Dynamics of dissolved organic matter composition in Scottish rivers and headwater streams – resolving environmental and biogeochemical process interactions

by:

Sara Trojahn

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Heriot Watt University, The Lyell Centre
School of Energy, Geoscience, Infrastructure and Society

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Abstract

Dissolved organic matter (DOM) has a wide range of chemical structures that give it a multifunctional role in the natural environment. Although the role of DOM in aquatic ecosystems has been the focus of previous work, a comprehensive understanding of the compositional behaviour of DOM under different environmental processes is still incomplete. New field-based geochemistry data is presented from a two-year study (03/2017- 03/2019) in Scottish headwaters and a 9-month study in large Scottish rivers. This research shows that the DOM mobilisation follows seasonality with enhanced exports of DOM during winter months compared to the summer. At a larger spatial scale, the seasonal trend is overprinted by the catchments soil type. Size-Exclusion Chromatography combined with high-resolution time series of DOM variables reveal that precipitation events preferentially mobilise humics from the surrounding soils, while humics concentration decline during low flow conditions. Furthermore, the data show that non-UV absorbing ("invisible") low molecular weight (LMW) neutrals (iDOM) contribute up to 50 % to the total DOM pool in headwaters, especially during low flow conditions, and on average 13 % to the DOM in larger river systems. The source of iDOM was found to be the topsoil of peatland and peaty podzols. Consequently, more labile OM can be leached from soils into the aquatic environment in the future through disturbed soils promoting instream microbial growth and act as a nutrient source for aquatic plants.

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Abbreviations

DOM/ DOC	Dissolved organic matter/ Dissolved organic carbon (interchangeable, but DOC usually a bulk parameter, while DOM describes an organic matter pool including carbon, nitrogen and other elements)
TOC/ TOM	Total organic carbon (describing the whole organic carbon pool, dissolved and particulate)/ Total organic matter
C/N	Carbon and Nitrogen ratio
HOC	Hydrophobic organic carbon (relatively insoluble, nonpolar compounds that have large octanol-water partition coefficients (log $K_{ow} > 2$)
POM/ POC	Particulate organic matter/ Particulate organic carbon (see DOM/DOC)
DON	Dissolved organic nitrogen
SOM	Soil organic matter
WEOM	water extrable soil organic matter
TerrOM	Terrestrial organic matter
LMW	Low molecular weight (a small molecule/organic matter with a molecular weight of < 900 daltons)
HMW	High molecular weight (large molecule/organic matter with a molecular weight of > 900 daltons)
HS	Humic substances, interchangeable with 'humics'
BB	Building blocks (degradation products of humics)
BP	Biopolymers (polymers produced from natural sources either chemically synthesized from a biological material or entirely biosynthesized by living organisms
iDOM	Invisible dissolved organic matter, interchangeable with LMW neutrals (low molecular organic matter that does not or only weakly absorb or fluorescent any light)

CDOM	Coloured or chromophoric dissolved organic matter (optically measurable component of dissolved organic matter in water
Mn	Molecular weight
SUVA	Specific ultraviolet (UV) absorbance, usually at 254 nm absorbance (SUVA$_{254}$)
LC-OCD-OND-UVD	Liquid Chromatography with organic carbon detector, organic nitrogen detector and UV detector, short form is LC-OCD or LC-OCD-OND (used to measured DOM fractions)
SEC	Size Exclusion Chromatography (chromatographic method in which molecules in solution are separated by their size/molecular weight)
GHG	Greenhouse Gases, dominantly carbon dioxide (CO_2) and methane (CH_4)
LOD/LOQ	Limit of detection/ Limit of quantification (defined as the lowest concentration of the analyte that can be reliably detected and quantified, respectively)

Chapter 1 - Introduction

1.1. The global carbon cycle

In Earth's biogeochemical cycles elements transfer between chemical forms between major global reservoirs (e.g. biosphere, lithosphere, and atmosphere); between smaller ecosystems (forest to river in a landscape); or between reservoirs within an ecosystem (soil to plant, etc.) [2]. Carbon is unequally distributed among three major reservoirs: terrestrial, oceanic and atmospheric. Simplified representations of the global carbon cycle consider oceans and land as the two main biologically active reservoirs connected through gas exchange via the atmosphere [3, 4]. Sundquist et al. [5] argues that carbon transfers through different 'pathways': plants and animals, soils, rocks, and sediments.

The carbon cycle combines many processes including the seasonal cycle of plant growth and decay, and the geologic cycle of sediment burial and weathering. These processes are ultimately linked to exchange of carbon dioxide (CO_2) with the atmosphere [5]. Figure 1.1 [2] shows a representation of the global carbon cycle including the estimated release of CO_2 due to anthropogenic activity. The increase of atmospheric CO_2 is of growing concern as it causes significant warming which affects the balance between the surface and atmosphere [5].

The different carbon stores are connected through various fluxes, including weathering and sedimentation, photosynthesis, gas exchange with the atmosphere, and riverine transport, moving on timescales from seconds to millennia. A fundamental role in the carbon cycle is played by the terrestrial biosphere, providing direct and indirect feedbacks to the climate. Since the beginning of the industrial age, there have been changes in the way carbon is transported between the major carbon stores, thereby creating new pathways and potential feedback mechanisms [6, 7].

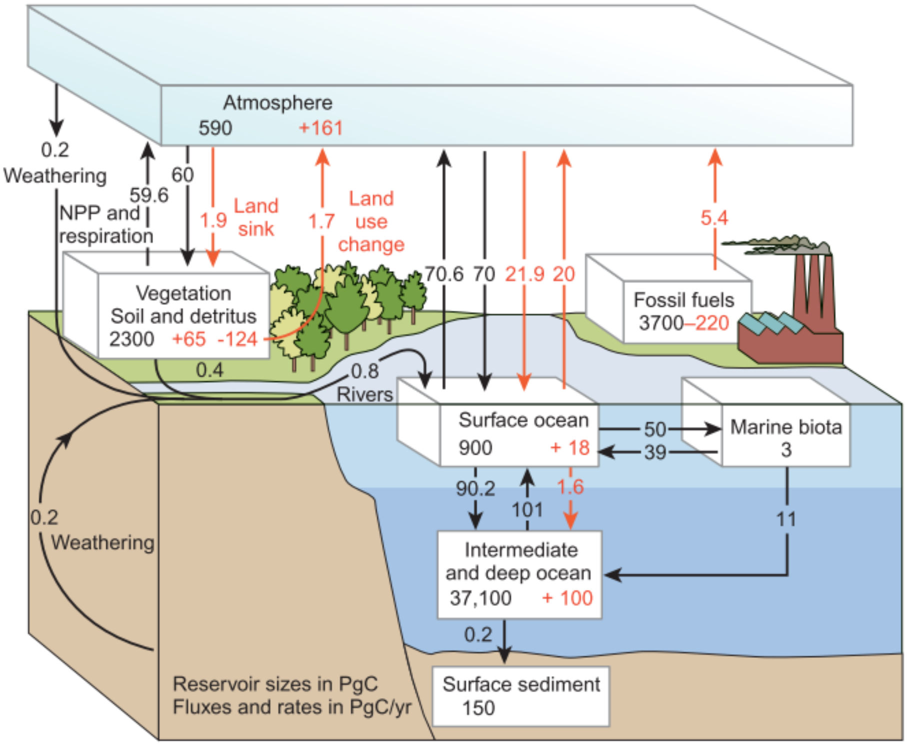

Figure 1.1: Simplified representation of the global carbon cycle. The values inside the boxes are standing stocks (in Pg C); the arrows represent annual fluxes (Pg C/y). The black arrows and numbers show the preindustrial values of standing stocks and fluxes; the red arrows and numbers indicate the changes due to anthropogenic activity [1].

1.2. The importance of peatlands in the carbon cycle

Peatlands contain more than 550 gigatons of carbon, representing 42% of all soil carbon exceeding the carbon stored in all other vegetation types, including the world's forests, which, if destabilised, could result in significant positive feedbacks to climate warming [8]. Northern peatlands are a globally important soil carbon (SC) store [9, 10], with aquatic systems draining peatland catchments receive a high loading of dissolved organic carbon (DOC) and particulate organic carbon (POC) from the surrounding terrestrial environment [11, 12]. Once incorporated into the aquatic environment, internal processes occur in-situ as well as with the atmosphere to modify the carbon pool [12]. Understanding the spatial and temporal variability of controls on peatland carbon cycling is essential to project the effects of future environmental change. While there is understanding of individual drivers of carbon cycling, the effect of multiple drivers [13–15] on the carbon transformations in peat-dominated catchments are poorly understood. The main cause is that the characteristics and concentration of carbon from these unique ecosystems depend on various factors like catchment type, climate, weather conditions and hydrology [16].

Scottish peatlands are historically considered as important carbon sinks in Europe [17]; this function seems to be changing as trends over the last decades show increasing concentrations and fluxes of organic carbon (OC) in Scottish rivers draining carbon from these peatland areas [13, 18–22].

The turnover of aquatic carbon into atmospheric CO_2 plays an important role in the mobilisation and degradation process of carbon in peat-dominated catchments, directly affecting the carbon cycle [23–25]. It is therefore critical to determine the quantity, composition and potential bioavailability of riverine organic matter (OM), comprised of carbon and nitrogen (N) components, during key periods of the hydrological cycle, and investigate the changing role of OM from the soil-headwater interface downstream into the larger rivers.

1.3. The role of rivers in the carbon cycle

Inland waters including rivers play a fundamental role in the land-ocean interactions, which directly impact the global nutrient and carbon balances [26]. A study by Drake et al. [27] estimated that 5.1 Pg of terrestrial carbon is added to inland waters each year which includes oceanic export (0.95 Pg C/yr), storage (0.6 Pg C/yr), outgassing (3.9 Pg C/yr), and subtracting non-terrestrial-C fixation (0.3 Pg C/yr). Generally, three key processes affect the C mobilisation and/or storage at the aquatic – terrestrial interface: a) mineral interaction in the soil (sorption and desorption processes) [26], b) microbial turnover [28], and c) photo-oxidation after mobilisation [29]. In combination, these processes determine the mobilisation and storage of carbon in soil and river systems, with the mineralisation of OM contributing to the outgassing of CO_2 into the atmosphere [14, 30–32].

When inland aquatic systems are included in global models, it is usually only for the transport of carbon through the riverine pipe. This delivery of terrestrial carbon through the riverine drainage network is in fact the end result of a number of transformations and losses in aquatic systems [3]. Understanding carbon transfer across the terrestrial landscape and into aquatic ecosystems is important, especially considering the unprecedented anthropogenic environmental changes we are experiencing in the biosphere, lithosphere, and atmosphere.

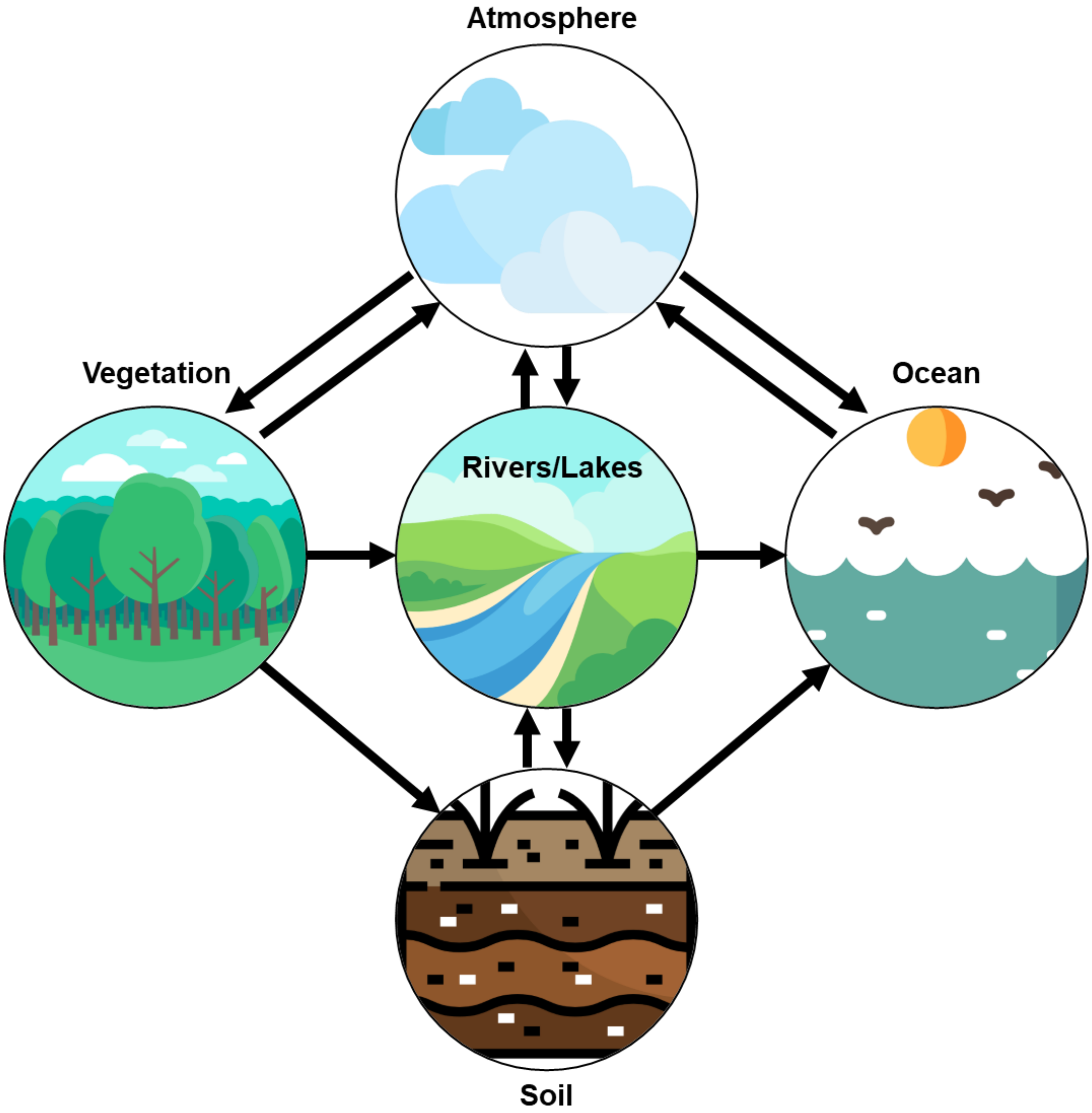

Figure 1.2: Schematic diagram showing the coupling of land (vegetation, soil), oceans, atmosphere and rivers/lakes (inspired by Battin et al. [1]) in the carbon and water cycle.

The role of carbon in terrestrial ecosystems has been the focus of previous work [3, 33–38], but a comprehensive understanding of the variation in the composition of freshwater DOM is currently partially lacking, especially for headwater river systems. There is limited knowledge of the temporal variability of carbon cycling at the headwater scale even though the impact of soil organic matter (SOM), vegetation and land use is dominant at this smaller scale, linking the hydrological and climatic processes to the carbon and nutrient cycle of river and terrestrial catchments [39, 40].

Headwaters represent a “hotspot” of biogeochemical cycling but there are still large uncertainties of the processes that control carbon supply to the river and subsequent riverine outgassing [41, 42]. Overall, the majority of inland waters (96 %) are oversaturated with respect to CO_2, relative to the atmospheric concentration [43], therefore CO_2 is degassing to the atmosphere.

Known examples of CO_2 oversaturated inland waters are peatland headwater streams because they are consistently supersaturated with respect to gaseous C and are known to degas CO_2 and methane (CH_4) to the atmosphere [25, 44].

This CO_2 potential is significant and justifies a focussed effort to better understand and quantify the role of headwater streams, which account for 70 - 80 % of the total river network [45] on only 20 % of the global land surface [41]. Furthermore, it has been shown that a large proportion of the annual C export (36 to >50 %) in headwaters is focussed on short and high-intensity rainfall events [39, 46–48]. However, DOC inputs from precipitation events are strongly regulated by the succession and severity of storm events (increased hydrology through precipitation events) [35, 49], dry periods [50, 51], the dominant soil type [52] and antecedent environmental conditions e.g. soil moisture, snow cover [53, 54].

1.4. Dissolved organic matter – classification, sources and significance for the global carbon cycle

Natural organic material (NOM), also called total organic matter (TOM), is part of terrestrial environments and aquatic systems and defined as weathering material from plants and animals as well as their degradation products. The simplest characterisation of NOM can be operationally defined as fractions of DOM and POM [55–57]. DOM represents the OM that passes through a filter (filters generally range in size between 0.7 and 0.22 µm). Conversely, POM is OM that is too large and is filtered out of a sample [58–60].

DOM represents one of Earth's largest exchangeable reservoirs of organic material [60]. It is a diverse mixture of C, N and Phosphorus (P) and other nutrients [55]. The sources of DOM can be classified into two categories: allochthonous (or pedogenic) and autochthonous (or aquagenic). Allochthonous sources derive from precipitation and exchange of water with soils, mobilising soil organic matter (SOM), e.g. from the riparian zone. Autochthonous DOM instead is released by organisms living within the river system [57, 61]. Therefore, bulk DOM contains many compounds that exhibit a range of reactivity and turnover times. The most labile DOM compounds turn over on time scales of minutes to weeks [62–64], while at the other extreme end long-lived DOM compounds resist microbial degradation, turning over on timescales of centuries to millennia [65, 66].

DOM plays a central role in aquatic environments because it is highly reactive in terrestrial and aquatic ecosystems and has a strong influence on the functioning of these ecosystems by controlling microbial food webs and biogeochemical reactions [67, 68].

Estimates of the impact of DOM on the terrestrial carbon balance mostly rely on the fluxes measured in river waters [69] but the concentrations and fluxes of DOM are affected by soil properties, hydrological conditions, biotic factors and land use of the catchment [70]. The amount and character of DOM in terrestrial and aquatic ecosystems can, however, vary considerably over time due to seasonal processes that regulate its production, consumption, and transport [71].

Dissolved organic nitrogen (DON) represents a significant pool of soluble N in many soils and freshwaters. Further, the low molecular weight (LMW) component of DON represents an important source of N for microorganisms and can also be utilised directly by some plants [72, 73]. Although the quantitative significance of DON as well as nitrate in the nitrogen cycle of streams and rivers has been recognised for decades [74–78], the drivers controlling its production and consumption remain vague [79, 80].

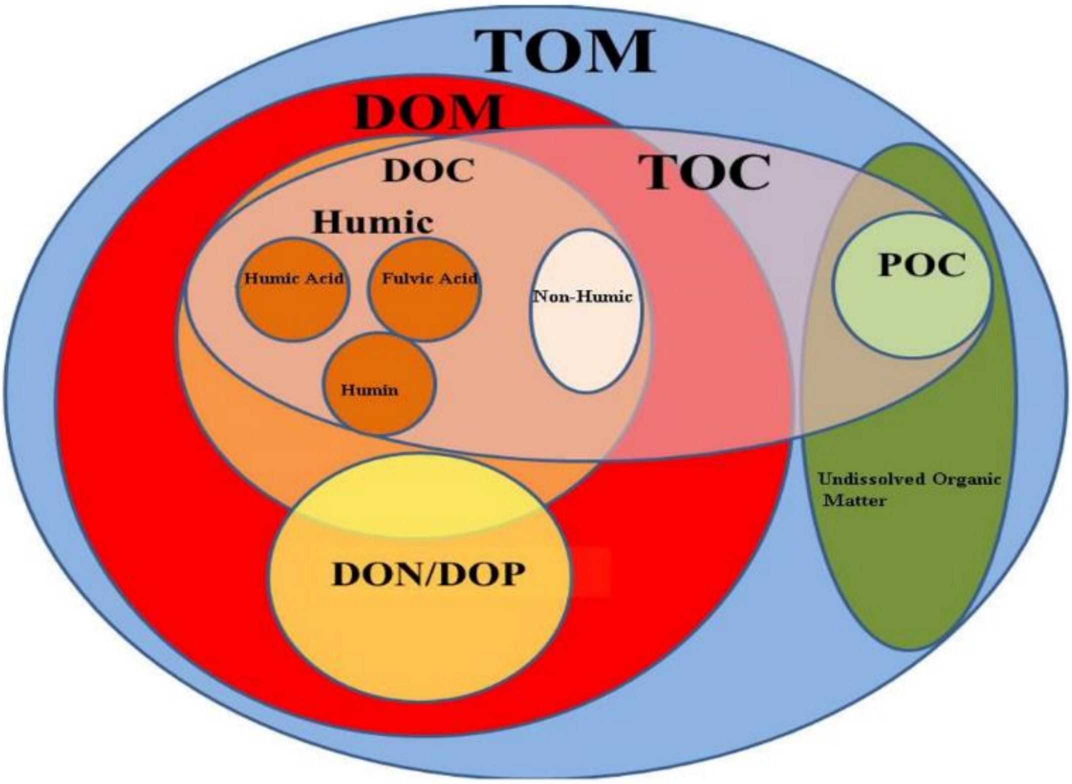

Figure 1.3: Simplified Venn representation of the various forms of organic matter found in natural waters. Total Organic Matter (TOM), Total Organic Carbon (TOC), Dissolved Organic Matter (DOM), Dissolved Organic Carbon (DOC), Particulate Organic Carbon (POC)), Dissolved Organic Nitrogen (DON), and Dissolved Organic Phosphorus (DOP) are represented. DOC can be further broken down to its humic (humic acid, fulvic acid, and humin) and non-humic material, while new analytical methods continue to reveal more molecular-level detail [2].

DOC is an intermediate of DOM in the global carbon cycle [28, 67] and plays an important role in freshwater ecology by providing substrate for microbial respiration, determining UV light penetration and influencing water acidity, and the solubility of metals and nutrients.

It can be degraded, both biologically and photo-chemically, liberating CO_2, CH_4 and CO (carbon monoxide) into the atmosphere once in the aquatic system [3, 81, 82].

The measurement of DOC concentrations and fluxes in small headwater streams can provide information on the factors that influence the cycling and transport of DOC in terrestrial systems [38, 38] but carbon is not just transported in from of DOC, also POC and gaseous forms (Greenhouse gases, e.g. CO_2 and CH_4) are important carbon sources. The importance of each depend on a range of catchment characteristics (e.g. slope, catchment area, soil coverage) [14]. The quantity and quality of stream DOC are related to watershed attributes including soil type, wetland coverage, agricultural land use, and urban point-source inputs. Given the wide variety of sources and changing contributions over time and in different catchment systems, it is essential to better understand how the composition of DOM varies, to identify the underlying drivers behind this variation and consequences thereof as part of dynamic aquatic systems.

1.4.1. Current challenges in dissolved organic matter characterisation and implications for temperate peat-dominated river systems

Regardless of the importance of aquatic DOM and much work on DOM characterisation, a comprehensive understanding of the variations of DOM under different environmental conditions remains vague. One reason is that DOM is often present in low concentrations (1 – 20 mg/L) in water and high concentrations of inorganic compounds, which can adversely affect chemical analyses [83]. A second reason is the heterogeneity of OM structures and molecular weights included within aquatic DOM [84], which complicates isolation and analysis approaches.

Measurements of UV-visible absorption [85, 86] fluorescence characteristics values [87, 88] and C/N and isotopic values [89] on bulk DOM samples provide information on a large portion of the DOM pool, but do not give detailed structural information. Approaches such as Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and mass spectrometry (MS) can provide functional group, compound class, and elemental formula information on DOM [90–92], but usually require an isolation and concentration step prior to use, and thus a fractionation of the DOM pool [90, 93]. Size-Exclusion Chromatography (SEC) [94, 95] has been found to be very effective in fractionating DOM as it allows samples analyses without chemical alterations and links the DOC mass balancing approaches and qualitative DOC methods [96].

