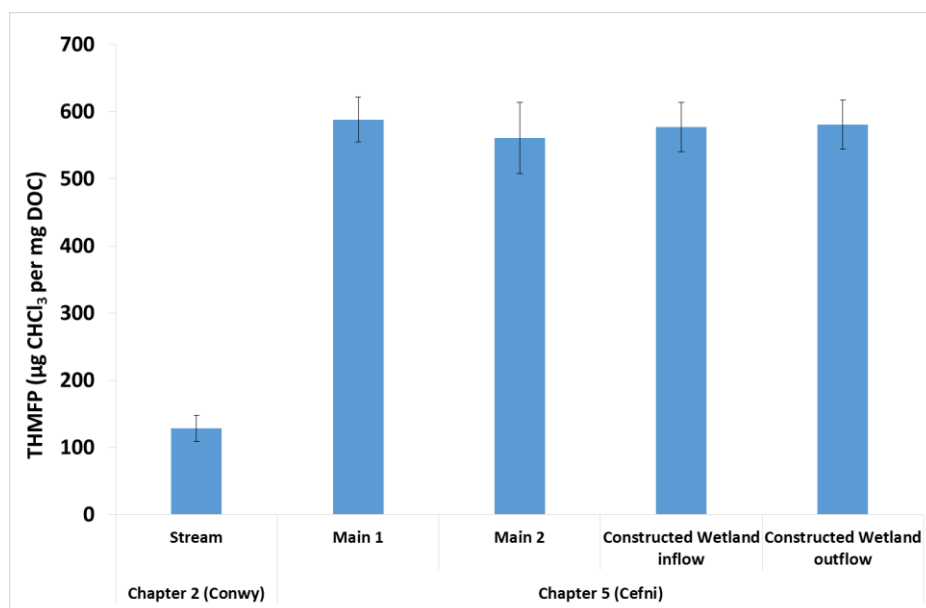


Figure 7.11. Mean total THM formation potential concentrations ($\mu\text{g/L}$) of stream site compared to Cefni site. Error bars represent variance between data.

Previous studies show that Llyn Cefni has consistently high DOC concentrations year round, resulting in higher THM formation, and published works show that compliance for THMs at this site has been historically poor (Balch, 2005). Our data suggest that the relationship between THMFP ($\mu\text{g}/\text{mg}$ DOC) and DOC concentration appears to be random.

7.3. Impact of thesis findings

Environmental issues, most importantly climate change, are not the only concern relating to the issue of rising DOC in freshwaters. It can also lead to higher treatment costs in water treatment plants, and/or higher potential disinfection by-product formation. It is important to control or reduce the concentration of THMs in finished water. This can be achieved most effectively by removing DOC prior to chlorination. 80-100% DOC removal can be achieved using membranes (Fu *et al.*, 1994; Lin *et al.*, 1999). Other studies have shown that coagulation by aluminium sulphate can remove 10-40% of DOC (Volk *et al.*, 2000), 40-60% by ferric chloride (Dennett *et al.*, 1995) and ozonation (Goel *et al.*, 1995).

7.4. Suggestions for future work

It would be useful to investigate the cause of the difference in THMFP between bog and fen Cefni, control and drought samples. To achieve this it would be necessary to further analyse the characteristics of the DOC. Nuclear magnetic resonance (NMR) and Mass Spectroscopy could be used to identify specific functional groups. Another important variable is the relative importance of allochthonous and autochthonous sources of DOC. In the Cefni reservoir, algae is known to be an important source of THMFP. However, the amount of DOC produced by algae is not known. Quantification of different DOC sources could help to explain variations in THMFP between different sites. In this case, if the majority of the DOC in the reservoir is algae-derived, then the water companies can help the local farmers surrounding the reservoir to reduce the levels of nutrients entering the reservoir from the catchment which stimulates algal growth. However, if the DOC is largely formed outside the reservoir (allochthonous) then the water companies should invest in the management of Cors Erddreiniog fen in the Cefni catchment.

7.5. Final conclusion

- Eutrophic reservoir and its inflowing streams were monitored for parameters including DOC, nutrients and THMFP.
- Higher DOC concentrations in the lake than the 2 dominant inflowing streams, demonstrate that there is significant production of DOC from algae within the lake.
- There was no difference in the THMFP between the streams and reservoir indicating no differences in DOC characteristic in terms of reactivity with chlorine.
- The constructed wetland reduced DOC concentration by 18% on average representing an additional benefit.
- The constructed wetland sequestered nutrients, removing 9 and 21% of nitrate and phosphate, which was presumably taken up by algae and plants within the constructed wetland, but the extent of sequestration was much lower than during the winter
- The constructed wetland is benefiting the water quality of Llyn Cefni reservoir by reducing the nutrients going to the lake, and thereby reducing the amount of algae and DOC building up in the lake.
- The DOC concentration in the different streams was highly variable, reflecting different process happening within each stream and its catchment.
- The most important graph of this study shows the amount of THMs you would expect to form per mg of DOC and there is no difference between the main inflows and the outflow, so there is no impact of the constructed wetland in this respect.
- The pH of all streams were relatively constant and ranged from 7.3 to approximately 7.8 because the site is lowland and is influenced by groundwater.
- The reservoir showed the highest pH because algae generate alkalinity.
- Drought generally lowered pH, especially for the fen peat. This is because the fen receives its nutrients from groundwater, so cutting off the nutrient supply will reduce pH
- Drought lowered concentrations of DOC and phenolics for both peat types. This will have resulted in the complete breakdown of soil organic matter to CO₂, rather than incomplete breakdown to DOC, due to the opening of the enzymic latch.
- The loss of DOC and phenolics due to drought can be attributed to oxygenation and improved conditions for decomposition in the soil.
- Evidence of greater THMFP in the drought treatment shows that peatland drought changes the characteristics of the DOC to make it more likely to form THMs. However, this is more than offset by the much lower DOC concentrations.

- The DOC concentration of Llyn Conwy changed little throughout the study period, despite the DOC concentration of the streams and ditches varying throughout the year, because Llyn Conwy lake is a large lake and has a high volume.
- The higher SUVA of the Ditches is most likely to be due to the release of more complex, humified DOC from the deep soil layers.
- Long-term experimental drought in peatlands has a strong impact on porewater DOC concentration but no significant effect on THMFP.

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