The concentration of DOC in most river waters has a strong relationship with chromophoric DOM (CDOM, coloured or chromophoric DOM) from humic substances [97] as DOC quantity and quality is often characterised by UV absorbance measurements [16, 85, 98–101]. However, the use of SEC allowed Pereira et al. [102] to find that optically invisible DOM (iDOM or LMW neutrals) constitute a large proportion (4 – 89%) of the total DOC besides CDOM, at least in tropical headwater rivers. The iDOM pool is characterised by non UV-absorbing organic compounds (mono- and oligosaccharides, alcohols, aldehydes, ketones and amino sugars) which originate from terrestrial plant OM sources and are likely bioavailable [102, 103]. This finding formed the ambitions of this project to ascertain the compositional behaviour of DOM over different timescales (seasons, interannual, monthly, event-based) in temperate river systems with a focus on organic rich peat environments.

1.4.2. The significance of DOM fractionation and compound group identification in fluvial organic matter research

Because of its heterogeneity, DOM characterisation requires the use of multiple analytical techniques, one of them being Size Exclusion Chromatography (SEC) with instruments like the LC-OCD (Liquid Chromatography with organic carbon detector). The five main fractions (biopolymers, humics, building blocks, LMW acids and LMW neutrals) that can be characterised with the LC-OCD have different chemical characteristics that will affect the riverine biochemistry in various ways, hence it is important to characterise and quantify all of these fractions beside bulk DOC.

The biopolymers fraction is characterised by a molecular weight of 10 kDa or higher. The fraction includes polysaccharides with some contribution from nitrogen-containing material such as proteins or amino sugars [95]. Polysaccharides are considered to be the dominating material of Extracellular Polymeric Substances (EPS) [104]. EPS are responsible for the cohesion of microorganisms and adhesion of biofilms to surfaces, influencing spatial organisation, allowing interactions among microorganisms, and acting as adhesives between cells [105]. These functions are important for the establishment and biological activities of biofilms and flocs, hence increased concentrations of biopolymers may indicate enhanced microbial activity and/or a change of OM source from allochthonous to autochthonous.

Humics and their degradation products, building blocks, can be defined as being naturally occurring, biogenic, heterogeneous organic substances that can generally be characterised as being yellow to black in colour, of high molecular weight, and more refractory in the environment [86].

Humic substances are formed by the organic breakdown of plant materials [106] and the decay of dead organisms [107]. In previous studies it was observed that humic substances in streams have different characteristics to humic substances in soils or the ocean, and marine humic substances have some definite aromatic character [108]. Furthermore, humic substances, particularly fulvic acids, can be generated by algae and contributed to the multitude of diverse compounds that comprised the dissolved humic substances [109]. Additionally, in-channel and streambed processing of soil-derived DOM is an important source of CO_2 in the stream network, wherein especially humic-like aromatic DOM is likely selectively photo-mineralised along the stream continuum [110–113].

LMW acids can occur in the course of functioning of soil or aquatic biota. They can continuously enter the soil with plant remains, root exudates, and products of microbial metabolism. They are actively involved in transformation and dissolution of minerals, as well as in migration of several chemical elements, and affect the great geological cycle of chemical elements and the behaviour of nutrients and pollutants in soils and landscapes. Some LMW acids are involved in signalling functions, chemotaxis, and detoxication of aluminium and heavy metals, as well as in stimulation or inhibition of plant growth and development [114].

LMW neutrals are characterised by low ion density, being hydrophilic to amphiphilic and their properties point to LMW alcohols, aldehydes, ketones, sugars, but also amino acids [95]. The material shows no or very little response in UV absorbance and fluorescence, hence it is referred to as optically “invisible” DOM (iDOM) [102]. Optical techniques typically employed to characterise DOM are not able to identify or resolve LMW neutrals and it is unlikely to be affected by photodegradation [115]. Although the LMW neutrals fraction has been recognised in water treatment research as e.g. a potential source of bacterial regrowth and biofilm formation [116, 117] its role in the fluvial environment needs further research. In tropical headwaters it was observed that LMW neutrals have the highest abundance during rainstorm events and that they are preferentially mobilised from the litter/surface soil pool [102], creating a scope for the present study.

1.5. Aim, objectives and hypotheses

The overarching aim of this study is to gain a deeper understanding of the quantitative and in particular qualitative dynamics of DOM in temperate Scottish fluvial systems. Many previous studies ascertained DOC concentrations, fluxes and exports from temperate rivers and headwaters and while some information exists about the compositional behaviour of DOM, especially in regard to UV absorbing DOM, there is a large uncertainty on the range of variability in DOM composition and its role in the aquatic environment. Consequently, it is necessary to examine the entire DOM pool, UV absorbing and UV invisible, and its interactions with different environmental processes, like precipitation, to better understand the mobilisation and fate of carbon and nutrients in rivers.

The three key hypotheses to be tested within this study are:

I. DOM flux and composition varies between river catchments as a function of the dominant soil type with higher export of high-molecular weight humic-like DOM from peat-dominated catchments compared to overall higher low-molecular weight DOM fluxes from non-peat catchments.
II. DOM composition is highly variable in all studied headwater streams depending on seasonal and hydrological conditions, with higher inputs of terrestrial DOM in winter during peak flow conditions than in summer with low flow conditions.
III. Precipitation events will favour the input of more recalcitrant, higher molecular weight DOM into the water column over more labile and lower molecular weight DOM by mobilising OM from the surface and the upper humic rich soil horizons rather than infiltrating deeper soil horizons.

To address the aim and the hypotheses the main objective is to assess the riverine DOM quality with well-known spectrophotometric methods as well as novel chromatography analysis within the seasonal hydrological cycle, in regard to their dominant soil type, and in response to precipitation events. The first hypothesis has been addressed in Chapter 4 with a more detailed peat versus peat-podzol study in Chapter 5. The second hypothesis has been tested in Chapter 5; however, seasonal hydrological conditions are also addressed in Chapter 6. The last hypothesis about precipitation events has been addressed in Chapter 6, with a link to soil analysis within Chapter 5.

1.6. Thesis outline

This thesis is composed of seven chapters starting with a general introduction that presents the background literature and the general framework of this thesis. An overview of the key methods and field sites used to generate the data presented in this thesis follows in Chapter 2, with further details provided in the respective results and discussion chapters. The four main research chapters (Chapters 3 to 6) of this thesis include a chapter addressing the stability of DOM quantity and quality over time while supporting the methodological approach of the three main results chapters each addressing a hypothesis listed above. The last chapter (Chapter 7) of this PhD thesis combines the core research conclusions and discusses ideas for future research based on the findings of this work. Figure 1.4 shows a simple schematic of the spatial and temporal variability between the chapters and how they are all connected with each other.

Chapter 3 - Assessing the advantages and disadvantages of freezing and pasteurisation for dissolved organic matter concentration and composition preservation

In this chapter the effects of freezing, fast freezing (with Liquid Nitrogen) and pasteurisation were compared on the DOM concentration and composition of river water to quantify and identify DOM changes due to these preservation methods as well as storage times. Additionally, this chapter looks into the quantity and quality changes of DOM of untreated river water over time to provide a credible temporal framework for the sampling strategies of Chapter 4 to 6.

Chapter 4 - The influence of dissolved organic matter flux and composition in Scottish Rivers for ecosystem function and climate feedback

In this chapter the compositional behaviour of DOM in large Scottish river systems was investigated in relation to hydrological conditions and dominant soil type. Monthly samples over a time of 10 Months from 10 Scottish rivers were analysed for their DOM quantity and quality using novel chromatographic and well know spectrophotometric methods. This chapter is a first look into the compositional variability, based on LC-OCD fractionation rather than relying on only optical parameters, of Scottish rivers and the importance of soil types and seasonal classification.

Chapter 5 - Insights on the DOM dynamics and composition of Scottish headwater catchments – influence of hydrology and seasonality

In this third research chapter the concepts of Chapter 4 were taken a step forward by looking at smaller spatial scale and a longer time period.

The DOM quantity and quality was observed in a lowland peatland and upland peat-dominated headwater catchments from a seasonal (Summer/Winter) perspective over a 2-year period to assess systematic changes that could largely be applied to different but widespread peat landscapes. In addition, soil samples from the two headwater catchments were analysed to characterise its composition and identify different soil horizons as a source for the riverine DOM fractions. Moreover, this chapter addresses the applicability of the Carter (2012) and Adams (2018) carbon model for peaty headwaters and identify shortfalls in current models to examine how carbon models can be utilised to investigate DOC composition and turnover.

Chapter 6 - Effect of extreme precipitation events on the dissolved organic matter composition in peat-dominated headwaters streams

The last research chapter builds on Chapter 5 and highlights the impact of precipitation events on the DOM composition from the two Scottish headwater catchments of Chapter 5. The effect of precipitation events was tested during a dry summer period (June-July 2018) and a wet winter (November 2018) to continue the seasonal trend analysis from previous chapters, as well as examine the compositional behaviour of DOM under different catchment dynamics (e.g. dry soils, rewetting etc.).

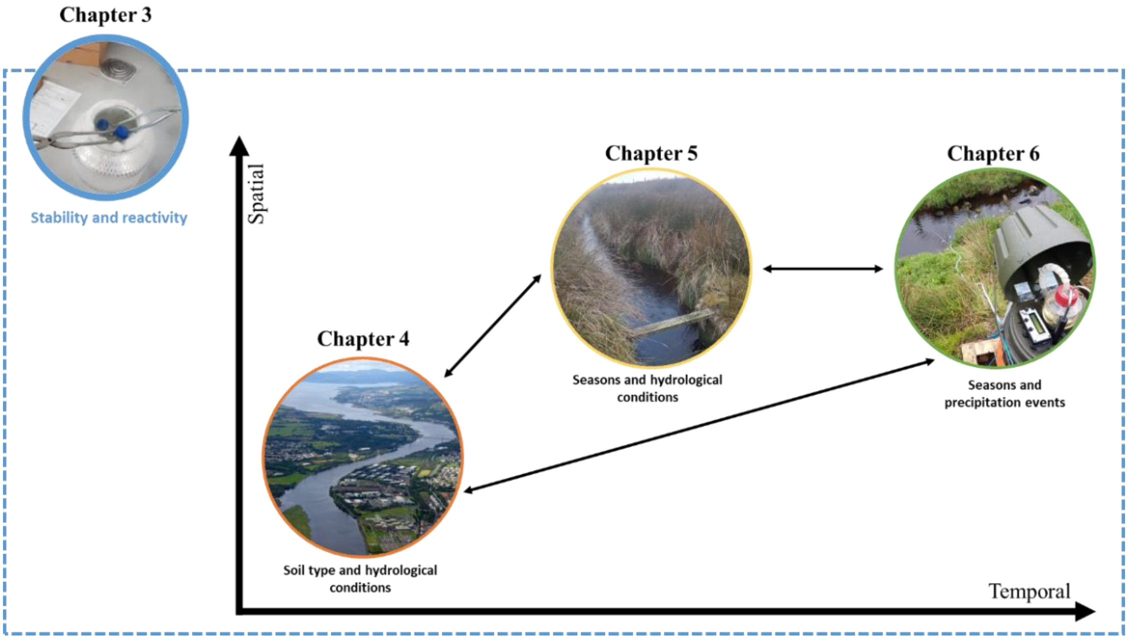

Figure 1.4 : Conceptual diagram showing the key research themes/questions of this thesis that investigates the interconnectivity of DOM and water over different temporal resolutions and spatial settings, detailed in chapters.

Chapter 2 - Materials and Methods

2.1. Study areas

2.1.1. Large Scottish rivers

The following 10 Scottish rivers were explored in this study: Halladale River, River Thurso, River Conon, River Cree, River Dee, River Deveron, River Forth, River Clyde, River Spey and River Tay. The proportional distribution of the land cover, the soil type and the catchment area from 10 Scottish rivers was obtained from the site description in the National River Flow Archive (https://nrfa.ceh.ac.uk/data/station/spatial/). The catchment area includes the main river and important headwaters (Figure 2.1).

River Clyde

The River Clyde catchment drains an area of approximately 1900 km^2, stretching from the Lowther Hills in the Scottish Borders to the Clyde estuary on the west coast of Scotland. The catchment is predominantly rural, comprising moorland hill terrain in its upper headwaters and agricultural land in the middle reaches. In contrast, the lower Clyde is urbanised with one of the UK's foremost historical industrial centres, the city of Glasgow situated close to the tidal limit [118]. Past and present industries of Glasgow and surrounding conurbations include shipbuilding, iron and steel manufacture, engineering, coal and other mining chemical production, as well as paper and textile manufacture [119].

River Conon

The Conon river system is composed of the River Meig and the stream which flows through Loch Luichart. The catchment area of the Conon river and its tributaries is around 960 km^2. The catchment is dominantly Mountain/Heather/Bog (74.2 %), Grassland (11.3 %) and Woodland (9.9 %) with till (unsorted glacial sediment), Alluvium (unconsolidated sediment that has been eroded and reshaped by water) and Peat being the superficial (geological youngest) deposits (UK National River Flow Archive).

River Cree

The River Cree is located in the Dumfries and Galloway Region, Scotland (West Region), (54°57′N; 4°28′W), with a catchment area of 366 km^2 [119]. A considerable part of this catchment is covered with woodland (around 50 %) and grassland (approximately 40 %). The catchment is characterised by highly siliceous granitic formations covered by organic rich acidic soils like peat and peaty gleys (UK National River Flow Archive).

It has become one of the most acidic catchments in south-west Scotland, with pH values as low as 5.0 being recorded occasionally in the river [119]. There are many sewage effluent discharges entering the river along its course; most of which have small flow rates [119]. The soils, derived from greywackes and shales, and the surface waters draining river Cree as an upland catchment are generally more responsive to changes in atmospheric deposition and afforestation [120].

River Dee

The River Dee is a river in Aberdeenshire, Scotland with a catchment area of approximately 1833 km^2. It rises in the Cairngorms and flows through southern Aberdeenshire to reach the North Sea at Aberdeen. The majority of the catchment is covered by mountain/heath/bog (63 %) and woodland (16 %) with dominantly humus-iron podzols and peaty podzols as soil types (UK National River Flow Archive). The Dee originates at 1220 m in the Cairngorms massif, and approximately half of the catchment area lies within the boundary of the Cairngorms National Park. The catchment is predominantly upland in character with 60 % of the total area lying above 300 m [121]. Average annual precipitation ranges from over 2000 mm in the mountainous western part of the catchment to 700 mm on the coast [122]. The Dee catchment is characterised by a ‘flashy’ runoff regime [123], although the precise hydrological regime of different sub catchments varies depending on the distribution of soils and glaciogenic drift cover [124].

The geology of the catchment is dominated by large lower Palaeozoic granite intrusions that form areas of high relief, whilst areas of low relief are underlain mainly by a mixture of metamorphosed sediments, quartzites, and schists [125].

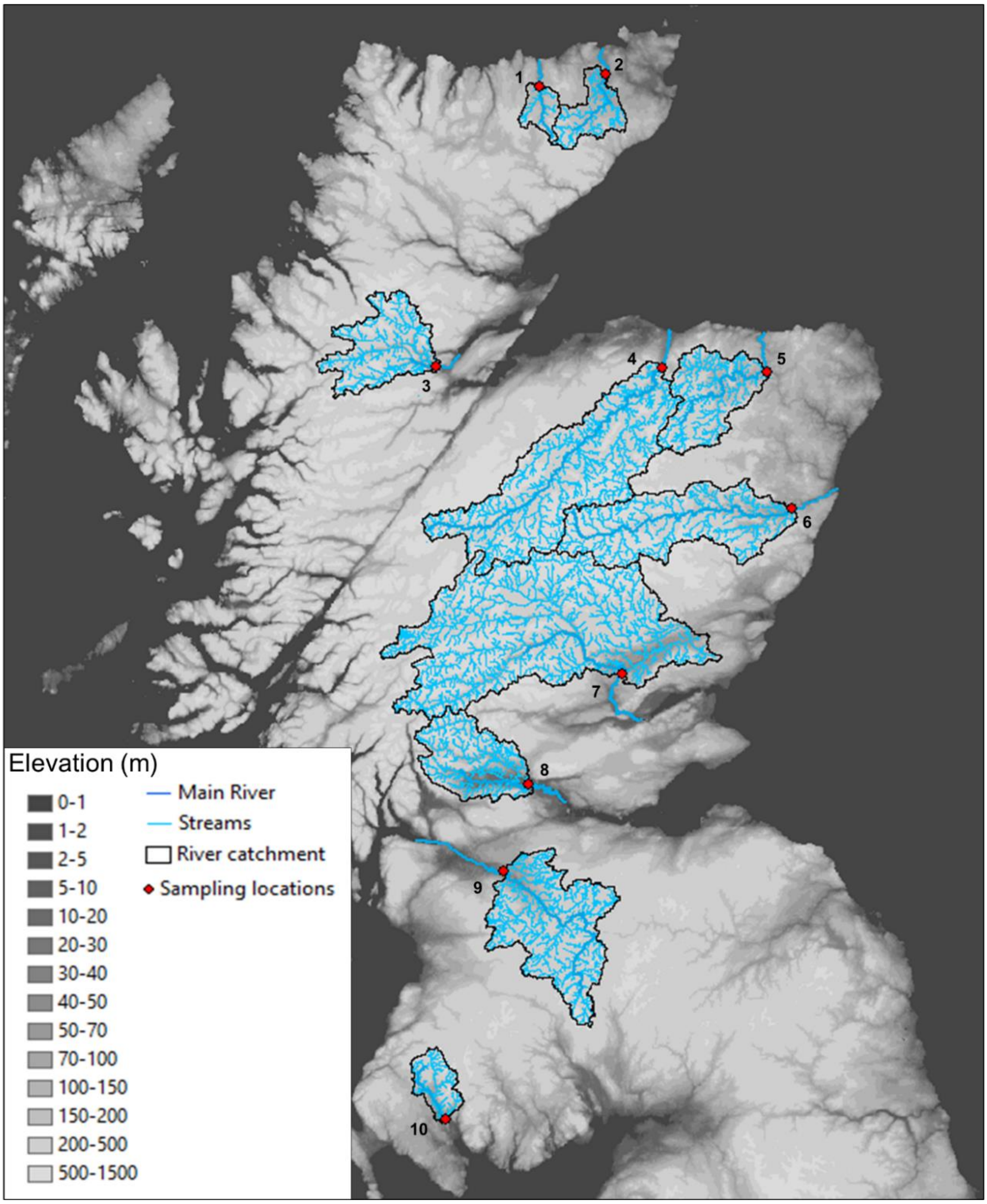

Figure 2.1: Elevation map of Scotland (GEBCO) with ten of the LOCATE rivers, their streams and headwaters (OS open rivers map) and their catchment outline (Data from the UK National River Flow Archive*)* The following rivers are shown in the map: 1) Halladale River, 2) River Thurso, 3) River Conon, 4) River Spey, 5) River Deveron, 6) River Dee, 7) River Tay, 8) River Forth, 9) River Clyde and 10) River Cree. Red dots are locations of rainfall and discharge station as well as water sampling location.

River Deveron

The River Deveron catchment includes the major tributaries of the River Bogie, River Isla, and Turriff Water and covers an area of 961 km^2. The Deveron rises in west Aberdeenshire and then flows through Huntly and Turriff before joining the North Sea at Banff. The catchment includes areas of moorland, farmland, and forestry, and also has a number of distilleries [126]. The main soil type are humus-iron podzols and brown earth.

The Deveron and its tributaries are important salmon, sea trout and brown trout fisheries. The catchment is underlain by a number of groundwater bodies and the water environment has been monitored on an ongoing basis. The majority of these are classified as being at good status for both water quality and quantity, but the eastern catchment around Turriff has issues relating to over abstraction and diffuse pollution [126].

River Forth

The River Forth catchment encompasses a large area – approximately 1029 km^2. Fresh water enters the Forth estuary from eight major rivers. Land-use within the catchment is predominantly rural, comprising managed forests and farmland with brown earth and mineral gleys as the dominant soil type (UK National River Flow Archive). In the lower-lying reaches, a greater degree of urbanisation can be seen with the City of Stirling and surrounding villages, interspersed with agricultural holdings [127]. Fresh water enters the estuary from eight major rivers. The combined freshwater discharges from the Rivers Teith, Forth and Allan, which enter the head of the estuary, constitute on average 75 % of the total freshwater flow to the estuary [128].

River Halladale

The Halladale River is a privately-owned salmon fishing river managed by the Strath Halladale Partnership and is located within the Caithness Flow Country blanket bog system of northern Scotland; the UK's largest (~ 4000 km^2) terrestrial carbon store. The Halladale River drains the hills to the southeast of Forsinard and flows northwards into the Pentland Firth at Melvich bay and has a catchment area of around 205 km^2 [129]. The catchment is covered mainly by bog (76 %) with peaty gleys and peaty podzol as the dominant soil types (UK National River Flow Archive).

The Flow Country of Caithness and Sutherland is the UK's largest (400,000 ha) blanket bog, of which about 150,000 ha are 'severely affected' by landscape scale drainage of the bog and non-native conifer planting [130]. So far, 2,200 ha of forestry have been felled and drains have been blocked across 16,500 ha of bog in Forsinard Flows [131].

River Spey

The River Spey catchment, located in north-east Scotland, has an area of approximately 2852 km^2, and flows into the Moray Firth (57°40N, 3°30W). Land cover is dominated by heather moorland, pastoral and arable agriculture, forestry, and native woodland [132]. Water-based industries include abstraction from four upper sub-catchments for hydro-electricity generation schemes in neighbouring catchments, and one commercial aquaculture site for rainbow trout. Water is also abstracted by whisky distilleries, public and private water supplies, and some irrigation [133].

River Tay

The Tay River is the longest river in Scotland (188 km) and it is the largest river in the UK by measured discharge. The Tay river catchment is one of Britain's largest, draining approximately 4587 km^2 of the Scottish Highlands and extending west to Ben Lui and Tyndrum, north almost to Dalwhinnie and east to Forfar [134]. The mountainous upper catchment is mainly covered by heather and bog whereas the main stream is dominated by woodland and grassland (UK National River Flow Archive. The river is of high biodiversity value and is both a Site of Special Scientific Interest (SSSI) and a Special Area of Conservation [135].

Generally, the catchment is characterised by thin soils and an impermeable bedrock contributing to high runoff rates, while Lochs Tummel, Tay and Lyon significantly reduce the flooding impact of heavy rains and snowmelt in the higher catchment by attenuating flood peaks [134].

River Thurso

The River Thurso has a catchment area of approximately 414 km^2 which is dominated by heather and bog (69 %) as land cover and peat is the main soil type (UK National River Flow Archive). The Thurso is, together with Halladale river, part of the Flow Country of Caithness and Sutherland, which is the UK's largest single peatland region [136]. A key feature of this area is the low, gently contoured expanse of peatland, with peat depth ranging from 0.4 m to 1.5 m. The dominant plant communities within the pristine areas of blanket bog are the *Sphagnum* Mosses and dwarf shrubs [136].

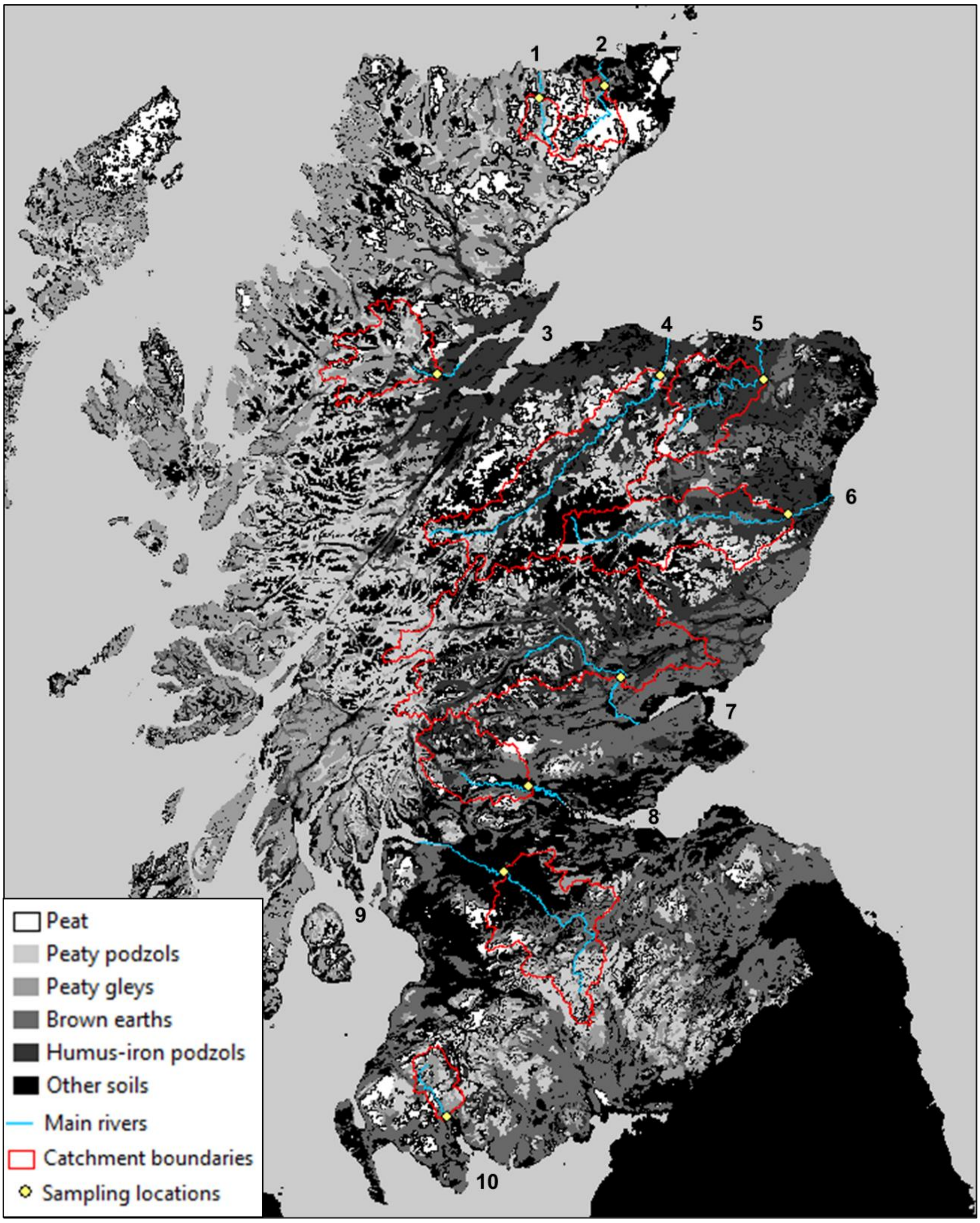

Figure 2.2: Soil type map of Scotland (GEBCO) with ten of the LOCATE rivers and their catchment outline (Data from the UK National River Flow Archive). The following rivers are shown in the map: 1) Halladale River, 2) River Thurso, 3) River Conon, 4) River Spey, 5) River Deveron, 6) River Dee, 7) River Tay, 8) River Forth, 9) River Clyde and 10) River Cree. Yellow dots are locations of rainfall and discharge station as well as water sampling location. Other soils include soil classification like immature soils, magnesian soils, mineral gleys, montane soils and alluvial soils.

2.1.2. Scottish headwater catchments

Headwaters (i.e., springs and intermittent, first and second-order streams defined by Strahler [137]) are abundant components of a river network. There is increasing evidence that the water quality, biodiversity, and ecological health of freshwater systems depend on functions provided by headwater streams.

Therefore, this study chose two Scottish headwaters, representing lowland and upland Scottish headwater catchments, covering common Scottish soils (peaty podzol and peat, [138]) and demonstrating different hydrometeorological pattern to investigate their water quality changes under diverse environmental processes. Auchencorth Moss was chosen as it is part of the long-term monitoring site of the UK Centre for Ecology & Hydrology and offers a historical record for comparison. Menstrie was chosen as a contrastable catchment to investigate the role of different soil types, water colour and topography on DOM functioning.

Auchencorth Moss

Auchencorth Moss is located in South East Scotland (55°47’36” N, 3°14’41” W). It is an ombrotrophic peatland with an extensive fetch at an elevation of 270 m, lying 18 km SSW of Edinburgh. The superficial deposits consist of a blue grey boulder clay overlain by peat deposits from < 0.5 m to 5 m thickness. Histosols (peats) cover approximately 85 % of the catchment, Gleysols (9 %), Humic Gleysols (3 %) and Cambisols (3 %) occur along the catchment margins [13].

The catchment has a long term data set including both terrestrial and aquatic carbon species, which includes a complete, inter-annual carbon and greenhouse gas (GHG) budget of CO_2, CH_4 and Nitrous oxide (N_2O) from 2008 onwards [139]. The site is used for sheep grazing occasionally but livestock is not kept on the site the whole year round so there are usually only a few tens of sheep on the bog, resulting in a very low livestock density of less than one livestock unit per hectare [140]. Since 2017 the site is used for peat extraction in the north-west of the catchment [141].

The vegetation cover over the main body of the peatland consists of grasses, rushes and sedges [140]. The peat catchment is drained by the Black Burn, a small stream with a width of ~1 m and a depth from peat surface to channel bed of ~1.2 m. The stream extends for 4700 m from source to the catchment outlet.

The Burn is characterised by low pH (~3 - 6), low temperature conditions and a dynamic hydrological response to rainfall events [23, 25]. Stream water is typically highly coloured and mean long-term DOC concentrations are 28.4 ± 1.07 mg/L [142].

It has a single land use over a small spatial area (6.7 km^2, Figure 2.3), which is ideal to investigate the response of DOM fluxes during hydrological events. The flume sampling point (long-term monitoring site) captures an area of ~3.3 km^2.

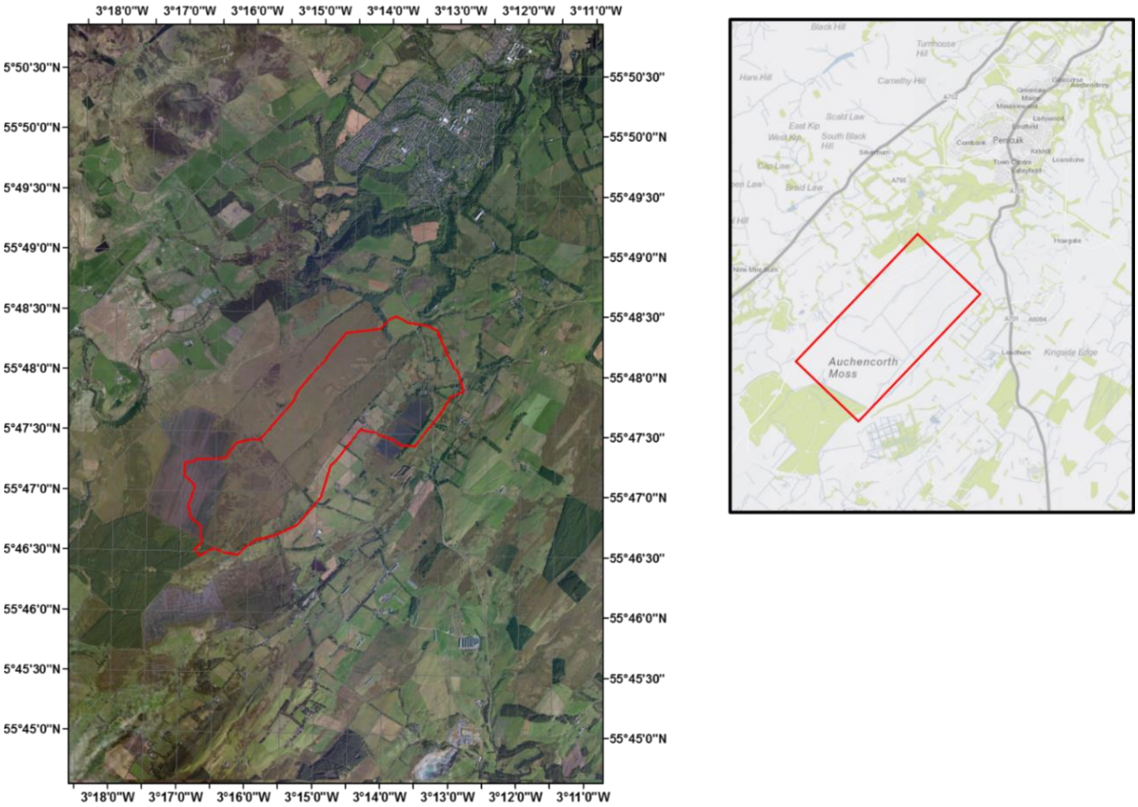

Figure 2.3: Location of Auchencorth Moss shown in the right panel (Open Street Map) and catchment area from the Black Burn stream shown in the left panel (Satellite Open Street Map).

Menstrie catchment

The Menstrie Burn catchment (56°09’43.7” N, 3°51’24.0” W) is a sub-catchment of the River Devon, a confluence to the Forth River. Menstrie Burn flows through Menstrie village, before its confluence to River Devon and has three tributaries named: Inch 1, Inch 2 and Inch 3. The whole catchment area is approx. 12 km^2 (Figure 2.4) whereby the main catchment with the three Inches is around 6 km^2 and the studied Inch 1 catchment is 0.9 km^2. The Menstrie catchment is not a typical peatland but the main soil type is peaty podzol, what covers large areas in Scotland. The river morphology is characterised by a steep slope with low deposition (mostly suspended load).

The Menstrie catchment area has four types of data monitoring due to another project led by Prof Lindsay Beevers and Martina Egedusevic from the Institute for Infrastructure and Environment, Heriot Watt University, which is focused on Natural Flood Management (NFM) and impact of initial site cultivations.

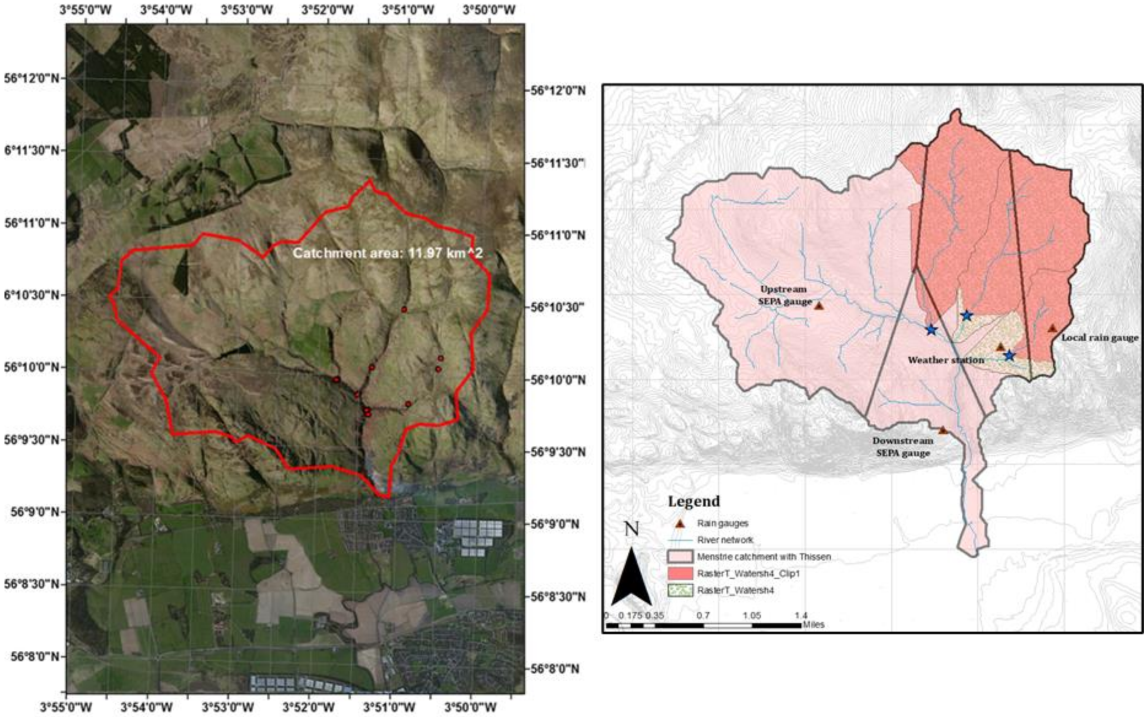

Figure 2.4: Menstrie Burn catchment boundary in a satellite map (left) (Open Street Map). On the right the Menstrie Burn catchment boundary, the catchment boundary from Inch 1,2 and 3, the installed rain gauges and weather station (red triangle) as well as the three sampling locations within the Inches (Blue stars) are shown.

The Menstrie field site has been dominated by extensive grazing mainly by sheep but also cattle. Woodland cover is sparse (< 3 %) and generally restricted to remnant riparian woodlands along the steep and least accessible lower sections of the Menstrie Burn. The upper catchment with the three Inches that run into the main stream is defined by six different cultivations: unplanted (natural grasses/mosses), hand screefing (small trees planted by hand), ploughed ground (trees planted in line with man-made trenches), excavator mound (mounds made by machinery by scooping mud out of holes), hand turfing and Scarifier (removing dead Moss and other debris before tree planting). The river water of the Menstrie burn is low in DOC (1 - 15 mg/L) and has almost no visible colouration. All presented Menstrie results in this chapter are from Inch 1, the furthest south tributary, due to its location between unplanted and planted areas with no impacts of ploughing (channels manually cut into the landscape).

2.2. Water level and river discharge

2.2.1. Large Scottish rivers (LOCATE)

Gauged daily flow data for the 10 observed Scottish rivers was downloaded from the UK National River Flow Archive (stations: 97002, 15006, 8006, 96001, 18011, 9002, 12002, 81002, 4001, 84013). The Gauged Daily Flow is the mean river flow in cubic metres per second in a water-day, (09.00 to 08.59 GMT, for example, 09.00 1st December to 08.59 2nd December) or, where indicated, a calendar day.

2.2.2. Headwater catchments

A Level TROLL 300 (manufactured by In-Situ Inc.), an absolute (non-vented) instrument that monitors and records changes in water head pressure and temperature was installed in the Black Burn in 2016. The barometric pressure was measured at the Auchencorth Moss Atmospheric Observatory (AMo). To calculate water level, the equation for Level Surface Elevation (Ls) was used, which uses a reference to measure water level with respect to surface-water elevation (staff gauge in streams or wetlands):

$$Ls = ((0.703073 * ((Pm-Bm) – (Pr-Br))) / SG) \qquad [143]\ [1]$$

Ls = Level Surface in meters
Pm - Total Pressure measured in PSI
Bm - Barometric Pressure measured at the time Pm was taken in PSI
Pr - Pressure Head at Reference in PSI
Br - Barometric Pressure measured at the time Pr was taken in PSI
SG - Specific Gravity

The measured water level data from the Level Troll 300 sensor in 2018 showed anomalously low water levels which lead to an installation in 2019 of a new level logger next to the old sensor to produce more reliable water level data and to be able to correct the older measurements. The following calculations were used to correct the Ls from the old level logger:

$$Ls_{corr} = Ls - WL_{new} \qquad [2]$$

$$TSL = Ls – Mean\ (Ls_{corr}) \qquad [3]$$

Ls_{corr} - Corrected Level Surface in meters
Ls - Level Surface from old sensor in meters
WL_{new} - Water level from new sensor in meters
TSL - True level surface in meters

Velocity is the other component needed to calculate discharge.

Manual velocity measurements were only taken in 2017, thus for comprehensiveness the Manning's equation was used for 2017 - 2019 to calculate cross-sectional average velocity flow:

$$V = (R_h^{2/3} * S^{1/2})/n \qquad [144]\ [4]$$

V - cross-sectional average velocity (m/s)
n - Manning coefficient of roughness ($s/[m^{1/3}]$)
R_h - hydraulic radius (m)
S - slope of stream (m/m)

Hydraulic radius can be expressed as:

$$R_h = A / P_w \qquad [5]$$

A = cross sectional area of flow (m)
P_w = wetted perimeter (m)

Wetted perimeter is calculated as follows:

$$P_w = \Sigma_i (l_i) \qquad [6]$$

where l_i is the length of the side i, and the sum runs over all of the sides in contact with the water.

The slope is 0.001 (m/m) in the Auchencorth Moss catchment, which was measured in the field. A Manning's coefficient n of 0.04 was used for water levels <1.5 m and 0.115 for water levels ≥1.5 m [144]. The channel width of the stream is 0.82 m at the location of water level measurements. Discharge was calculated as follows:

$$Q\ (m^3/s) = V\ (m/s) * A\ (m^2) \qquad [7]$$

For the Menstrie burn and its tributary Inch 1 discharge was calculated with equation 7 by using the measured velocity and water level by locally installed Stingray 2.0 loggers (manufactured by Greyline, 5 min resolution flow measurements).

2.3. Baseflow index

The Base flow index (BFI) is a non-dimensional ratio which is defined as the volume of baseflow divided by the volume of total streamflow (or alternatively, as the ratio between the average discharge under the separated baseflow hydrograph and the average discharge of the total hydrograph) [145].

$$BFI = \frac{\sum_i b_i}{\sum_i d_i} \qquad [8]$$

with b_i base flow values (entire series)

d_i total streamflow values (entire series)

It describes the effect of geology on low flows and in catchments with high groundwater contribution to streamflow, BFI may be close to 1, but it is equal to zero for ephemeral streams [146].

2.4. Precipitation

2.4.1. Large Scottish rivers (LOCATE)

The catchment daily rainfall data was downloaded from the UK National River Flow Archive (stations: 97002, 15006, 8006, 96001, 18011, 9002, 12002, 81002, 4001, 84013). The data is a time series of the total rainfall averaged over the catchment in millimetres for each day. The catchment averaged daily rainfall data have been derived from the UK Centre for Ecology & Hydrology – Gridded Estimates of Areal Rainfall (UK CEH-GEAR) data, a 1 km gridded rainfall dataset generated from all daily and monthly observed rainfall data available for the UK from the Met Office [147].

2.4.2. Headwater catchments

Precipitation data for Auchencorth Moss was collected via tipping buckets run through the Auchencorth Moss Atmospheric Observatory (AMo) and downloaded from the Natural Environment Research Council's Data Repository for Atmospheric Science and Earth Observation [148].

The precipitation data from Menstrie was collected from existing Scottish Environment Protection Agency (SEPA) gauges in the lower catchment (Glenochil station) and one local rainfall station (lower Inch 1) and meteorological station in the upper catchment (lower Inch 2) installed as part of another Heriot-Watt PhD study. The local rainfall station is the HOBO Data Logging Rain Gauge RG3 combines a tipping bucket (0.2 mm) and a pendant data logger. The meteorological station is Vantage Pro2 Weather Station records data from wind direction, wind speed, rainfall (tipping bucket 0.4 mm), temperature, solar radiation, and humidity. During June - July 2018 and November 2018 a tipping bucket (0.1 mm resolution, ISCO 674 Rain Gauge) was installed together with an ISCO autosampler at the upper Inch 1 sampling location.

2.5. Sampling strategies

2.5.1. Water samples

Sample collection of the large Scottish rivers was conducted monthly by colleagues from the UK Centre for Ecology and Hydrology and the Environmental Research Institute in Thurso within the "Land Ocean CArbon TransfEr" (LOCATE) project. LOCATE is a multi-disciplinary project that undertakes coordinated sampling of 41 major rivers in Great Britain to establish how much carbon from soils is getting into rivers and estuaries and to determine what is happening to it [149, 150]. The water samples were collected close to the tidal limit, and where possible at long-term national hydrometric and water quality monitoring sites.

The headwaters were sampled monthly to biweekly from February 2017 to March 2019 in Auchencorth Moss and from May 2017 to March 2019 in the Menstrie catchment. Additionally, hourly to eight hourly samples were taken with the help of autosamplers in summer (1st June 2018 to 15th July 2018) and during winter (1st to 30th November 2018) in the Auchencorth Moss and Menstrie catchment. All autosamplers were equipped with 24 Polypropylene bottles (ISCO=1 L, EPIC=750 mL), which were cleaned two times with MilliQ water and twice rinsed with river water before usage. The Black Burn in Auchencorth Moss was sampled every four hours with a pre-installed Teledyne ISCO autosampler. The autosampler was configured to take water samples from the Black Burn every four hours.

Inch 1 in the Menstrie catchment was sampled with the help of an ISCO autosampler (manufactured by Teledyne ISCO, USA, Model: 6712 Full-size Portable Sampler) and the Menstrie Burn was sampled with an Epic 1011 autosampler (manufactured by Hach Lange Ltd, Manchester, UK). Both autosamplers were initially configured to take water samples every four hours. The ISCO autosampler was equipped with a rain gauge (manufactured by Teledyne ISCO, Model: 674, tipping bucket). In November 2018, the ISCO autosampler at the Menstrie catchment was triggered to sample every eight hours (12 bottles) plus collection of rainfall triggered (0.5 mm within one hour) samples (12 bottles).

For the preservation experiment (Chapter 3) approximately 20 L of water were collected using a carboy that was pre-cleaned with 10 % Hydrochloric acid (HCl), rinsed in copious amount of deionised (18.2 $M\Omega\ cm^{-1}$) laboratory water and then rinsed in sample water three times at the sampling site.

The 20 L sample was sealed and shaken for several minutes to ensure adequate mixing. This water was decanted on site in either 500 mL PET bottles or 15 mL PP tubes ensuring that headspace is minimised.

The bottles were then stored in the dark and returned to the laboratory within one hour where they were prepared for preservation using a sacrificial approach in triplicate.

2.5.2. Greenhouse gas samples

Concentrations of CO_2 and CH_4 in stream water were measured directly using a headspace equilibration technique [151]. A 40 ml water sample was collected in a 60 mL plastic syringe equipped with three-way valves and equilibrated with 20 ml of ambient air at stream temperature by shaking vigorously under water for 1 min. The equilibrated headspace was then transferred to a gas-tight 12 mL glass exetainer (Labco) and returned to the laboratory for analysis. On each sampling occasion, two replicate headspace samples were collected alongside a separate sample of ambient air.

2.5.3. Soil samples

Soil samples were taken in 2018 and 2019 from the Auchencorth Moss catchment and 2019 from unplanted area close to Inch 1 in the Menstrie catchment.

The soil samples from 2018 were taken vertically with an open-face soil auger (10 cm length) at four soil depths (see Table 2.1) in Auchencorth Moss. The soil auger was cleaned with MilliQ water between the samples. The soil samples were stored in labelled glass jars covered with clean aluminium foil and closed with a plastic lid.

In 2019 the soils were sampled horizontally with 10 cm PVC tubes from the 4 identified soil horizons in Menstrie and Auchencorth Moss (see Table 2.1). The samples were collected by creating a 30x30 cm hole with a maximum depth of 70 cm and pushing PVC tubes into the different soil horizons. The two open endings of the labelled PVC tube were than covered by clean aluminium foil.

Table 2.1: Location, coordinates, depth and soil classification for soils collected in March 2018 and 2019.

Location	Coordinates	Depth (cm)	Soil classification
Auchencorth Moss	55.792725 N -3.247605 W	0-10	topsoil, slightly reddish, mostly roots
		10-30	blackish soil, 10YR 2/1
		31-49	beige, 10YR 4/2 dark greyish brown, slightly sandy
		50-84	same as C-horizon but with more "iron" inclusions
Menstrie	55.792935 N -3.247891 W	0-20	Topsoil, vegetation cover
		20-25	black horizons with roots, uneven clay distribution, 5-10YR2/1
		26-45	relative consistent, some roots, some grey clay, 5YR 4-3/3
		46-65	potentially same as C-horizon with more rocks and "iron" inclusions, 5YR 3/2 dark reddish brown

2.6. Sample preparation

2.6.1. Water samples

Water samples from the large rivers were filtered immediately through 0.45 µm silver nitrate filters using rubber free syringes into HDPE bottles for DOC analysis and amber glass bottles for UV absorbance measurements by colleagues from UK CEH and ERI. The Samples were posted to the laboratory under cool conditions within 24 h of collection and stored at 4°C until further analysis.

All water samples from the focal headwaters were filtered with 0.45 µm Polyethersulfone (PES) syringe filters, as DOC is operationally defined as the organic carbon in water that has passed through a 0.45 µm filter [58]. PES filters were chosen as they are easy to clean, show minimal carbon leaching and have relatively consistent performance from filter to filter [152]. Water samples were immediately filtered in the field and brought to the laboratory at the Lyell Centre, Heriot-Watt University for storage at 4 °C and analysed within 24 hours of collection, if not differently described in the individual research chapters.

2.6.2. Soil samples

Soil samples were frozen in a -20 °C freezer before being freeze dried for 48 hours in their glass container. The soils were then ground to powder by using the Vibrating-disc mill (TD 750 Model) and stored in a new labelled and pre-cleaned glass container until further analysis. Soil leachate experiments were conducted on all collected soil samples from 2018 and 2019.

Water-extractable organic matter (WEOM) was made by mixing around 2 g of soil with 20 mL of ultrapure water. The mixture was shaken for 24 hours at a constant temperature of 25 °C at 150 rpm and put through a pre-combusted 0.7 µm GF/F filter. The soil extracts were analysed for DOM fractions on the LC-OCD-OND.

Table 2.2: Weight of soil samples

Sample name	Sampling date	Weight (grams)
Auch_1_2018_ground	23/03/2018	2.04
Auch_2_2018_ground	23/03/2018	2.17
Auch_3_2018_ground	23/03/2018	2.01
Auch_4 2018 ground	23/03/2018	2.80
Auch_5_2018_ground	23/03/2018	1.99
Auch_6 2018 ground	23/03/2018	2.00
Auch_7_2018_ground	23/03/2018	2.06
Auch_8 2018 ground	23/03/2018	2.02
Auch_9 2018 ground	23/03/2018	2.00
Auch_B_2_110319_ground	11/03/2019	2.00
Auch_C_2_110319_ground	11/03/2019	2.03
Auch_D_1_1103319 ground	11/03/2019	2.03
Auch_topsoil_110319_ground	11/03/2019	2.02
Men_250118_HS_deep_ground	25/01/2018	2.00
Men_250118_HS_surface_ground	25/01/2018	2.00
Men_250118_PG_deep_ground	25/01/2018	2.01
Men_250118_PG_surface ground	25/01/2018	2.00
Men_250118_UP_deep_ground	25/01/2018	2.00
Men_250118_UP_surface ground	25/01/2018	2.03
Men_B(+C)_110319_ground	11/03/2019	1.99
Men_C_2_110319_ground	11/03/2019	2.01
Men_D_1_110319_ground	11/03/2019	2.04
Men_topsoil_110319_ground	11/03/2019	1.99

2.7. Sample analysis

2.7.1. Dissolved organic and inorganic carbon

DOC and DIC were measured using a total organic carbon (TOC) Analyser (Sievers M5310 C) from GE Analytical Instruments. The TOC analyser oxidises organic compounds to CO_2 using UV radiation and a chemical oxidising agent (ammonium persulfate). CO_2 is measured using a sensitive, selective membrane-based conductometric detection technique.

For each DOC measurement, the concentration of inorganic carbon species (CO_2, HCO_3^-, and CO_3^{-2}) is determined and, after oxidation of the organic compounds, the total carbon (TC) content of the sample is measured.

The concentration of the organic compounds is then calculated from the difference between the concentrations of TC and total DIC:

DOC = TC – DIC.

The instrument is also calibrated using certified TOC Standards (1, 5, 10, 25, 50 mg/L TOC as Potassium hydrogen phthalate) and IC Standards (10 mg/L IC as Na_2CO_3) purchased directly from GE SUEZ (list part no STD 77000-01). Drift of the instrument was checked using a sucrose standard, which was used as a standard for accuracy and reproducibility of the system. Sucrose crystals (weights 0.4 - 1.2 mg) were dissolved in 30 mL MilliQ water in a 40 mL glass vial. Standard deviations and relative standard deviations are based on a GE Sievers automated "leave one out" principle where the three closest measurements out of four measurements from the same vial are presented. Sucrose DOC concentrations ranged from 4.5 - 14.2 mg/L with a standard deviation (Std. Dev.) of maximum 0.5 mg/L and a relative standard deviation (RSD) of 0.03 - 7.5 %. The DIC Std. Dev. was below 0.01 mg/L and the RSD being 0.2 - 0.9 %. The RSD from water samples ranged between 0.03 - 20 % for DOC and 0.03 - 15 % for DIC.

2.7.2. Greenhouse Gases

GHG are defined as trace components of the atmosphere that absorb infrared radiation emitted by the Earth's surface. The GHG compounds considered in this research are CO_2 and CH_4 which occur naturally in the atmosphere. The GHG are measured with the TRACE 1300 Gas Chromatograph (Thermo Scientific) which consists of two separate channels with 1/8" in stainless steel packed columns (HayeSep Q 80/100). The first channel employs two valves with Flame Ionization Detector (FID). This channel provides the flexibility for CO_2 in varying levels. Low level CO_2 can be converted to CH_4 through the methaniser and measured by FID.

Another micro-ECD channel with two valves is dedicated to measuring N_2O and Sulphur hexafluoride (SF_6); however, these GHG were not considered in this study due concentrations below used gas standards. Pre-columns direct heavier components (mainly water) to be back-flushed to vent 1 and vent 2, hence Oxygen (O_2) should be excluded from the methaniser and micro-ECD. The installed instrument method had the following settings: Oven temperature is 70 °C, equilibration time is 0.10 minutes, Valve oven temperature is 70 °C, Methaniser temperature is 375 °C, Front Detector (ECD) temperature is 350 °C, Makeup Gas is 5.0 mL/min and Back Detector (FID) Temperature is 250 °C, Makeup Gas is 30 mL/min.

The two used standards are gas mixtures of CO_2, CH_4, N_2O and SF_6 in different concentrations: High concentration standard (1000 ppm CO_2, 1000 ppm CH_4, 500 ppm N_2O and 5 ppm SF_6) and low concentration standard (10 ppm CO_2, 10 ppm CH_4, 10 ppm N_2O and 500 ppb SF_6). Every standard calibration included at least three measurements of each standard before samples were measured.

2.7.3. Soil organic matter composition

The soil organic matter (SOM) data (total and organic carbon, total nitrogen, $\delta^{13}C_{org}$ and $\delta^{15}N$) was obtained at Iso-Analytical Ltd, Cheshire UK, an external service laboratory. The laboratory procedure for sample processing is described below.

Weighed samples are placed in universal tubes, acidified with 2M hydrochloric acid (HCl), mixed, oven heated at 60 °C for two hours and left for 24 hours to allow all carbonate to be liberated as CO_2. The sample fractions are then isolated by centrifugation and the acid is decanted. The samples are then washed twice using distilled water and centrifuged again before being oven dried at 60 °C.

The samples are then analysed using an Elemental Analyser - Isotope Ratio Mass Spectrometry (EA-IRMS). In brief, tin capsules containing ground sample or reference material are loaded into an auto-sampler on a Europa Scientific elemental analyser, from where they were dropped in sequence into a furnace held at 1,000 °C, where they are combusted in an oxygen rich environment, raising the temperature to ~1,700 °C. The gases produced on combustion are swept in a helium stream over combustion catalyst (Cr_2O_3), copper oxide wires to oxidise hydrocarbons and silver wool to remove sulphur and halides. The resultant gases, N_2, NO_x, H_2O, O_2 and CO_2 are swept through a reduction stage of pure copper wires held at 600 °C. This step removes O_2 and converts NO_x species to N_2. A magnesium perchlorate chemical trap removes water. CO_2 is separated from nitrogen by a packed column gas chromatograph held at an isothermal temperature of 100 °C. The resultant CO_2 chromatographic peak enters the ion source of the Europa Scientific 20-20 IRMS where it is ionised and accelerated. Gas species of different mass are separated in a magnetic field and then simultaneously measured using a Faraday cup collector array to measure the isotopologues of CO_2 at m/z 44, 45, and 46. Both references and samples are converted and analysed in this manner. The analysis proceeds in a batch process, whereby a reference is analysed followed by a number of samples and then another reference.

The reference material used during analysis was IA-R001 (wheat flour, 40.20 %C and 1.88 %N). For quality control purposes check samples of IA-R001, IA-R005 (beet sugar, 42.0 %C) and IA-R069 (tuna protein formula, 41.27 %C and 10.53 %N) were analysed during batch analysis of the samples.

2.7.4. Chromophoric and fluorescent dissolved organic matter

Samples for coloured (chromophoric) DOM (CDOM) and fluorescent DOM (FDOM) were measured with the Aqualog Spectrophotometer (Horiba). The Aqualog is the only instrument to simultaneously measure both absorbance spectra and fluorescence Excitation-Emission Matrices (EEMs).

CDOM absorbance was measured in a 1 cm path length quartz cuvette from 230-500 nm at medium speed, at 2.1 nm wavelength resolution, at room temperature (20 °C) and blank corrected using deionised (18.2 MΩ cm^{-1}) laboratory water prior to analysis. Absorption coefficients a(λ) were calculated from absorbance (A_λ) values as:

$$a(\lambda), m^{-1} = 2.303 * A_\lambda / L \qquad [9]$$

where L is the path length of the cuvette (0.01 m).

As CDOM absorption is exponential, the absorption spectral slope S (nm^{-1}) was determined by a standard equation by linear regression of log-transformed absorption spectra against the wavelength:

$$a(\lambda) = a_0 e^{-S(\lambda - \lambda_0)} \qquad [10]$$

With $a(\lambda_0)$ being the absorption coefficient at a reference wavelength λ_0. Multiple 20-nm wavelength intervals in a stepwise (1 nm) linear regression analysis was used according to Loiselle et al (2009) [153]. S (Spectral Slope) computed between 275 and 295 nm, S1, has been shown to correlate with changes in CDOM aromaticity and inversely related with DOM molecular weight. Additionally, a multiple 50-nm wavelength interval in a stepwise (1 nm) linear regression analysis was used to determine spectral slope between 350 and 400 nm (S2) [85]. S1 and S2 were used to calculate the spectral slope ratio (S_R) (275 – 295 nm slope divided by 350 – 400 nm slope), a useful indicators of CDOM composition [85]. Lower S_R values (≤ 2.0) indicate terrestrially derived or microbially altered CDOM while higher S_R values (≥ 2.0) may indicate lower molecular weight of the DOM pool [85].

The specific UV absorbance at 254 nm ($SUVA_{254}$) has been shown to be strongly correlated with the hydrophobic organic acid fraction of DOM [154], where $SUVA_{254}$ is calculated according to DOC values ($SUVA_{254}$ = a(254) / DOC mg L^{-1}) and is a useful proxy for DOM aromatic content [97].

In this study the UV absorbance at 254 nm was measured with the Aqualog and normalised with DOC measured with the LC-OCD-OND system by integrating the bypass which represents the bulk sample before it goes through the column.

For FDOM, 3-D fluorescence spectroscopy with the data assembled into EEMs. Samples were acclimatised and scanned at a fixed 20 °C temperature in 1 cm path length quartz cuvette. Scans were performed using an excitation range (Ex) of 230 - 500 nm with 2.1 nm increments and recorded emission (Em) in the range 244 - 825 nm with 2.4 nm increments. Samples were run in a mode of 5 nm slit for both excitation and emission and 1.5 s integration time.

The 3-D recorded spectra were internally corrected for the instrumental biases both for excitation and emission ranges. Additionally, spectra were corrected against Deionised (18.2 MΩ cm^{-1}) laboratory water prior to analysis to remove water Raman peaks. Fluorescence spectra were normalised to Raman Units (RU) by integrating the Raman peak of 350 nm excitation and 382 to 407 nm emission extracted by deionised (18.2 MΩ cm^{-1}) laboratory water.

The fluorescence index (FI) (the ratio of the emission intensity at a wavelength of 450 nm to that at 500 nm at an excitation wavelength of 370 nm) provides information about the source (> 1.8 = microbial or lower molecular weight and < 1.2 = terrestrial or higher molecular weight) or degree of degradation of DOM because it reflects the relative contribution of aromatic vs. nonaromatic DOM [155, 156].

The humification index (HIX) was calculated as the ratio H/L of two spectral region areas of the emission spectrum scanned at 254 nm excitation. Area L is calculated between the emission wavelengths 300 nm and 345 nm, and area H between 435 nm and 480 nm [157]. HIX informs on the degree of DOM aromaticity as the emission spectrum at excitation 254 nm is shifted towards the red (longer wavelengths), implying an increase in H/L ratio and in HIX. High HIX implies maximum fluorescence intensity at long wavelengths and therefore the presence of complex molecules like higher molecular weight aromatic DOM [158].

2.7.5. Dissolved organic matter fractionation

General description

The Model 9 of the Liquid Chromatography system with Organic Carbon, Organic Nitrogen and UV Detector (LC-OCD-OND-UVD) was used to analyse DOM concentration and composition. Size-Exclusion Chromatography (SEC) was performed using a HW50S column (Tosoh, Japan, 250 x 20 mm) with a mobile phase at a flow rate of 2 mL/min comprising of a mild phosphate buffer (potassium dihydrogen phosphate 1.2 g/L plus 2 g/L di-sodium hydrogen phosphate x 2 H_2O, pH of 6.58). Before entering the pump, the buffer was purified by on-line exposition to about 20 W of UV-light (254 nm, 185 nm) for 1 hr to remove all organic impurities. The UV reactor also produces small amounts of stable peroxides which suppress the microbial activity in the chromatographic column. A sample injection volume of 1 mL for river water and 4 mL for deionised (18.2 $M\Omega\ cm^{-1}$) laboratory water was used. The LC system has in total three detectors for (a) organic carbon (OCD), (b) UV-absorption at 254 nm (UVD) and (c) nitrogen (OND).

DOM compound groups

DOM compound groups identified are: HOC (hydrophobic organic carbon) and CDOC (chromatographic DOC, hydrophilic organic carbon), which consists of biopolymers (including proteins based on OND), humics (including molecular weight and aromaticity measurements), building blocks (lower molecular weight humic substances), low molecular weight (LMW) acids and LMW neutrals which are combined DOC [95].

Additionally, in the UV-reactor, organic carbon is converted to carbonic acid while organically bound nitrogen (e.g. bound to humic substances or biopolymers) and inorganically bound nitrogen (ammonium, nitrite and urea) is converted to nitrate while primary nitrate remains unaltered. Nitrate absorbs strongly in the deep UV-range, which is used to quantify nitrate in a UV-detector at 220 nm [95].

Specific UV absorbance ($SUVA_{254}$)

Besides the organic carbon detector, the system also incorporates a UV detector, which is used to assess the aromaticity of the bulk sample by processing its respective SUVA values. The specific UV absorbance at 254 nm ($SUVA_{254}$) is calculated by dividing the special absorbance coefficient (SAC), with the DOC concentration, both derived from the bypass. Low $SUVA_{254}$ values of 1.3 - 1.8 L/(mg*m) are comparable to values reported for the hydrophobic organic acid fraction (HPOA) isolates from microbial-dominated end-members [97] and aquatic systems with little vascular plant input [159].

Higher $SUVA_{254}$ values of 3.2 - 5.3 L/(mg*m) were reported for HPOA isolates from allochthonous-dominated endmembers [97] and aquatic systems with significant vascular plant inputs [159, 160].

The LC-OCD system also allows the calculation of additional SUVA values by measuring the SAC and organic carbon concentration from the hydrophilic (chromatographic DOC; CDOC), UV amenable fractions - humic substances, building blocks, LMW acids and amphiphiles; referred to as $SUVA_{CDOC}$ in this study. $SUVA_{CDOC}$ represents the aromaticity of the UV amenable DOM fractions that are measured through the column, whereas $SUVA_{254}$ represents the bulk DOC pool from the bypass including HOC. Additionally, SUVA from humic substances ($SUVA_{HS}$) and SUVA from building blocks ($SUVA_{BB}$) were used alongside the bulk $SUVA_{254}$ and $SUVA_{CDOC}$ values to describe compositional changes of DOM. Overall, $SUVA_{254}$ values have been positively correlated to the percent aromaticity of DOM as measured by 13C-NMR; however, sample pH, nitrate, and iron were found to influence SUVA measurements [97]. The LC-OCD-OND systems also allows to calculate the nominal average molecular weight (Mn-value) from the humics fraction which can be plotted against the SAC/OC ratio ($SUVA_{HS}$) in the Humification pathway [95]. The humics fraction is calibrated using the Suwannee river Standard III humic (HA) and fulvic (FA) acids from the International Humic Substances Society (IHSS) [161].

Quality Assurance

The limit of detection (LOD) of the OND and OCD were calculated as three times the standard deviation of the mean area of the noise for six blank injections (Milli-Q water), and the values were converted to concentration units using calibration curves. The limit of quantification (LOQ) was calculated in a similar way, except that it was taken as 10 times the standard deviation of the mean area of the noise. The LOD for OCD and OND were 82 ppb/C and 53 ppb/N, respectively. The corresponding LOQ were 273 ppb/C and 176 ppb/N, respectively. The LOD for OCD for biopolymers were 11 ppb/C and LOQ were 38 ppb/C. Building blocks have an LOD of 10 ppb/C and a LOQ of 33 ppb/C. LMW acids have an LOD of 3 ppb/C and an LOQ of 10 ppb/C. LMW neutrals have a LOD of 38 ppb/C and a LOQ of 128 ppb/C. The LOD for DON biopolymers (DON_{BP}) was 5 ppb/N and LOQ was 18 ppb/N.

The reproducibility of OCD and OND was tested by injecting eight times river water from Menstrie with a mean of 2.7 ppm/C and 0.5 ppm/N and four times river water from Auchencorth Moss with 26.7 ppm/C and 64 ppb/N, respectively. For OCD, the relative standard deviations (RSDs) varied from 2.7 % for 2.7 ppm/C to 2.8 % for 26.7 ppm/C. For OND, the RSDs varied from 1.5 % for 0.5 ppm/N to 6.1 % for 64 ppb/N.

RSDs for the DOC fractions were as follow: biopolymers varied between 5.3 % for 7.3 ppm/C and 11.8 % for 0.2 ppm/C, humics varied between 1.8% for 21 ppm/C and 5.7 % for 2.2 ppm/C, building blocks varied between 3.5 % for 1.2 ppm/C and 6.2 % for 0.2 ppm/C, LMW neutrals varied between 4.8 % for 2 ppm/C and 3.4 % for 0.3 ppm/C, lastly LMW acids varied between 6.9 % for 0.6 ppm/C and 22.9 % for 0.1 ppm/C.

2.8. Dissolved organic matter flux calculation

DOC fluxes were calculated by using ‘‘method 5’’ from Littlewood et al. [162]:

$$\text{Flux} = K\frac{\sum_{i=1}^{n}[C_i Q_i]}{\sum_{i=1}^{n} Q_i}\ Q_T \qquad [11]$$

where K = conversion factor allowing for period of sampling (days, hours, minutes)

C_i = concentration of determine and in sample i

Q_i = instantaneous discharge at sampling time i

Q_T = mean river discharge over the period

n = number of samples.

Using DOC concentration from sample in parts per million (ppm), instantaneous discharge at sampling time and mean river discharge over the time period in cubic meters per seconds, as well as a ‘k’ factor to correct for time (seconds to hours).

$$\text{Area normalised Flux} = \frac{\text{Flux}}{\text{Catchment area}} \qquad [12]$$

Area normalised flux was calculated following formula 12. The unit of catchment area was either hectare (ha) or square kilometre (km^2).

2.9. Statistical analysis

2.9.1. Standard deviation, Standard error and t-test

Standard deviation (SD) calculation was used for normal distributed datasets to measure the dispersion of the data from its mean. It measures the absolute variability of a distribution; the higher the dispersion or variability, the greater is the standard deviation and greater will be the magnitude of the deviation of the value from their mean [163]. Standard deviations were calculated by using 'Stdev.S.' function in Microsoft Excel after the following formula:

$$\mathrm{SD} = \sqrt{\frac{\Sigma(\mathrm{x_i}-\bar{\mathrm{x}})^2}{\mathrm{n}-1}} \qquad [13]$$

x_i - takes on each value in the set

$\bar{x}$ - is the average (statistical mean) of the set of values

n - is the number of values

The standard error (SE) was used to inform how accurate the mean of any given sample from that population is likely to be compared to the true population mean [163]. When the standard error increases it becomes more likely that any given mean is an inaccurate representation of the true population mean. The SE was calculated using the following formula:

$$SE = \frac{SD}{\sqrt{n}} \qquad [14]$$

SD – Standard deviation of sample

N – sample size

The T-test, specifically the paired t test, was used to compare two groups with the condition that the two groups are dependent on each other [164] and normally distributed. T-tests were carried out using Microsoft Excel using the following function:

T.TEST (array1, array2, tails, type) [15]

Array1 - The first data set.

Array2 - The second data set.

Tails - Specifies the number of distribution tails. Tails = 2

Type - The kind of t-Test to perform. Type 1 - Paired

The t-test result or p-value is a value is expressed in decimal to support or reject the null hypothesis. The following statistical significance levels were defined:

Significant: $p \leq 0.05$

Insignificant: $p > 0.05$

2.9.2. Non-parametric statistical test

The chosen non-parametric for analysing discharge-precipitation patterns as well as discharge-DOM relationships is the Mann Kendall (MK) test. Non-parametric MK test data has been implemented in previous studies as a suitable method to identify trends in the hydro-metrological observations that do not require regular distribution of data points [165, 166]. The theoretical background and calculations of the MK test can be found in a number of publications, e.g. Libiseller et al. [167] and Da Silva et al. [168]. IBM SPSS Statistics were used to calculate the Kendall's tau-b (τb) correlation coefficient, which is the nonparametric measure of the strength and direction of association that exists between two variables measured on at least an ordinal scale. In this study, significance levels $p = 0.01$ and $p = 0.05$ were used to determine the

2.9.3. Total least square regression

Total least squares (TLS) regression (Williamson-York Iterative Bivariate Fit) based on Cantrell [169] was used to correct the DOC concentrations from the GE Analyser to infill temporal data by blending DOC data from the GE Analyser and the LC-OCD-OND. The TLS method creates a linear regression line based on best fit, assuming that an error can be found in the x and y data. The accuracy of this approach is dependent on the size of the data set. As an example, the average error in the slope decreases from 19 % to 6 % to less than 1 % as the number of data points goes from 100 to 1000 to 10000 (an approximate $\sqrt{n}$ relationship). The specific error of this approach on the data sets of this thesis are reported within the research chapters.

2.10. 3-component UV absorbance model

Tipping et al. [170] described a two-component model of DOM, which accounted for optical absorbance in terms of the linear sum of two components (A and B) each with its own fixed absorbance spectrum. Carter et al. [98] adopted the same approach, with one modification, which is the inclusion of a third component (C) that does not absorb light and is present at the same concentration in all water samples. Thus, the DOC concentration in a given sample is given by

$$DOC = DOC_{AB} + DOC_{C} \qquad [16]$$

where DOC_{AB} refers to the light-absorbing components. Therefore

$$DOC = A_{\lambda}/E_{AB,\lambda} + DOC_{C} \qquad [17]$$

Where A_λ is the absorbance of the sample at wavelength λ (nm) in the UV range, and $E_{AB,\lambda}$ is the extinction coefficient (absorbance cm^{-1} DOC_{AB}^{-1}) of the light-absorbing DOM.

The fixed parameters were the extinction coefficients from each component A and B at 270 and 350 nm based on Carter et al. [98] (E350(A) = 30 L/g*cm and E350(B) = 0 L/g*cm). These assumptions are based on findings indicating 35 L/g*cm as a possible upper limit on E340 and the consistency during fitting of a low absorption for the second (B) component at 350 nm. Thus, here component B is assumed to absorb light only in the UV range and to be completely transparent to light above 350 nm [171]. Carter et al [98] used the average (fixed) value of 0.80 mg/L for DOC_C, while Adams et al. [172] evaluated the extinction coefficients of DOC_C in the UV range of algae-derived DOM.

In the third research chapter (Chapter 5) the Carter and Adams models were tested with the DOC and UV absorbance data from Auchencorth Moss and Menstrie. Furthermore, three additional model approaches were tested to improve the relationship between measured and modelled DOC from the original Carter model:

1. using measured LMW neutrals concentration, representing iDOM, as a variable non-UV absorbing DOC_C,
2. using CDOC from the 4 UV-absorbing fractions (humics, building blocks, LMW acids and amphiphiles) derived from LC-OCD-OND as measured DOC together with measured LMW neutrals concentration (DOC_C) and
3. including non-UV absorbing biopolymers fraction in the measured DOC_C concentration.

Chapter 3 – Assessing the advantages and disadvantages of freezing and pasteurisation for dissolved organic matter concentration and composition preservation

3.1. Introduction

Dissolved organic matter (DOM) is a major concern in drinking water treatment since it creates harmful carcinogenic compounds when chlorinated [173] and it has a significant effect on the biogeochemical processes in the aquatic systems [174, 175]. DOM is biologically and chemically reactive [176], leading to important emissions of carbon dioxide to the atmosphere [3, 177]. As a result, DOM quantity and quality has been examined across a diverse range of environments including tropical [31, 101, 102], arid [178], temperate [179–181] and arctic [44, 110] systems.

DOM in water is subject to a variety of changes with regard to its physical, chemical or biological state at varying rates from the time of sampling, due biotic and abiotic processes [182, 183]. Therefore, DOM water samples are ideally analysed immediately after sample collection to closely reflect in-situ stream water solute concentrations and composition [58, 61]. However, it is often necessary to store water samples before processing due to logistical constraints associated with analysing samples collected in remote locations or at fine temporal scales during storm sampling.

How a sample is stored and preserved can be just as important as the analysis of the sample itself and variations in results can be due to methods of containment and preservation, and not simply from differences in instrumentation or water mass [184]. Consequently, various attempts have been made to preserve samples through acidification (< pH 2), cold storage (4 °C) and freezing (-20 °C and -80 °C), although each method has its limitations with potential impacts on DOM concentration and composition.

Preservation of water samples through acidification using phosphoric acid (H_3PO_4) [185], Formalin, silver nitrate ($AgNO_3$) or mercuric chloride ($HgCl_2$) [186] has been suggested to reduce or inhibit biological activity for the analysis of dissolved organic carbon (DOC) concentration. However, pH changes can severely modify the DOC concentration and molecular DOM structure by increasing polymers and colloids abundance with increasing pH [185, 187, 188]. Alternatively, it can lead to abiotic decarboxylation (and loss to CO_2), humic acid precipitation, or residual microbial population DOM remineralisation via the addition of phosphate (via H_3PO_4) [185]. Therefore, acidification as a preservation method is not favourable for DOM composition studies.

Cold storage (4 °C) and freezing of water samples at -20 °C or fast-freezing with Liquid Nitrogen have been shown to have some potential for the long-term preservation of DOC concentration [189–191] and other nutrient concentration [192, 193]. However, cold storage has an impact on the sample integrity. In temperate and tropical rivers, DOC and dissolved organic nitrogen (DON) concentrations have been shown to decrease with the -20 °C freezing of water samples [190, 194–196], whereas DOM from peatland headwaters, soil porewaters, and estuarine settings appear unaffected by these freezing processes [197], suggesting that original composition of DOM has a critical role in the preservation. Furthermore, some of these studies observed significant changes in the absorbance spectra of the samples due to alterations on the chemical quality of DOC by removal/breakdown of the higher aromatic and higher molecular weight fraction [190, 191, 195, 197], aggregation of DOM during the freezing process [196, 198] and/or pH changes within the water [195]. Thieme et al. [199] demonstrated that DOC concentrations are not affected by freezing with Liquid Nitrogen (-80 °C, fast freezing) or freezing at -20 °C; however, alterations of DOM structure are possible due to the thawing process in support with re-crystallisation of ice crystals in frozen state [200, 201].

Heinz and Zak [191] found overall no change of DON concentration in -20 °C frozen samples but some individual replicates showed a decrease of DON due to -20 °C freezing but the changes were not statistically significant in both studies ($p > 0.05$). Further work found only a small change in DON concentrations with freezing when DOC and DON concentrations were low (< 3000 ppb C; < 1000 ppb N) [190]. Chapman and Mostert [192] observed that nitrate was successfully preserved by -20 °C freezing and no increase was detected on storage, in agreement with MacDonald and McLaughlin [193]. Given the relative importance of DOM in aquatic ecosystems and the large fluxes of DOC and DON that comprise DOM it is notable that there is only some guidance concerning sampling and analysis protocols. Clearly improved preservation methods are required to address the weaknesses of widely used cold storage methods.

Pasteurisation offers a potential alternative for the extended preservation of DOM in water. Pasteurisation is widely used for improving the drinking water quality [202, 203], or to preserve dairy products [204] by heating water/milk to 65 - 70 °C for 6 - 10 minutes by limiting microbial activity. However, this method has never been investigated for the preservation of organic matter in water samples.

In this chapter river water, as well as MilliQ water, was analysed over a month with the liquid chromatography - organic carbon detection – organic nitrogen detection (LC-OCD-OND) system to determine changes in the bulk DOC and DON concentration and the DOM composition [95]. The aim of this study is to determine the stability and reactivity of DOC, DON and DOM fractions [95] over time. The main research question to be addressed was 'Are freezing and/or pasteurisation effective and sensible preservation methods for DOM concentration and composition?'. Additionally, this methodological study was conducted to give a better insight on the temporal quality and quantity changes of DOM from peat-dominated waters under controlled laboratory conditions to support the following field-based studies regarding analytical time delay as well as storage effects.

3.2. Material and Methods

3.2.1. Sampling and analyses

The experimental procedure was conducted in the laboratories of the *Lyell Centre*, Heriot-Watt University, Edinburgh, UK. River water from the Menstrie Burn, a small headwater stream in Scotland, was investigated. A full description of the field site can be found in Chapter 2 section 2.1.2 and a detailed explanation of the sampling strategy is described in section 2.5.1. The first analyses ("Day zero") were performed within 12 hours after sampling and filtered through a 0.45 µm polyethersulfone (PES) filter through the LC-OCD system. Subsequent analyses were carried out after 1, 2, 4, 8, 16 and 32 days after sampling, see Figure 3.1.

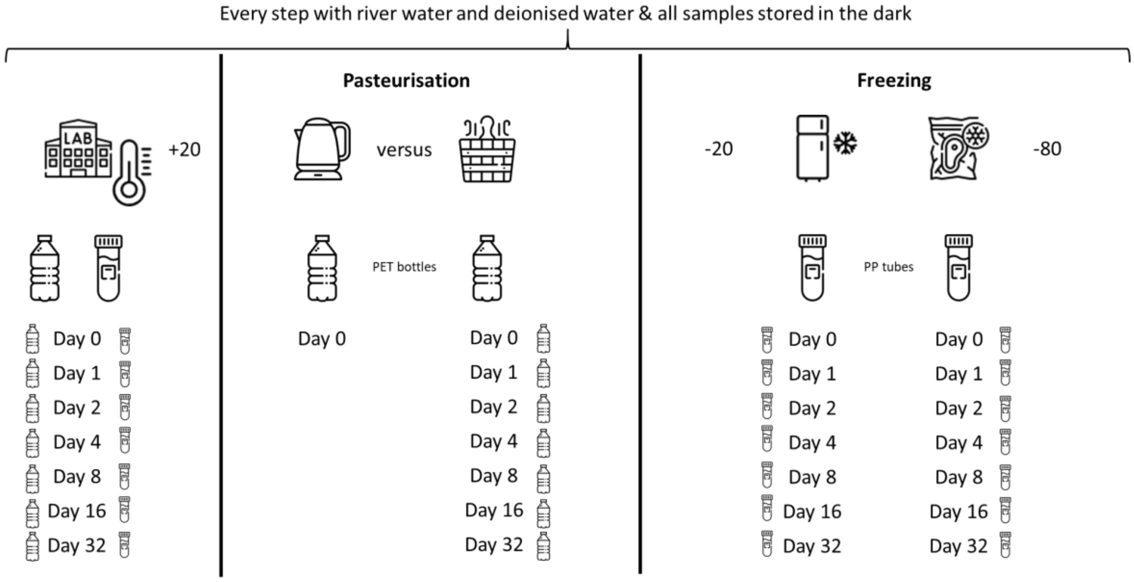

Figure 3.1: Overview of methodological set-up for the pasteurisation and freezing experiment.

Deionised (18.2 MΩ cm^{-1}) laboratory water (passed through a dual wavelength UV lamp to remove organic substances) was used as a system blank to identify potential leaching from sample bottles and carry over between analyses.

The river and deionised water were analysed on the LC-OCD-OND Model 9 for DOC and DON, five DOM fractions (humics, biopolymers, building blocks, LMW acids and neutrals) were identified and quantified, with $SUVA_{254}$ from DOC, chromophoric (hydrophilic) DOC ($SUVA_{CDOC}$), humics ($SUVA_{HS}$) and building blocks ($SUVA_{BB}$) providing characterisation of UV absorbance amenable DOM moieties.

3.2.2. Pasteurisation

In order to make DOM water sampling more widely available without specialist equipment, commercially available polyethylene terephthalate (PET) bottles (500 mL) were filled with non-carbonated mineral water to assess their viability for environmental sampling. The use of PET bottles assumes that it is of similar quality worldwide, regardless of whether PET is juvenile or recycled [205].

PET is produced by the polymerization of the petroleum monomers terephthalic acid and ethylene glycol by antimony-, titanium- or germanium-based catalysts [206]. PET is characterised with high hardness, stiffness and strength in thermoplastic and is stable at service temperatures from -40 °C to 100 °C [207, 208]. It is resistant to degradation with water at room temperature, dilute acids, neutral and acidic salts, alcohol, ethers, oils, fats, aromatic and aliphatic hydrocarbons. However, it is not resistant to alkalis, superheated steam, phenols, esters, oxidizing acids and chlorinated hydrocarbons [207, 209]. A review of PET bottle suitability for storing water suggested that the concentration of phthalates in the contents of PET bottles varied as a function of the contents of the bottle, with phthalates leaching into lower pH products more readily than into bottled water. Temperature also appears to influence the leaching both of phthalates [210] and antimony [206] from PET, with greater leaching at higher temperatures. However, due to the high sensitivity range of the LC-OCD-OND low concentration of phthalates would be measured.

Two pasteurisation protocols were developed. The first utilised 500 mL PET bottles and a standard, commercially available, household kettle of approximately 2 L volume. This is big enough to hold a 500 mL PET bottle with the kettle lid kept open.

This protocol was developed with the aspiration that it could be applied across the world using commonly available equipment. The second protocol utilised a 10 L water bath (Fisher Scientific, UK) for the pasteurisation of more samples at a time.

Water was added to the “max” line of the kettle reservoir and was then brought to the boiling point with the heater automatically shutting off. The sample was left for 10 min in the boiler and then removed. The kettle was emptied, and the process repeated for the next sample. For the water bath, the same procedure was followed with six bottles processed at a time. The temperature profiles inside the PET bottles from the kettle and water bath are shown in Figure 3.2. The temperature of the water was approximately 20 °C in both experiments prior to pasteurisation. The water temperature in the bottle increased to 41 - 44 °C after 4 min when the kettle turned off automatically. The residual heat in the surrounding water increases the temperature in the PET bottle to > 70 °C for about 12 min and to > 80 °C for about 5 min. The water bath was heated to a maximum of 80 °C. The temperature in the bottle reaches 75 °C within 10 min but the residual heat remains longer in the water bath (15 min) than in the kettle. Such conditions are sufficient to pasteurise the sample [204].

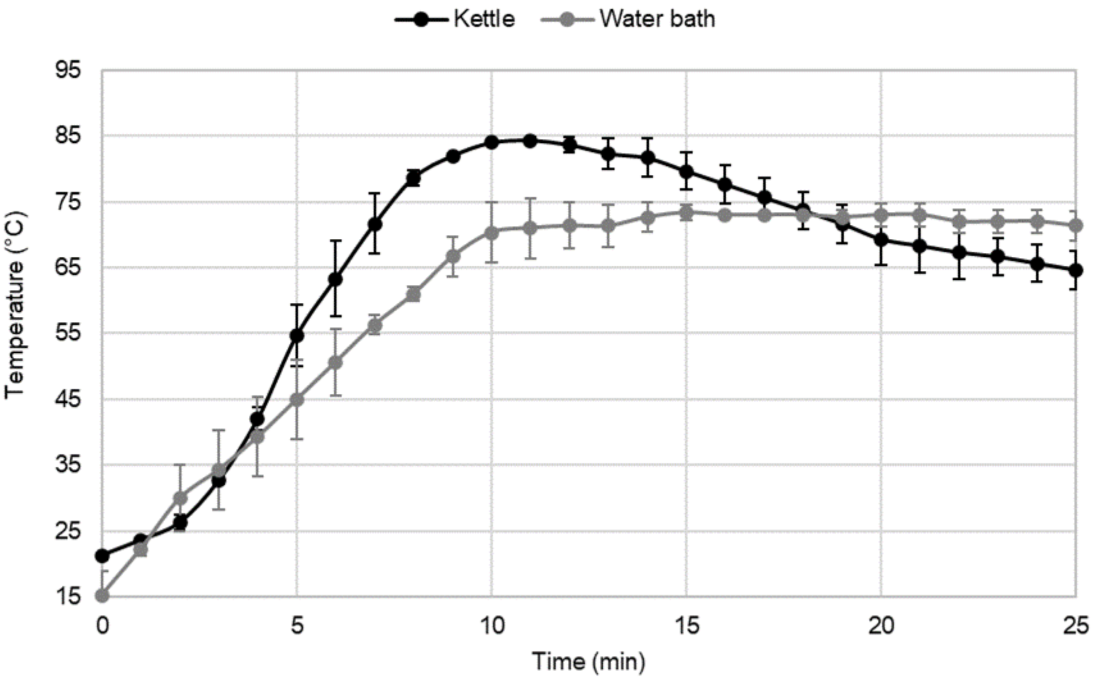

Figure 3.2: Temperature profile of the water in the PET bottle from the kettles and the water bath. Error bars are Standard deviation from n=3.

Figure 3.2 suggests that the pasteurisation process is independent of the volume, or insulation of the kettle or water bath. The energy input will affect the upslope temperature gradient and the water volume will affect the downslope gradient but the critical temperature window for sanitisation will always be achieved.

All pasteurised and non-pasteurised bottles were kept in the dark at room temperature ranging from 20 - 25 °C for analysis. Multiple samples were prepared to sacrifice at each time point, every time point and condition were replicated in triplicate for quality assurance and control purposes.

3.2.3. Freezing

The freezing experiment started 9 Days after the pasteurisation experiment to ensure that LC-OCD-OND-UVD samples were analysed in a timely manner with water from the same location in Menstrie Burn but with a different starting concentration and composition. The DOC concentration was 2.5 ± 0.1 ppm in the initial water taken for the freezing experiment, that is 0.2 ppm less DOC than in the initial water sample for the pasteurisation experiment.

With regard to the composition, the humics concentration was 0.2 ppm lower in the initial unfrozen sample than the unpasteurised samples, the building blocks concentrations were 0.1 ppm lower; biopolymers were 0.04 ppm higher and LMW neutrals concentration were on average 0.1 ppm lower in the initial unfrozen water samples the unpasteurised samples. LMW acids were not quantifiable in either of the initial water samples.

For the freezing experiments 10 % HCl pre-cleaned 15 mL polypropylene (PP) centrifuge tubes were used to freeze smaller amounts of water as suggested in a previous study by Thieme et al. [199]. Samples were either frozen in a -20 °C freezer, fast frozen with liquid nitrogen and stored in a -80 °C freezer or stored in the dark under room temperature (20 - 25 °C) as a control. Samples frozen with liquid nitrogen will be referred to as ‘fast-frozen/fast freezing’ whereas the samples frozen in a -20 °C will be referred to ‘-20°C frozen/freezing’ and if both methods are reported it will be stated simply as ‘freezing’ or ‘frozen’ in this study to keep a uniform nomenclature.

For each preservation method, three samples per water type for each time point allowed triplicate measurements for quality assurance and control purposes. All samples for Day 0 were measured within 12 hours after sampling and freezing. To thaw frozen samples each bottle was transferred into a beaker filled with water (25 °C) and located in the dark for up to 2 hours before measurement. All of the sample was defrosted prior to measurement.

3.3. Results

3.3.1. Pasteurisation and freezing of deionised water

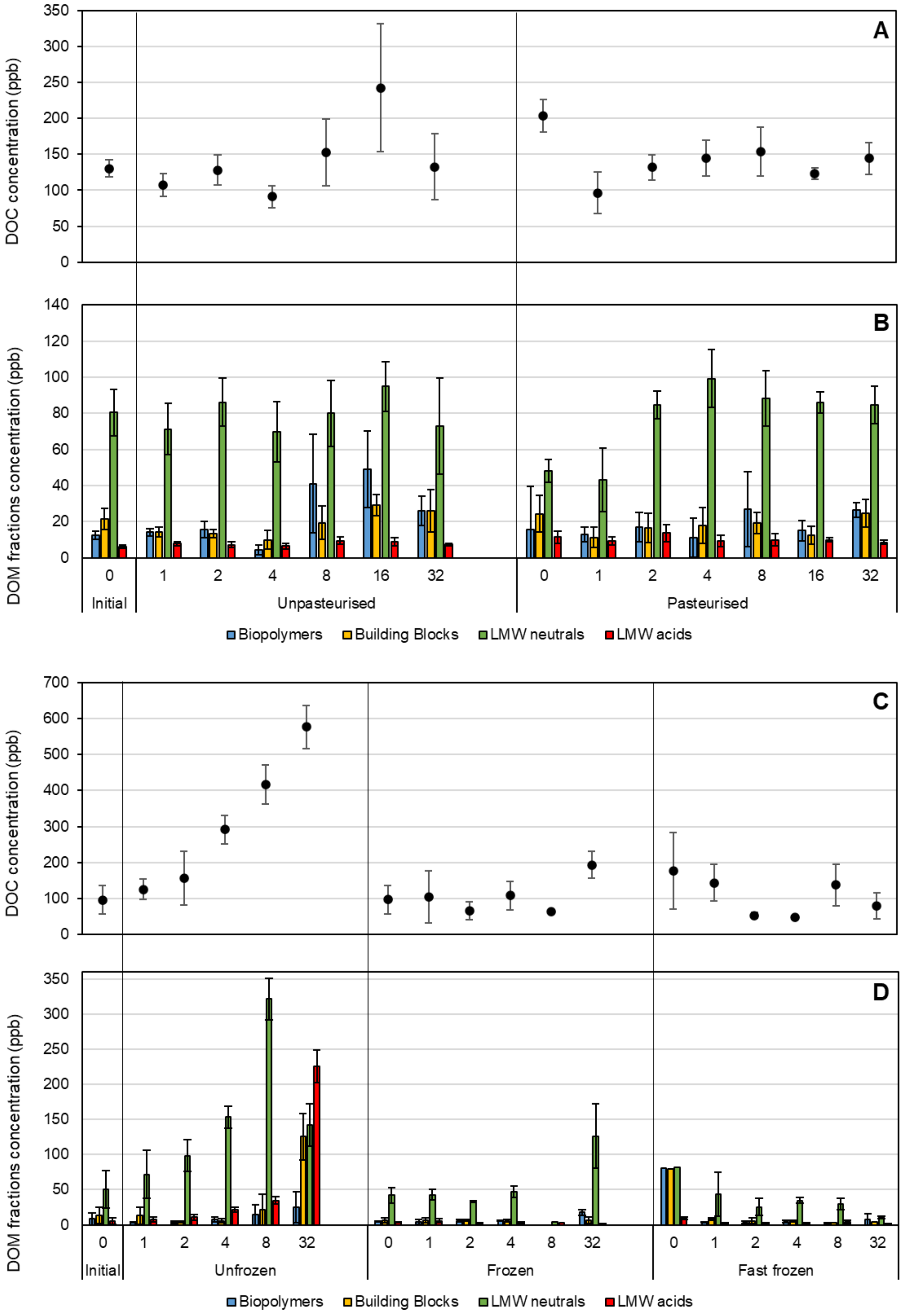

Figure 3.3: LC-OCD-OND derived results for unpasteurised and pasteurised MilliQ water over 32 days of A) DOC concentration in ppb, B) concentration of DOC fractions (biopolymers, humic subst., building blocks, LMW neutrals). LC-OCD-OND derived results for unfrozen, frozen and fast-frozen MilliQ water over 32 days (excluding Day 16) of C) DOC concentration in ppb and D) concentration of DOC fractions (biopolymers, humic subst., building blocks, LMW neutrals). Error bars represent standard deviation based on n = 3 - 6.

The average changes in DOC, biopolymers, building blocks, LMW neutrals and LMW acids concentrations from all untreated and treated (pasteurised, -20 °C frozen, fast frozen) deionised (18.2 MΩ cm^{-1}) laboratory water samples over time are shown in the 4 panels of Figure 3.3.

The results from the pasteurisation and freezing experiment show that deionised water is not free of DOM, although concentrations can vary depending on storage time and preservation method. Possible DOM fractions that could be added through deionised water are biopolymers, building blocks, LMW acids and LMW neutrals. Humic substances were not observed in the original (Day 0) deionised water, suggesting that the DOC removal mechanisms are primarily focussed on humic substances. Therefore, caution needs to be taken when non-humic DOM is investigated and deionised water is used for dilution.

The results from the deionised water were used to correct the LC-OCD-OND results from the river water for potential bottle leaching. The DOC and DOM fractions data from the unpasteurised and unfrozen river samples were corrected for bottle leaching (PET bottles and PP tubes) by subtracting the difference in concentration between Day 0 to the Days 1, 2, 4, 8, 16 and 32. Samples showing a loss in concentration from Day 0 to the following days, as seen on Day 1 and Day 4 of unpasteurised samples, were not affected by bottle leaching. In regard to the pasteurised and frozen samples, the concentrations of DOC and its fractions were corrected for maximum bottle leaching by subtracting the maximum measured concentration of the treated (pasteurised, -20 °C frozen and fast-frozen) deionised (18.2 MΩ cm^{-1}) laboratory water from Day 0 from the treated river water. Although, deionised water is used in the LC-OCD system it was not used to dilute the river water samples, hence no direct input of organics should have affected the samples. Additionally, system blanks were run before any samples were measured to minimise any contamination from the deionised water on the sample run.

3.3.2. DOC concentration and composition of unpasteurised and pasteurised river water

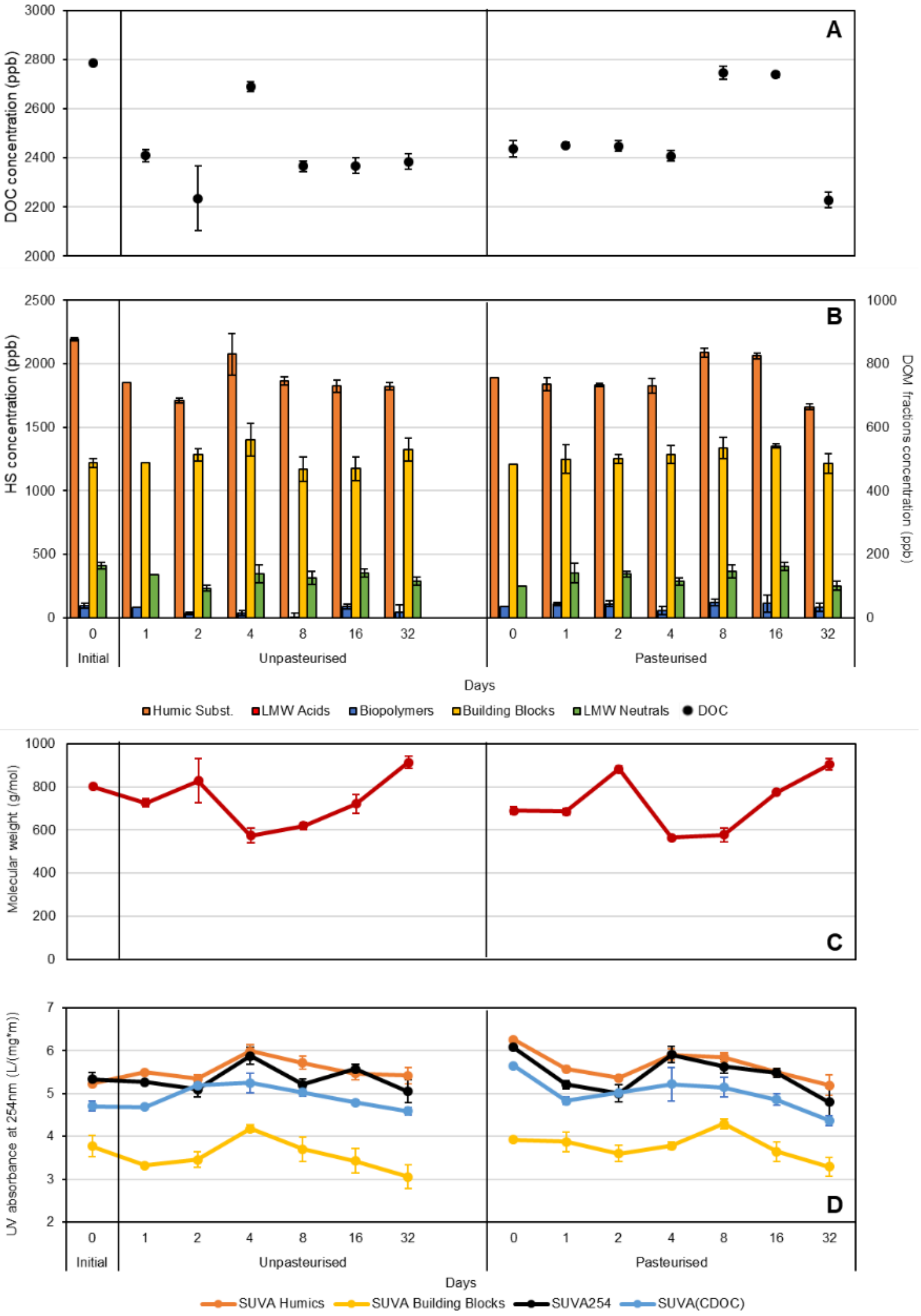

Figure 3.4: LC-OCD-OND derived results for unpasteurised and pasteurised water samples over 32 days of A) DOC concentration in ppb, B) concentration of DOC fractions (biopolymers, humic subst., building blocks, LMW neutrals), C) molecular weight in g/mol and D) SUVA from the Bypass (SUVA$_{254}$), from CDOC (SUVA$_{CDOC}$), from humics (SUVA$_{HS}$) and from building blocks (SUVA$_{BB}$) in L/(mg*m). Error bars represent standard deviation based on n = 5 - 6.

The maximum and minimum concentrations of DOC, biopolymers, building blocks, LMW neutrals and LMW acids from unpasteurised and pasteurised river water samples as well as deviation between unpasteurised and pasteurised Day 0 samples are shown in the supplementary material, Appendix 3.1.

The initial DOC concentration mean was 2785 ppb (± 17 ppb, n = 6) which decreased after pasteurisation to 2437 ppb (± 34 ppb, n = 6). The pasteurised river water samples had stable DOC concentrations over the first 4 days between 2408 ppb (Day 4, ± 20 ppb, n = 6) and 2452 ppb on Day 1 (± 12 ppb, n = 6). Day 8 pasteurised showed elevated DOC concentrations of 2748 ± 26 ppb (n = 6) in comparison to the previous 4 days.

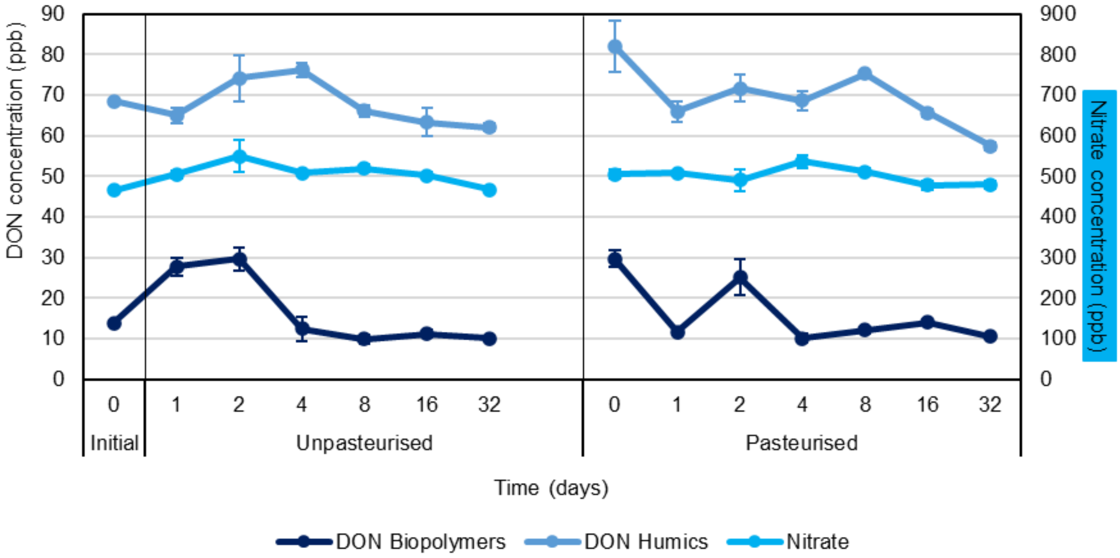

Figure 3.5: Average dissolved organic nitrogen (ppb) from humics and biopolymers and nitrate concentration derived from organic nitrogen detector from the LC-COD system in parts per billion (ppb) for unpasteurised and pasteurised river water samples over time. Error bars are standard deviation from n=5-6.

Humic substances are the dominant DOC fraction in the tested river water with starting concentration of 2195 ± 13 ppb (n = 5) decreasing to 1889 ppb (± 50 ppb, n = 6) after pasteurisation. DON concentrations of humic substances (DON_{HS}) were initially 69 ppb (± 2 ppb, n = 5) which increased after pasteurisation to 82 ± 6 ppb (n = 6). DON_{HS} in the unpasteurised and pasteurised samples are stable over the whole 32 days of the experiment with concentration losses and gains of < 20 ppb ($p > 0.05$).

Humic substances concentration experienced a decrease of 344 ppb over the first 24 hours of the experiment and an increase from Day 2 (1712 ± 163 ppb, n = 6) to Day 4 (2076 ± 33 ppb, n = 6) of 364 ppb in the unpasteurised samples. The humic substances concentration in pasteurised samples was stable over the first 4 days with values between 1825 ppb (± 35 ppb, Day 4, n = 6) and 1888 ppb (Day 0) but increased to 2088 ± 23 ppb (n = 6) on Day 8.

The second most abundant DOC components are building blocks with an initial concentration of 487 ± 14 ppb (n = 5) which decreased to 482 ± 46 ppb (n = 6, $p > 0.05$) in the pasteurised samples. Building blocks concentration in untreated water samples increased from Day 0 with 487 ppb to Day 4 with 560 ± 38 ppb (n = 6, $p < 0.05$). On Day 8 the concentration dropped to 467 ppb (± 38 ppb, n = 6), which is a statistically significant decrease ($p < 0.05$). The pasteurised samples had stable building blocks concentration over the first 16 days ($p > 0.05$) but a clear decrease from 540 ± 30 ppb (n = 6) on Day 16 to 485 ± 20 ppb (n = 6) on Day 32 (Figure 3.4 B).

Biopolymers had an initial concentration of 37 ppb (± 7 ppb, n = 5) in the original river water which decreased to 34 ppb (± 5 ppb, n = 6) in the pasteurised samples ($p > 0.05$). Unpasteurised river water samples from days 0 to 4 and Day 32 are above LOD but all days are below biopolymers LOQ of 38 ppb. Pasteurised water samples had concentrations above LOD on all days but only on Day 1, 2, 8 and 16 concentrations were above LOQ with concentrations between 42 ± 9 ppb on Day 2 and 48 ± 27 ppb on Day 8. DON from the biopolymers (DON_{BP}) is ranging between 10 and 30 ppb for unpasteurised and pasteurised water samples which is below LOD (53 ppb/N) and LOQ (176 ppb/N) for DON hence any observed fluctuations are not reportable.

LMW neutrals initial concentration is 164 ± 11 ppb (n = 5) which decreased significantly to 99 ± 31 ppb (n = 6, $p < 0.05$) after pasteurisation. Unpasteurised water samples showed a decrease of 72 ppb (± 18 ppb) over the first 48 hours ($p < 0.05$).

The pasteurised samples showed an increase of LMW neutrals of 41 ppb ($p < 0.05$) in the first 24 hours but the concentration stabilises until Day 8 where an increase of 32 ppb was observed from 114 ppb (Day 4, ± 20 ppb) to 146 ppb (Day 8, ± 13 ppb).

LMW acids were below LOQ (10 ppb/C) and LOD (3 ppb/C) after blank correction in the unpasteurised and pasteurised water samples at all time points.

The initial molecular weight is 801 ± 25 g/mol but it gets reduced to 690 ± 17 g/mol after pasteurisation. The highest molecular weight in the unpasteurised samples was measured on Day 32 with 913 g/mol (± 30 g/mol, n = 6) which is a significant increase from Day 16 with 720 ± 43 g/mol. The lowest molecular weight on Day 4 is 564 g/mol (± 34 g/mol), a massive decline from Day 2 827 ± 102 g/mol. In the pasteurised samples the molecular weight increased after 48 hours from 684 g/mol (± 15 g/mol) to 882 g/mol (± 16 g/mol) but decreased significantly on Day 4 to 564 g/mol (n = 6). From Day 8 to Day 32 the molecular weight increased by 328 g/mol, a large gain of humic substances with higher molecularity.

Figure 3.4D shows the different SUVA trends that can be obtained from the LC-OCD-OND system, which is explained in detail in the methods. $SUVA_{254}$, representing the bulk DOM pool, increased from initially 5.34 L/(mg*m) (± 0.16, n = 5) to 6.08 L/(mg*m) (± 0.1) after pasteurisation. None of the $SUVA_{254}$ changes in unpasteurised samples are statistically significant ($p > 0.05$).

In the pasteurised samples, $SUVA_{254}$ decreased after the first 24 hours by 0.87 L/(mg*m) and it decreased from Day 16 (5.48 ± 0.32 L/(mg*m)) to Day 32 (4.8 ± 0.14 L/(mg*m)) of 0.68 L/(mg*m). However, $SUVA_{254}$ is representing the bulk DOC pool measured from the Bypass, including hydrophobic DOC (HOC) and hydrophilic DOC (CDOC). $SUVA_{CDOC}$ is the UV absorbance at 254 nm and DOC from all UV amenable DOC fractions (see Methods), excluding HOC.

$SUVA_{CDOC}$ had an initial value of 4.71 ± 0.12 L/(mg*m) which is 0.5 L/(mg*m) lower than $SUVA_{254}$, which is linked to the negative HOC values that decreased DOC concentrations in comparison to CDOC concentrations. After pasteurisation $SUVA_{CDOC}$ increased to 5.65 ± 0.09 L/(mg*m), a rise of 0.94 L/(mg*m). Unpasteurised river water samples showed no substantial changes in $SUVA_{CDOC}$. $SUVA_{CDOC}$ from the pasteurised samples reduced notably after 24 hours to 4.83 L/(mg*m), a loss of 0.82 L/(mg*m). After the rapid decrease of $SUVA_{CDOC}$ in the first 24 hours it increased slightly to 5.22 ± 0.23 L/(mg*m) on Day 4. SUVA values from humic substances ($SUVA_{HS}$) showed a similar trend as the $SUVA_{254}$ with an initial value of 5.23 L/(mg*m) (± 0.05) which increased by 1.02 L/(mg*m) to 6.25 L/(mg*m) (± 0.07) after pasteurisation suggesting a gain of higher aromatic humic substances. SUVA from building blocks ($SUVA_{BB}$) of the pasteurised samples was 3.93 L/(mg*m), which is an increase of only 0.15 from the initial 3.77 L/(mg*m) in the untreated samples.

Initial nitrate concentration was 465 ± 8 ppb which increased in the pasteurised samples to 505 ± 12 ppb. In the first 48 hours the nitrate concentration increased to 549 ± 40 ppb in the untreated samples (n = 6, $p < 0.05$); however, it decreased to 467 ± 5 ppb (n = 6, $p < 0.05$) from Day 2 to Day 32. Pasteurised water samples showed no significant changes in their nitrate content with concentrations of 477 ppb (± 10 ppb) on Day 16 to 536 ppb (± 16 ppb) on Day (Figure 3.5).

3.3.3. DOC concentration and composition of unfrozen and frozen river water

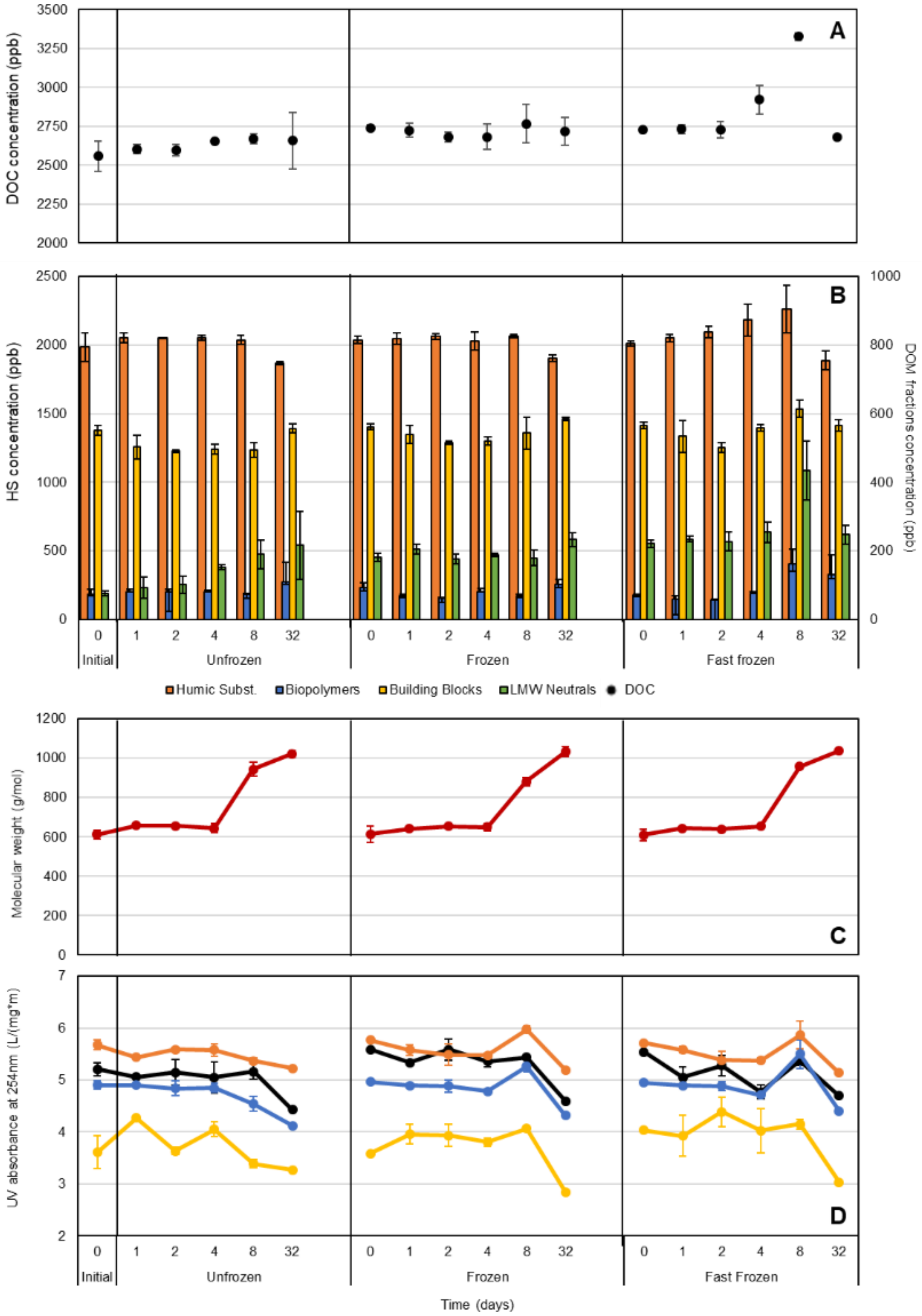

Figure 3.6: LC-OCD-OND derived results for unfrozen, -20 °C frozen and fast-frozen water samples over 32 days of A) DOC concentration in ppb, B) concentration of DOC fractions (biopolymers, humic subst., building blocks, LMW neutrals), C) molecular weight in g/mol and D) SUVA from the Bypass ($SUVA_{254}$), from CDOC ($SUVA_{CDOC}$), from humics ($SUVA_{HS}$) and from building blocks ($SUVA_{BB}$) in L/(mg*m). Error bars represent standard deviation based on n = 5 - 6.

The maximum and minimum concentrations of DOC, biopolymers, building blocks, LMW neutrals and LMW acids from unfrozen and frozen (-20 °C and fast-frozen) river water samples as well as deviation between unpasteurised and pasteurised Day 0 samples are shown in supplementary material, Appendix 3.1.

DOC concentration was 2558 ppb (± 98 ppb, n = 3) in the original sample, which increased to 2740 ± 19 ppb (n = 3) in the -20 °C frozen samples and to 2727 ± 1 ppb (n = 2) in the fast-frozen samples. Unfrozen water samples showed a gradual increase of 100 ppb over 32 days to 2658 ppb (± 182 ppb, n = 2). The DOC concentration in the frozen samples varies show no critical changes over time. The fast-frozen samples increased from 2730 ppb on Day 2 (± 2 ppb, n = 3) to 3326 ppb on Day 8 (± 1 ppb, n = 2) before it declined to 2682 ± 4 ppb on Day 32 (n = 2) (Figure 3.6).

The initial humic substances concentration in the frozen and fast frozen samples was 2036 (± 26 ppb) and 2012 ppb (± 17 ppb) which is higher than the unfrozen concentration of 1984 ± 106 ppb. Humic substances showed no change in the unfrozen and -20 °C frozen samples over the first 8 days. Humic substances concentration in fast frozen water samples were stable in the first 2 days but increased to 2262 ± 174 ppb on Day 8, an increase of 170 ppb in 6 days. From Day 8 to Day 32 the humic substances concentration dropped to 1886 ppb (± 68 ppb) in the fast-frozen water samples.

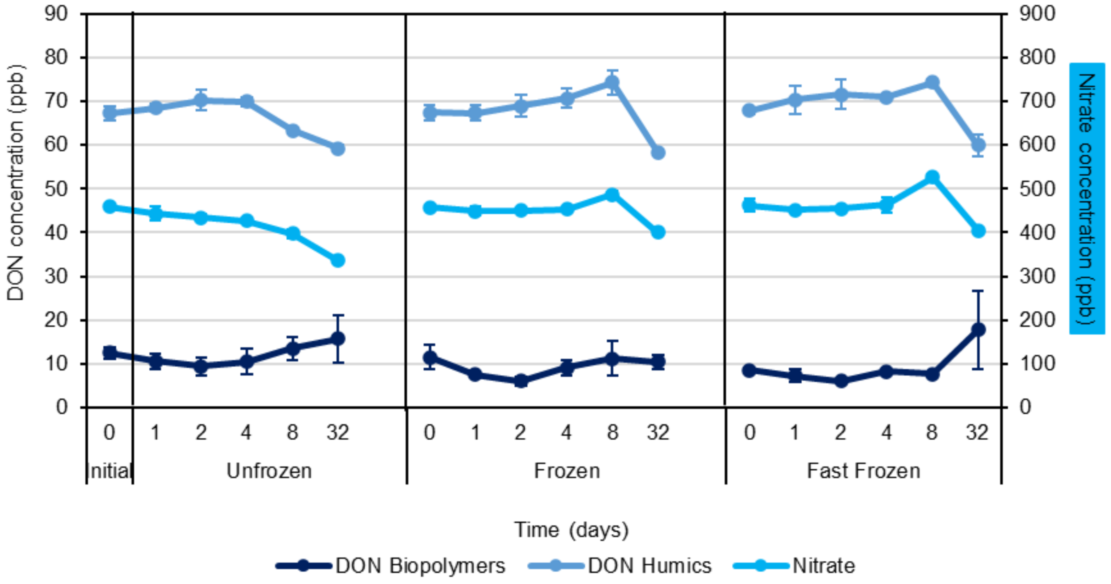

Figure 3.7: Dissolved Organic Nitrogen (DON) from humics and biopolymers and nitrate concentration derived from organic nitrogen detector from the LC-OCD-OND system in parts per billion (ppb) for unfrozen, frozen and fast-frozen river water samples over time. Error bars are standard deviation from n = 3.

The DON_{HS} concentration was initially 67 ppb (± 2 ppb) with no observed change after freezing (67 ± 2 ppb) or fast freezing (68 ± 1 ppb) (Figure 3.7). No changes in DON_{HS} concentrations was observed between the days ($p > 0.05$); however, all three samples sets showed a decline in DON_{HS} over time (loss of 8 ppb in all three preservation methods).

Building blocks had an average starting concentration of 550 ± 14 ppb in the unfrozen samples, 561 ± 9 ppb in the -20 °C frozen and 565 ± 10 ppb in the fast-frozen samples (Figure 3.6B). The -20 °C frozen samples showed no clear changes in the first 4 days ($p > 0.05$). The fast-frozen water samples showed a decline in the first 2 days of 64 ppb from 561 ppb to 501 ppb (± 15 ppb) in the building blocks concentration and a small increase ($p = 0.05$) from Day 2 (501 ± 46 ppb) to Day 8 (614 ± 25 ppb).

Biopolymers concentration was initially 74 ± 15 ppb in the unfrozen samples, 71 ± 3 ppb after fast freezing and 91 ± 14 ppb after -20 °C freezing. Overall, the concentration of biopolymers was stable over 8 Days in the unfrozen (74 – 82 ppb) and frozen (62 - 93 ppb) samples ($p > 0.05$). The fast-frozen samples had stable concentrations over 4 days and an increase on Day 8 of 85 ppb from initially 76 ppb on Day 4 (± 1 ppb). All three sample sets show no significant changes in their DON_{BP} concentration besides all DON_{BP} concentrations were below the LOD of 56 ppb/N.

LMW neutrals had an initial concentration of 75 ± 8 ppb ($n = 3$), increased to 182 ± 12 after -20 °C freezing and to 221 ± 11 ppb after fast freezing. LMW neutrals concentration increased exponentially to 216 ± 99 ppb on Day 32 ($n = 2$) in the unfrozen samples. The -20 °C frozen samples had overall stable LMW neutrals concentrations over time. LMW neutrals concentrations in fast frozen samples showed a notable increase from Day 4 (254 ± 30 ppb) to Day 8 of 179 ppb and decrease on Day 32 to 247 ± 27 ppb.

LMW acids were not quantifiable in unfrozen and frozen samples with concentrations below LOQ of 10 ppb/C.

The molecular weight of humic substances in unfrozen water samples was 612 ± 9 g/mol which marginally increased in the -20 °C frozen samples to 615 ± 21 g/mol and decreased to 610 ± 19 g/mol in the fast-frozen samples. No notable changes in molecular weight was observed in the first 4 days of the experiment in neither of the samples sets ($p > 0.05$).

On Day 8 all samples showed a large increase in molecular weight, adding 300 g/mol in the unfrozen samples (from 644 to 944 g/mol), 231 g/mol in the -20 °C frozen samples (from 649 to 880 g/mol) and 304 g/mol (from 654 to 958 g/mol) in the fast-frozen samples.

$SUVA_{254}$ was initially 5.21 ± 0.12 L/(mg*m) and increased to 5.59 ± 0.04 L/(mg*m) after -20 °C freezing and to 5.54 ± 0.20 L/(mg*m) after fast freezing (Figure 3.6D). The $SUVA_{254}$ from unfrozen samples was stable over the first 8 days as well as the -20 °C frozen samples; but a decline to 4.59 ± 0.16 L/(mg*m) on Day 32 was observed in -20 °C frozen samples. The fast-frozen samples showed an overall decreasing trend but no strong change in $SUVA_{254}$ over time.

$SUVA_{CDOC}$ was initially 4.90 ± 0.08 L/(mg*m), which is 0.3 L/(mg*m) lower than $SUVA_{254}$, increased to 4.97 ± 0.05 L/(mg*m) in the -20 °C frozen samples and to 4.95 ± 0.05 L/(mg*m) in the fast-frozen samples. Although the increased $SUVA_{CDOC}$ on Day 8 interrupted the overall decreasing trend in $SUVA_{CDOC}$ of the two frozen sample sets it is not statistically significant ($p > 0.05$).

The original water sample had a $SUVA_{HS}$ value of 5.68 ± 0.09 L/(mg*m), decreasing to 5.52 ± 0.10 L/(mg*m) in the -20 °C frozen samples and increasing to 5.71 ± 0.06 L/(mg*m) after fast freezing (Figure 3.6D). Neither of the frozen sample sets showed considerable changes in $SUVA_{HS}$ over time ($p > 0.05$).

Initially the $SUVA_{BB}$ value was 3.61 ± 0.31 L/(mg*m), which decreased to 3.58 ± 0.19 L/(mg*m) after -20 °C freezing but increased to 4.03 ± 0.40 L/(mg*m) after fast freezing. Unfrozen samples showed a general decreasing $SUVA_{BB}$ trend regardless of the increase on Day 4. The -20 °C frozen and fast-frozen samples had stable $SUVA_{BB}$ values until Day 8 ($p > 0.05$) but a decrease on Day 32 (Figure 3.6D).

Nitrate concentration was initially 459 ± 5 ppb which decreased after -20 °C freezing to 457 ± 2 ppb and increased after fast freezing to 462 ± 5 ppb (Figure 3.7). The nitrate lost 91 ppb from Day 4 to Day 32 in the unfrozen samples and 86 and 121 ppb in the -20 °C frozen samples sets between Day 8 and Day 32, respectively.

3.4. Discussion

3.4.1. Impact of freezing and fast freezing on DOM quantity and quality

Over the whole time period of this study (32 days) the unfrozen and -20 °C frozen water samples had stable DOC concentrations, although the standard deviation increased over time. This stability in DOC of -20 °C frozen samples over time had been observed before by e.g. Norman and Thomas [211] who suggested that water samples can be stored at -20 °C for over a year without substantial DOC concentration changes. However, in the fast-frozen samples DOC increased after 8 days before the concentrations dropped again to the starting concentration suggesting that fast-freezing of water samples may lead to more uncertainty in preservation of the DOC quantity than conventual freezing at -20 °C.

This observation does not agree with the findings from Thieme et al. [199] who found good preservation of DOC concentration over 41 days in fast-frozen water samples. However, they unfroze their samples overnight under 5 °C, increasing the time until analysis but also thawing the samples less extreme as in this study under room temperature in 20 °C water.

Nevertheless, an important result to consider from the freezing experiment is the increase of DOC concentration in both frozen water samples in comparison to the samples stored at room temperature, especially since the frozen water samples are corrected for maximum initial bottle leaching due to freezing (see 3.3.1). This observation does not agree with the outcomes of previous studies who found either no significant change in DOC concentration due to freeze/thaw processes [196] or a decrease in DOC concentration after freezing and thawing [190, 191, 194, 199]. Hence the increase in DOC may be linked to different thaw protocols, rather than the freezing itself.

The small decrease in DOC concentration over 2 - 4 days of freezing time may be caused by aggregation and potentially particle formation [198] induced by partitioning and concentration effects during the freezing process [212, 213]. Conceivably it is possible that fine particulates that pass through a membrane filter disaggregate during the freezing and thawing process. This would be consistent with the sample water used that drains podzolic soils containing a high proportion of clays and especially fine organic detritus [214, 215]. Although, visual inspection of the samples suggest that no flocculation or aggregation was caused due to freezing, the filtration before measurement will have removed larger particles, potentially leading to a loss of DOC in the Day 32 sample.

Additionally, HOC was not quantified with the LC-OCD-OND in this samples nor inorganic colloids concentrations increased due to freezing, hence it suggests that particles larger than 0.45 µm have been created over 32 days of -80 °C storage.

In this study, neither the humic substances nor building blocks showed a significant increase after the first freeze/thaw process suggesting that no larger OM was coagulated during the freezing process. Peacock et al. [197] tested the effect of the freeze/thaw process on 17 samples (lake water and surface water from ditches) with DOC concentration < 25 mg/L, observing a DOC increase after freezing in 10 water samples from ditches and overland flow; however, only one lake water showed a statistically significant increase of > 1 mg/L Carbon. However, it is unknown what caused the substantial increase in one sample.

The most substantial compositional change observed between unfrozen and frozen samples was the increase in LMW neutrals concentration after 24 hours of storage in the freezer. This is in line with findings from Heinz and Zak [191] who found an increase in LMW DOC after freezing in some of their peat samples. Hudson et al. [194] demonstrate a decrease in fluorescence intensity after freezing/thawing which they suggested to be linked to a decrease in TOC during these processes. This observation agrees with findings from Spencer et al. [195], who showed an increase in DOC concentrations with decreasing fluorescence and absorbance values, indicating that the proportion of fluorescent and light absorbing DOM in this sample has decreased. These findings could be linked to an increase in non-UV absorbing LMW neutrals; however, neither of these studies looked into DOM size classes. Cook et al. [196] found that the freeze/thaw process lead to absorbance losses at 270 and 350 nm, indicating less aromatic DOM and this may result in the miscalculation of DOC concentrations based on UV absorbance [98] following freeze/thaw. However, DOC is preserved well when frozen at -20 °C samples, hence it is recommended to measure DOC rather than model it with UV absorbance carbon models due to the alterations of DOC composition during the freeze/thaw process.

Although, SUVA values were not changing significantly ($p > 0.05$) after freezing in this study, the observed increase in LMW neutrals does support the previous findings of an increase of less UV amenable DOM. This initial increase in LMW neutrals may indicate a DOM breakdown from HMW to LMW organic matter; however, no DOM fraction showed a significant decrease due to freezing. As corrections for bottle leaching were made it is unlikely to come from this source.

A study by Agten et al. [216] demonstrated that under aqueous conditions, the oxime reaction between a ketone and aminooxy group can be readily catalysed by freezing. They suggested that either a catalytic effect of the fine-structured growing ice crystals lead to oxime formation or the increase in salt concentration and associated change in pH and ionic strength could be reasons for rate acceleration. The reaction between ketones and ice crystals or salts during freezing could be an explanation for the increased LMW neutrals, since oximes are not affecting the HMW DOM pools with their low molecular weights and are overall less stable and more reactive than HMW DOM [216–218].

Nevertheless, both freezing methods increased the LMW neutrals concentration; however, since -20 °C freezing stabilised LMW neutrals for a longer time period than fast freezing it could be assumed that the thawing process has a larger effect on the LMW neutrals than previously recognised.

With the exception of initial changes in LMW neutrals concentrations from the freezing process, all four measured fractions are well preserved over 8 days in the unfrozen and frozen samples. At Day 32 humic substances concentrations were decreased in the unfrozen and -20 °C frozen samples whereas building blocks, biopolymers and LMW neutrals increased in both sample sets. Additionally, DON from humics and biopolymers decreased on Day 32 after being stable the first 8 days of the experiment. A gain of building blocks and LMW neutrals at the same time as a decline in humic substances (DON and DOC) suggests a breakdown of HMW humic substances over time, confirming previous observations by Heinz and Zak [191] who noticed HMW DOM decreasing with increasing LMW DOM and only minor changes in DON. Furthermore, Thieme et al. and Fellmann et al. [190, 199] observed an increase of aromaticity ($SUVA_{254}$) indicating a stronger removal of non-aromatic DOM and a decrease in the Humification index suggesting a preferential removal of humified CDOM during freezing without affecting the DOC concentration.

However, in this study a decrease in aromaticity of biopolymers and humic substances as well as bulk DOC, and an increase in molecular weight of humic substances was observed on Day 32. This indicates a gain of non-aromatic DOM with higher molecularity like biopolymers which is supported by the observed increase of biopolymers on Day 32.

Nevertheless, the -20 °C samples showed no compositional change from Day 1 to Day 8, suggesting that thawing of samples stored at -20 °C has a smaller effect on the DOC fractions than storage/freezing time. However, freezing samples at -20 °C did not improve the storage time in comparison to storage under room temperature (20 - 25 °C).

The fast-frozen samples showed a simultaneous increase of biopolymers, humic substances, building blocks and LMW neutrals over time together with the drop in humic substances and LMW neutrals on Day 32, which suggests a combination of processes in fast-frozen water samples are affecting the DOM composition. Deng et al. [189] found two proteins that precipitate upon thawing following fast freezing. They suggested that freezing/thawing damaged proteins through pH changes and that protein concentration increased due to removal from water ice crystals that can lead to denaturation and aggregation of these proteins. This incorporation from proteins, potentially from LMW neutrals binding (creating large molecules), could explain the biopolymers increase or the microbes that were dormant become active during the thawing process, hence creating more biopolymers.

The increase in humic substances together with the decreasing aromaticity and stable molecular weight suggests a breakdown of higher aromatic DOM within the humics pool but no loss of humics itself. On Day 8 the increase in building blocks and LMW neutrals together with humic substances and biopolymers suggests a combination of HMW DOM breakdown within the humic substances pool and beyond due to fast freezing. However, freezing does not affect SUVA notably over time in comparison to unfrozen samples, which is in contrast to previous work that showed either clear decreasing SUVA values due to freezing [190, 197] or increasing aromaticity [199] during freezing and thawing.

In regard to the molecular weight, freezing does not seem to have a negative effect since all three samples sets (unfrozen, frozen, fast-frozen) show stable molecular weight values over the first 4 days before all samples increased massively in their molecular weight on day 8 and day 32.

In regard to nitrate, freezing does not affect the nitrate concentration negatively; however, it does not extend the holding time compared to samples that were fast-frozen as a higher error was observed after 32 days than unfrozen samples. This finding is similar to previous observations by MacDonald and McLaughlin [193] who found no significant change due to freezing and Chapman and Mostert [192] who noticed that nitrate samples were generally successfully preserved by freezing, although the precision decreased slightly which can be observed in the fast-frozen samples.

3.4.2. Benefits and disadvantages of pasteurisation for quantitative and qualitative DOM preservation

The DOC concentration of pasteurised samples is stable for 4 days; however, directly after the initial pasteurisation the DOC concentration decreased by almost 400 ppb which suggests that the boiling of water removes or alters DOC which is a disadvantage for pasteurisation. This alteration of DOC can be observed in its composition with a decline of humic substances and LMW neutrals after pasteurisation, suggesting a simultaneous breakdown of HMW DOM and LMW DOM. Rijal and Fujioka [219] found that heating water up to 60 °C was the primary mechanism to reduce faecal indicator bacteria and enterococci to undetectable levels signifying that also other bacteria will be inactive to alter DOM. However, thermophilic organisms can survive and grow at temperatures of 45 – 50 °C [220]. These organisms can lead to a rapid decomposition of OM in peat [220] and potentially provide an explanation for the increase in humic substances (and DOC) in the pasteurised water samples. These heat resisting fungi may decompose OM within the humic substances pool, breaking aromatic rings and producing less aromatic humic substances.

The $SUVA_{254}$ values emphasise an increase in the aromaticity of humic substances initially after pasteurisation but a reduction in the overall molecular weight can be observed as well, which suggests a shift between lighter fulvic acids to heavier and more aromatic humic acids due to pasteurisation. Another potential cause for the increase of humics and DOC could be formation of particles due to abiotic synthesis within the DOC pool. Abiotic factors like temperature or nutrient availability could be a cause for formation of DOM although it is peculiar that the increase of humics did not happen in every pasteurised sample.

The heating process itself could have a large effect on the DOM composition. Thermal reactions caused by the heat during pasteurisation can lead to the observed decrease in humics and LMW neutrals by decomposition of specific carbon bonds. Although pasteurisation of waters did not reach temperatures over 80 °C organic compounds are extremely variable in their boiling points or ability to be broken down to volatile OC via thermal reaction [221]. The low boiling points of ketones and amino acids, who are part of the LMW neutrals fraction [222], could explain the decline in LMW neutrals after pasteurisation. In regard to humic substances, previous studies found that humic substances have a high thermal stability but within the humic substances pools the humic acids are more stable at higher temperatures than fulvic acids [223, 224].

Ioselis et al. [225] found that the ionization of carboxylic and especially phenolic groups can lead to an increase of the thermal stability of humic acids suggesting that thermal degradation during pasteurisation of humic substances is likely to affect the fulvic acids and less the humic acids. Moreover, the results suggest that building blocks are resistant to pasteurisation in contrast to humic substances, although building blocks are considered breakdown products of humic substances [95]. These may indicate that building blocks have more phenol groups than humic substances or broken-down humic substances are contributing to the building blocks pool after the initial pasteurisation.

Pasteurisation also increased the initial concentration of DON and nitrate and it does not improve the sample holding time of DON and nitrate compared to untreated waters. The observed fluctuations in DON and nitrate may have differing explanations. Nitrifying bacteria can oxidise inorganic N compounds leading to a potential decrease of nitrate or an increase by decomposing ammonium [226]. Moreover, Chernikov et al. [227] found that the exposure of water to heat results in the formation of nitrogen oxides (nitrite, nitrate, and nitrogen dioxide) and may explain the initial increases in nitrate concentration due to pasteurisation. However, in this study, as DON and nitrate concentrations showed no significant ($p > 0.05$) changes due to pasteurisation, it is recommended to leave the water samples unpasteurised to avoid any alteration of DON and nitrate.

3.5. Conclusions

The goal of this study was to investigate the transformation of DOM during freezing processes of river water based on DOM size fractionation and the ability of pasteurisation to preserve DOM quantity and quality. The following conclusions were drawn:

1) Deionised water is not completely free of DOM. It is free of humics but can consists small amounts of LMW neutrals, biopolymers and building blocks, as the initial Day 0 measurement demonstrates.
2) To investigate DOC concentration water samples should be stored in PP tubes in the dark either untreated under room temperatures or frozen at -20 °C for approximately 8 days.
3) To investigate the humic substances concentration of river water samples -20 °C freezing in PP tubes is recommended for up to 8 days.
4) Pasteurisation is suitable for investigations of LMW neutrals and biopolymers for up to 16 days. Although non-pasteurised samples may just be as stable, the decreases on Day 2 and Day 4 suggest that the untreated samples may undergo chemical alterations within the first 16 days.

Additionally, it needs to be considered that most of the earlier studies looked at large rivers, lakes and other surface waters with DOC concentrations > 10 mg/L that are under permafrost, urban impact or dominated by mineral soils in the nature, whereas this study considered river water with a DOC concentration of less than 10 mg/L, from humic dominated regions, which represents many rivers worldwide with DOC concentrations of < 10 mg/L [34, 228, 229]. Overall, this chapter demonstrated that different DOM pools need different preservation methods, with humic substances more suited to -20 °C freezing and LMW neutrals more suited to pasteurisation. However, even non-treated samples can be stable for at least 4 days, depending on the initial concentration and composition and on what parameters are under assessment.

Chapter 4 – The influence of dissolved organic matter flux and composition in Scottish Rivers for ecosystem function and climate feedback

4.1. Introduction

Inland waters — such as ponds, lakes, wetlands, streams, rivers and reservoirs — pervade terrestrial ecosystems and often shape the Earth's landscapes. Freshwaters, combining lakes and reservoirs with streams and rivers, cover a total surface area of about 3,620,000 km^2, which is only around 2.67 % of the Earth' surface [41], but their collective contribution to global carbon fluxes is important together with terrestrial and marine ecosystems [3, 22, 28, 36, 230]. Currently it is estimated that inland waters transport, mineralise and bury ~2.7 Petagram carbon per year (Pg/y); however, this number is under constant discussion and revaluation [3, 27, 28, 41].

The transport of terrestrial dissolved organic matter (DOM) through inland waters to the world's oceans is an important link in the global carbon cycle [175, 231, 232]. DOM is abundant in aquatic ecosystems and is often the predominant form of organic carbon (C), Nitrogen (N), and other nutrients like Phosphorus. It originates within aquatic ecosystems, arises from groundwater sources, and is imported from the surrounding terrestrial landscape. The quantity and composition of organic compounds that are available for aquagenic processes can affect the rate at which ecosystem function. However, understanding the role of DOM composition and abundance in regulating ecosystem function and structure remains a key question in stream ecology due to the compositional complexity of DOM and it's dynamic nature over space and time [71].

DOM provides a source of energy for microbes, controls absorption of light and photochemical activity, participates in nutrient cycling, buffers pH, sorbs metals and other organic pollutants, and interacts with nanoparticles [233–236]. Low molecular weight (LMW) DOM (e.g. amino acids, polyamines, nucleotides) have been shown to be directly available for plant and algal uptake [237, 238] and high molecular weight (HMW) DOM is known to be available for microbial assimilation in terrestrial and aquatic environments [62, 239–242]. Larger rivers are an accumulation of many headwater streams, and so are influenced by watershed and aquatic characteristics across broad spatial and temporal scales [243, 244]. River networks have been identified as active conduits through which DOM can be not only transported but also produced, buried in sediments, or mineralized and emitted to the atmosphere [3, 26, 41].

The need to monitor the quality and quantity of DOM and its carbon analogue, dissolved organic carbon (DOC), has recently become more apparent, partly due to the widespread increased in DOC quantity from 2003–2012 [233, 245–247]. These fluctuations in DOM concentration have been suggested to result from either or both of rising temperatures and catchment land use change driving the process by stimulating the export of DOM from peatlands [248–250]. With global climate change the rate of DOM changes is likely to increase further if global temperatures increase. However, the compositional dynamic complexity of aquatic DOM with differing chemical structures and reactivities impact our ability to understand and predict the ecological impact in terrestrial ecosystems.

The processes in controlling DOM are diverse and can include the degree of wetland and forest cover, urban and agricultural land use, precipitation, and hydrological processes [70, 251–253]. The composition and reactivity of aquatic DOM are highly dependent on carbon sources (vegetation, soil or microorganisms), transport pathways from source to sink and residence time in a watershed. Thus, the hydrologic regime and land cover distribution of a watershed substantially affect the biogeochemistry of aquatic DOM [67, 71]. For example, forests and wetlands can be a significant source of HMW, aromatic DOM while cropland and pasture can contribute amino acid-like, microbial-derived LMW DOM [254–256].

This study focuses on the DOM quantity and quality in river water of large Scottish river systems. The water samples were analysed on the LC-OCD to identify the compositional behaviour at the most downstream location of a river systems to get an integrated catchment DOM signal and to assess the DOM transport to the ocean. Furthermore, this study will explore the impact of the dominant soils (peat versus non-peat dominated catchments) and hydrological conditions (base and peak flow) on the DOM composition. Therefore, this study addresses the first hypothesis of this PhD study that DOM flux and composition varies between river catchments as a function of the dominant soil type.

Additionally, the following research question was addressed within this research chapter: ‘Do large river systems show a clear dominance of UV amenable HMW DOM [55, 257–259] or do the non-UV amenable DOM [102, 260] play a role in the DOM dynamics?’.

4.2. Methods and Material

River water samples were collected by the UK Centre for Ecology & Hydrology within the framework of the LOCATE project. LOCATE is a multi-disciplinary project that undertakes the first ever coordinated sampling of the major rivers in Great Britain. Water samples from ten Scottish rivers were collected on a monthly basis in 2017. All samples were collected at the end of the months with 3-4 weeks between sampling campaigns. More information about the sampling strategy can be found in section 2.6.1.

4.2.1. Sampling areas

A detailed description of each of the ten rivers can be found in section 2.1.1. Table 4.1 gives an overview about the ten rivers, their catchment size, dominant soil types [138], main land cover and the superficial geology defined by the National River Flow Archive.

Table 4.1: Overview of the ten observed Scottish rivers with their catchment size, dominant soil cover, main land cover and their superficial geology.

River	Catchment size (km^2)	Soil	Land cover	Superficial geology
Clyde	1903	Brown earths, Brown earths with gleying	Mountain (15 %), Grassland (54.9 %), Woodland (12.4 %)	Mixed permeability Till/Alluvium/Clay, 61.5 %)
Conon	962	Peaty gleys, peaty podzol	Mountain/Heath/Bog (74.2 %)	Mixed permeability (Till/Brickearth, 51 %)
Cree	368	Blanket peat, Peaty gleys	Grassland (39.7 %), Woodland (49.1 %)	Mixed permeability (Till/Brickearth, 38.1 %)
Dee	1844	Humus-iron podzols, Peaty podzols	Mountain (63 %), Grassland (13.8 %), Woodland (16.2 %)	Mixed permeability (Till/Alluvium/Clay, 55.9 %)
Deveron	955	Humus-iron podzols, Noncalcareous gleys	Mountain (19.3 %), Grassland (34.7 %), Woodland (21.0 %), Arable/horticultural (23.8 %)	Mixed permeability (Till/Alluvium/Clay, 73.3 %)
Forth	1036	Brown earths, Humus-iron podzols, Noncalcareous gleys	Mountain (25.2 %), Grassland (44.8 %), Woodland (22.0 %)	Mixed permeability (Till/Alluvium/Clay, 45.7 %)
Halladale	205	Blanket peat, Peaty gleys, peaty podzol	Heath/Bog (76.1 %)	Generally low permeability (Peat, 80.7 %)
Spey	2861	Brown magnesian soils, Peaty podzols	Mountain (63.3 %), Grassland (15.6 %), Woodland (18.1 %)	Mixed permeability (Till/Alluvium, 53.2 %)
Tay	4587	Humus-iron podzols, Peaty podzols	Mountain (48.1 %), Grassland (24.7 %), Woodland (15.9 %)	Mixed permeability (Till/Alluvium, 51.2 %)
Thurso	413	Blanket peat, Peaty gleys, peaty podzol	Heath/Bog (69.3 %)	Generally low permeability (Peat, 78.6 %)

4.2.2. Precipitation and hydrology

A detailed description of the precipitation data is described in section 2.4.1 and river discharge in section 2.2.1. The Base flow index (BFI), a non-dimensional ratio, was used to describe the effect of geology on low flows and access the contribution of groundwater to the streamflow, more details in section 2.3.

4.2.3. Sample analysis

All water samples were analysed on the LC-OCD system to measure the DOM composition and concentration, which was used to calculate fluxes. More information about the LC-OCD-OND analysis, flux calculation and the limit of detection (LOD) as well as the limit of quantification (LOQ) can be found in Chapter 2 section 2.7.5 and 2.7.6. All samples were measured within 2-4 days after sampling, which is an acceptable timeframe to secure a reliable dataset with minimal changes in the DOM quantity and quality, based on the results observed in chapter 3.

4.2.4. DOM flux calculations

Mean daily water flows from the 10 rivers monitored during 2017 were obtained from the Scottish Environmental Protection Agency (SEPA). Monthly DOC river fluxes were calculated using ‘‘method 5’’ of Littlewood et al. [162], detailed in Eq. 11, where k specifies a conversion factor for the duration of sampling, C_i refers to the DOC concentration at sampling time i, Q_i refers to flow at sampling time i, Q_T refers to the mean flow over the whole sampling period, and n is the number of samples taken. A more detailed description about the method can be found in Chapter 2, subchapter 2.8.

4.3. Results

4.3.1. Precipitation and Hydrology

The time series of precipitation and discharge for each river catchment investigated are shown in Figure 4.2 from April to December 2017, which captures the period of DOM sampling.

The furthest northern rivers, Halladale and Thurso, had an average daily precipitation of 4.7 and 4.5 mm, with maximum precipitation of 52.3 and 55.7 mm in June 2017. The highest discharge was measured in June 2017 in both rivers with values of 55.4 m^3/s (6th June 2017 in Halladale) and 107.2 m^3/s (7th June 2017 in Thurso), after a dry period with discharge values between 0.3 and 3 m^3/s, respectively (see Figure 4.2).

The average daily rainfall for River Conon was 5.6 mm with the highest precipitation in December 2017 of 30.6 mm leading to the highest daily mean discharge of 162 m^3/s. On average the River Conon had a discharge of 47 ± 29 m^3/s showing an increasing trend from April to December (Figure 4.2), but overall, no flashy discharge changes after heavy precipitation.

The River Spey and River Deveron in the northeast of Scotland showed comparable average daily precipitation of 3.3 and 3.7 mm; however, the maximum daily precipitation was 60.2 mm at River Deveron and only 41 mm at River Spey, both in June 2017. These large rainfall events in June 2017 lead to the largest discharge peak with river flows of 314 m^3/s in River Spey and 196 m^3/s in River Deveron. The average of mean daily discharge was 118 ± 23 m^3/s for the River Deveron and 53 ± 43 m^3/s for River Spey.

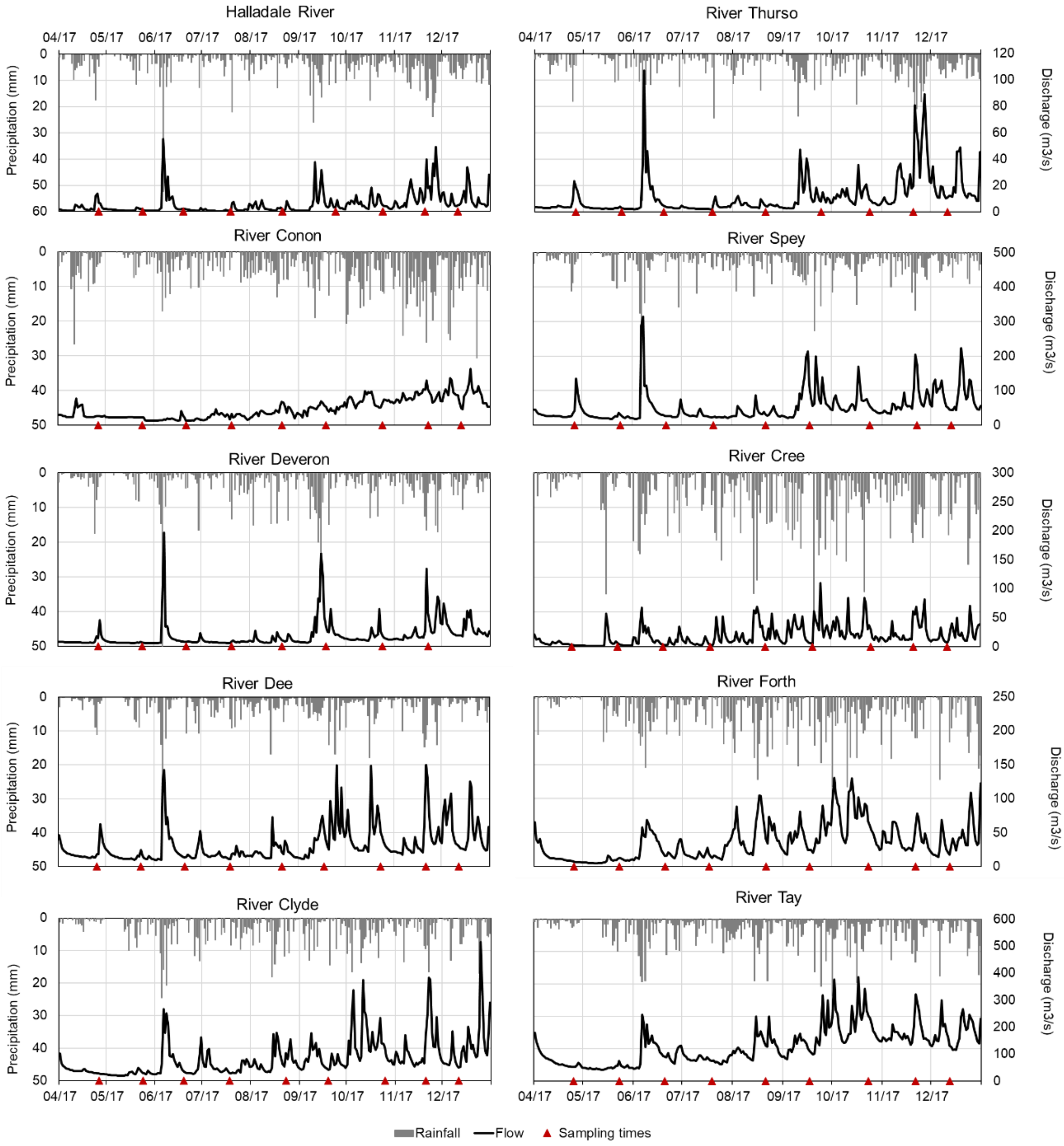

Figure 4.1: Mean daily discharge in m^3/s, total daily precipitation in mm and times of sampling from April 2017 to December 2017 for the Halladale River, River Thurso, River Conon, River Spey, River Deveron, River Cree, River Dee, River Forth, River Clyde, River Tay.

River Dee showed the lowest average daily precipitation of all observed rivers with 3 mm and a maximum of 28.8 mm in June 2017, which lead to a large discharge increase from 10 m^3/s on 5th June to 129 m^3/s on 6th June. However, in September, October and November three large discharge events were observed with values of 148 - 150 m^3/s. The longest Scottish river, River Tay, had a daily average precipitation of 3.9 mm between April and December 2017 and the highest daily precipitation of 23.6 mm was measured in October 2017.

The intensive rainfall in October lead to mean daily discharge values up to 385 m^3/s; however, on average River Tay had a mean daily discharge of 130 ± 68 m^3/s.

The urban River Forth had an average daily precipitation of 5.3 mm and a maximum daily precipitation of 26.5 mm in October 2017, leading to the highest mean daily average of 130 m^3/s. On average River Forth had a mean daily discharge of 36 ± 27 m^3/s. The other urban river and only west flowing river, River Clyde, had an average daily precipitation of 3.9 mm and the highest daily precipitation in June 2017 with 24.5 mm. However, the highest mean daily discharge was measured in December 2017 with a value of 256 m^3/s. On average the mean daily discharge was 41 ± 35 m^3/s between April and December 2017.

The most southern river, River Cree, had the highest daily average precipitation with 6.7 mm and the biggest rain event was in September 2017 with a total of 43.9 mm; however, the biggest mean daily discharge of 110 m^3/s was measured 4 days after the rainfall event. The River Cree had an average discharge of 19 ± 52 m^3/s.

The average, maximum and minimum catchment area normalised discharge in mm/day and the BFI of each river is shown below in Table 4.2.

Table 4.2: Base Flow Index (BFI), catchment area, average daily discharge and its standard deviation, maximum and minimum daily discharge normalised by catchment area from River Clyde, Conon, Cree, Dee, Deveron, Forth, Halladale, Spey, Tay and Thurso.

	BFI	Average discharge (mm/day)	Std. Dev. of average flow	Max. discharge (mm/day)	Min. discharge (mm/day)
Clyde	0.506	21.5	18.5	134.4	4.7
Conon	0.712	48.9	30.5	168.4	12.8
Cree	0.318	51.6	49.5	298.1	2.0
Dee	0.533	17.4	14.4	81.5	5.2
Deveron	0.469	18.7	23.6	205.3	4.8
Forth	0.479	34.8	25.9	125.8	4.7
Halladale	0.242	28.3	39.5	270.6	1.6
Spey	0.613	18.4	15.1	109.7	6.3
Tay	0.712	28.4	14.8	83.8	9.1
Thurso	0.398	27.4	35.1	259.7	4.6

4.3.2. DOC concentration and fluxes

Figure 4.3 shows DOC concentrations and area normalised DOC fluxes plotted versus time of each observed river with their respective linear trendline. Sum or averages fluxes of all 9 months of sampling are described as tonnes or kg/km^2, while the unit 'kg/km^2/month' or 'kg/km^2 per month' describes the average fluxes of a specific month that is named within the same sentence.

The two most northern rivers, Halladale and Thurso, and the most southern River Cree had highest average DOC concentration from April to December 2017 with 19.5 ± 2.2 mg/L (Halladale), 14.8 ± 1.7 mg/L (Thurso) and 12.9 ± 1.3 mg/L (Cree). These three peat-dominated rivers had also the highest area normalised DOC flux with average values of 1442 ± 344 kg/km^2 (Halladale), 1015 ± 217 kg/km^2 (Thurso) and 1823 ± 348 kg/km^2 (Cree), respectively. The three peat rivers transported a total of 38,523 kg/km^2 in the 9 months (April to December 2017) of this study, which is around 53% of all DOC exported from the 10 observed rivers (in total 72685 kg/km^2). The highest DOC flux in the Halladale River and Thurso River was measured in November 2017 (3475 kg/ km^2/month in the Halladale River and 2039 kg/km^2/month in River Thurso), whereas River Cree showed highest DOC flux in September 2017 (3419 kg/km^2/month).

The two more urban influenced rivers, Clyde and Forth, showed a stable DOC concentration over time with average values of 8.4 ± 0.9 mg/L in River Forth and 7.8 ± 0.6 mg/L in River Clyde. The DOC flux was on average 881 ± 233 kg/km^2 in the Forth and 455 ± 88 kg/km^2 in the Clyde with the highest DOC fluxes in October of 2233 kg/km^2/month (River Forth) and 969 kg/km^2/month (River Clyde) (see Figure 4.2).

Rivers Dee and Tay showed an overall increasing DOC concentration and flux from April to November 2017 but DOC dropped in December 2017 (see Figure 4.2). On average DOC concentrations were 4.7 ± 0.5 mg/L in River Tay and 6.8 ± 1.1 mg/L in River Dee. In River Tay the DOC flux increased from 0.9 kg/ha/month in May to 7.3 kg/ha/month in October 2017. In total both rivers transported a total of 64 kg/ha in the observed 9 months, with River Tay contributing 34.4 kg/ha and River Dee 29.6 kg/ha.

The two north running rivers Spey and Deveron showed the same DOC flux and concentration pattern with average DOC concentrations of 8.0 ± 1.4 mg/L and 9.1 ± 1.9 mg/L and a total flux in the 9 months of 3845 kg/km^2 and 3893 kg/km^2, respectively.

Both rivers showed increasing DOC fluxes over time with the highest flux in September 2017 with 1054 kg/km^2/month and 1317 kg/km^2/month but a drop in DOC flux in October.

The River Conon had the highest DOC flux from the seven rural non-peat rivers with an average of 903 ± 211 kg/km^2 and a total flux of 8127 kg/km^2. The DOC concentration was 6.5 ± 0.5 mg/L on average with the maximum concentration in November 2017 of 8.9 mg/L.

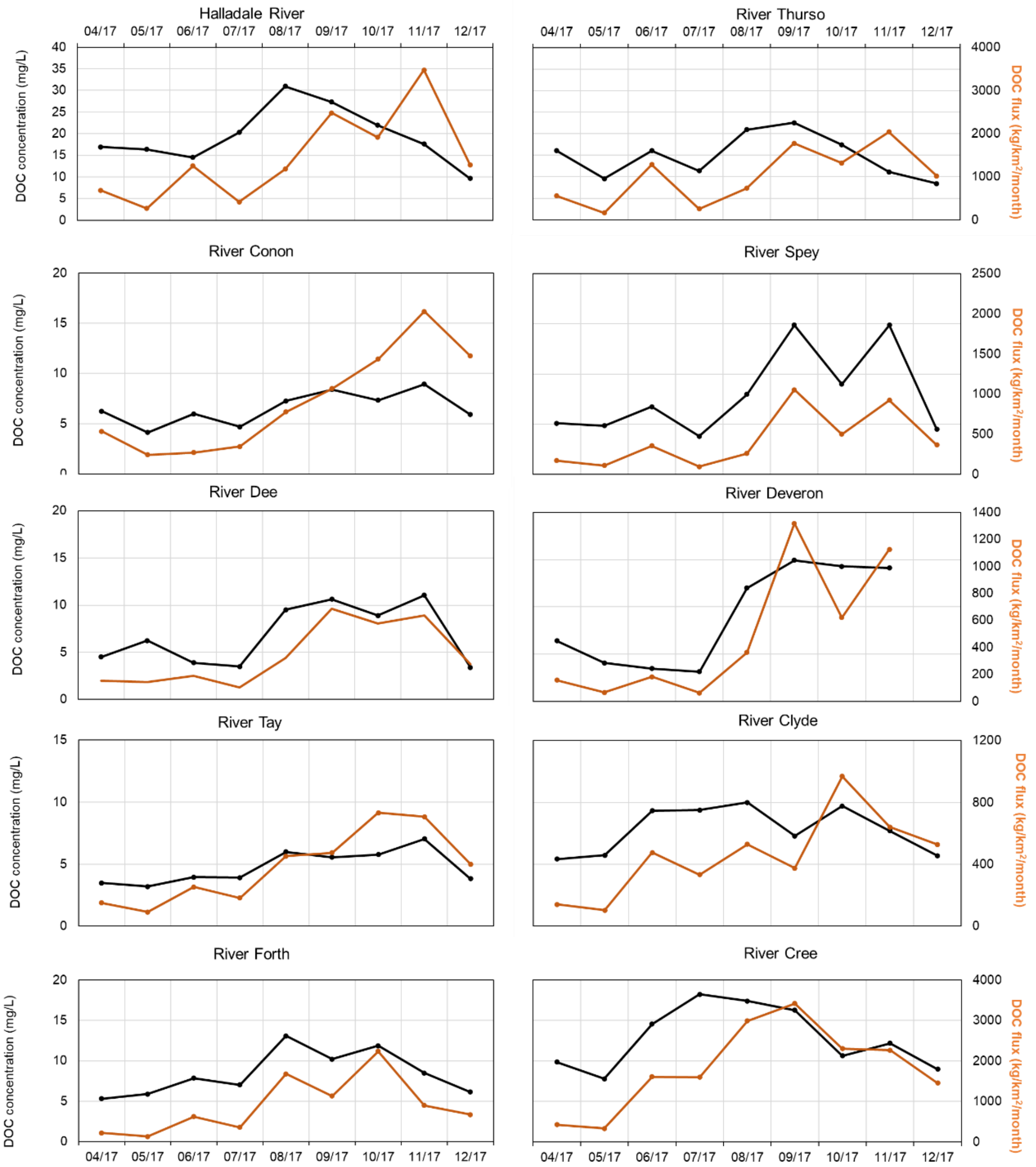

Figure 4.2: Dissolved organic carbon concentration in parts per million (ppm) and flux in tonnes per month (t/month) from the 10 studied rivers from April 2017 to December 2017

4.3.3. Spatial and temporal DOM composition

The DOM fractions and DON are reported in catchment area corrected fluxes (kg/month/ha) in Figure 4.3 and Figure 4.5 over time, flux calculations are described in Chapter 2. The total tonnes transported during the 9-month study period are displayed in the supplementary material (Appendix 4.3). LMW Acids concentrations were below LOQ (see Chapter 2), and not reported.

The two most northern rivers, Halladale and Thurso, showed the same pattern in their DOM composition with the lowest flux in May in all fractions and the highest fluxes in November 2017. Together with the most southern river Cree the three rivers transported around 8523 tonnes (26,522 kg/km^2 over 9 months) of humics from April to December 2017, which is around 69% of the bulk DOC pool with River Cree being the largest contributor. On average monthly humics flux was 968 ± 213 kg/km^2 in Halladale River, 720 ± 169 kg/km^2 in River Thurso and 1259 ± 240 kg/km^2 in River Cree. The second most abundant DOC fraction was LMW neutrals with a total of 1393 tonnes or 4658 kg/km^2, followed by building blocks with 1033 tonnes or 3200 kg/km^2 and biopolymers with 944 tonnes or 2922 kg/km^2.

River Forth and River Clyde followed a similar DOM composition pattern as River Thurso and Halladale with the highest humics flux in October and the lowest in May 2017 (Figure 4.3). River Forth showed monthly average humics fluxes of 580 ± 156 kg/km^2, whereas the River Clyde had monthly average fluxes of 290 ± 57 kg/km^2. In total both of these rivers transported 10327 tonnes or 7825 kg/km^2 humics in 9 months with River Forth contributing 67 % and River Clyde the other 33 %. The second largest DOM fraction are building blocks contributing 1728 tonnes (1267 kg/km^2 over 9 months) to the DOC pool of River Clyde and Forth followed by LMW neutrals with 1607 tonnes (1206 kg/km^2 over 9 months) and finally biopolymers with 1495 tonnes (1117 kg/km^2 over 9 months).

The River Conon showed an exponential increase in humics flux from May (145 tonnes) to November 2017 (1381 tonnes). On average the humics flux was 603 ± 150 kg/km^2, the highest humics flux from all non-peat rivers. Building blocks contributed 93 ± 24 kg/km^2 per month, whereas biopolymers were 69 ± 21 kg/km^2 per month and LMW neutrals had average fluxes of 104 ± 22 kg/km^2 per month. In total the River Conon transported over 9 months 5428 kg/km^2 humics, 933 kg/km^2 of LMW neutrals, 838 kg/km^2 of building blocks and 619 kg/km^2 biopolymers.

The contribution of DOM fractions over time in the River Spey and River Deveron is similar; however, the fluxes were higher in River Spey than River Deveron (Figure 4.4). River Deveron and River Spey had the highest contributions of humics in September with 866 kg/km^2 and 747 kg/km^2, respectively. In total 5187 kg/km^2 humics were transported in both rivers over 9 months.

River Spey transported 7451 tonnes of building blocks in 9 months and River Deveron 2476 tonnes, but area-normalised fluxes showed that both rivers transported in total 684 kg/km^2 over 9 months. In total both rivers transported 843 kg/km^2 LMW neutrals and 661 kg/km^2 biopolymers over 9 months.

The last two rivers, River Tay and River Dee had the lowest fluxes in all DOM fractions. humics contributed 1818 kg/km^2 in River Dee and 2106 kg/km^2 in River Tay. Building blocks were in total 665 kg/km^2 from both rivers, biopolymers contributed 470 kg/km^2 over 9 months and LMW neutrals had the second highest fluxes with 1025 kg/km^2 in total for both rivers combined. River Tay had the highest flux of LMW neutrals with 261 kg/km^2 in November 2017, the largest measured flux of LMW neutrals beside the three peat-dominated rivers.

On average $SUVA_{254}$ is 5.3 ± 1.1 L/(mg*m) with the highest value 11.1 L/(mg*m) measured in River Tay in June and the lowest value of 2.8 L/(mg*m) measured in River Dee in April 2017. Monthly variations in $SUVA_{254}$ values are shown in the supplementary material for each river (Appendix 4.8).

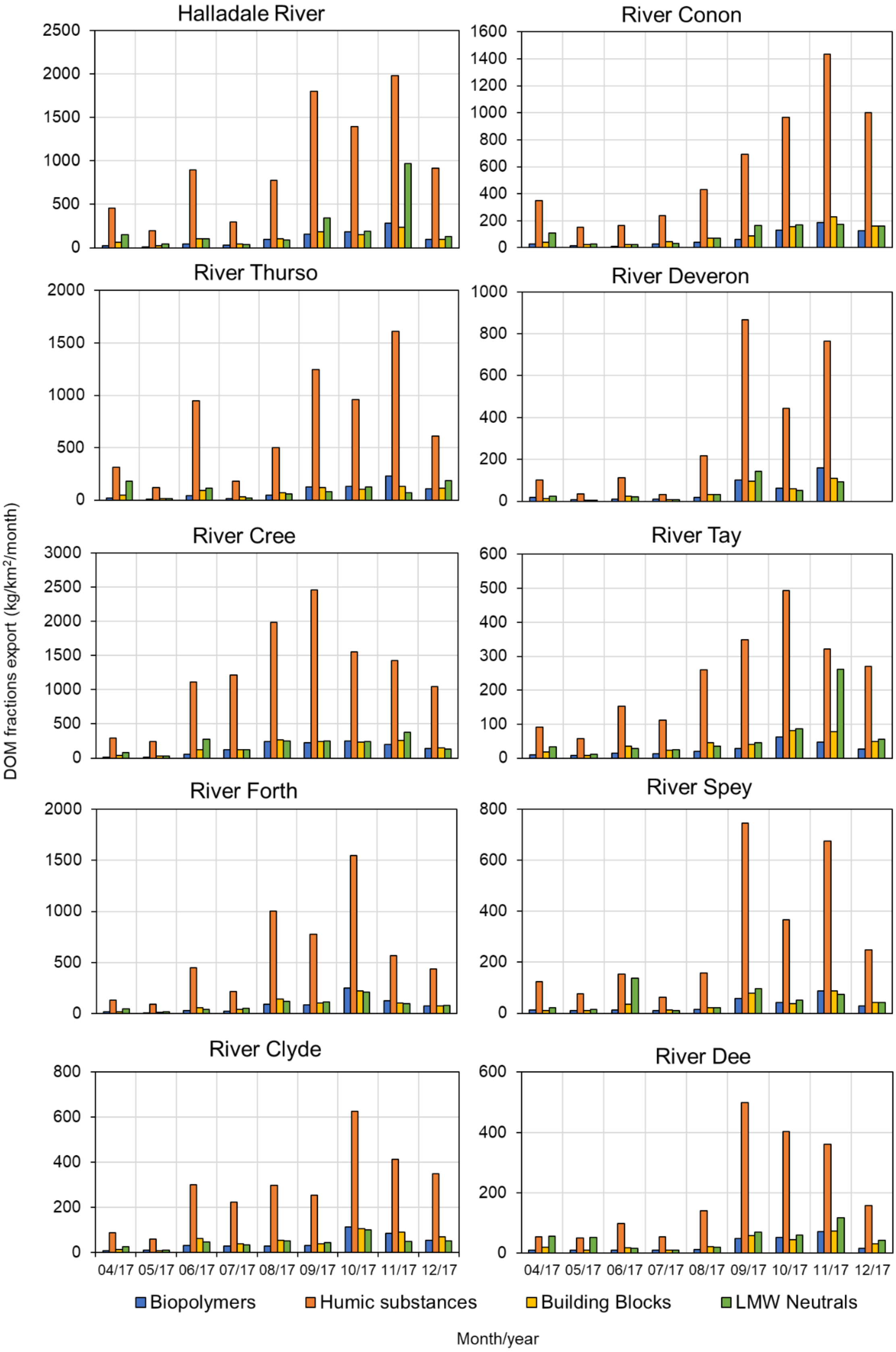

Figure 4.3: Fluxes from DOM fractions biopolymers, humics, building blocks, LMW neutrals and LMW acids in kilogram per month per hectare.

The Humification pathway (Figure 4.4) shows a trend towards higher proportion of more humified and higher molecular weight humic substances in the three rivers that are dominated by peat and peaty podzols, Rivers Cree, Halladale and Thurso. Overall, the biggest shift was observed in the molecular weight of the humics (720 to 991 g/mol), whereas the aromaticity fluctuates between 4.3 and 6.6 L/(mg*m).

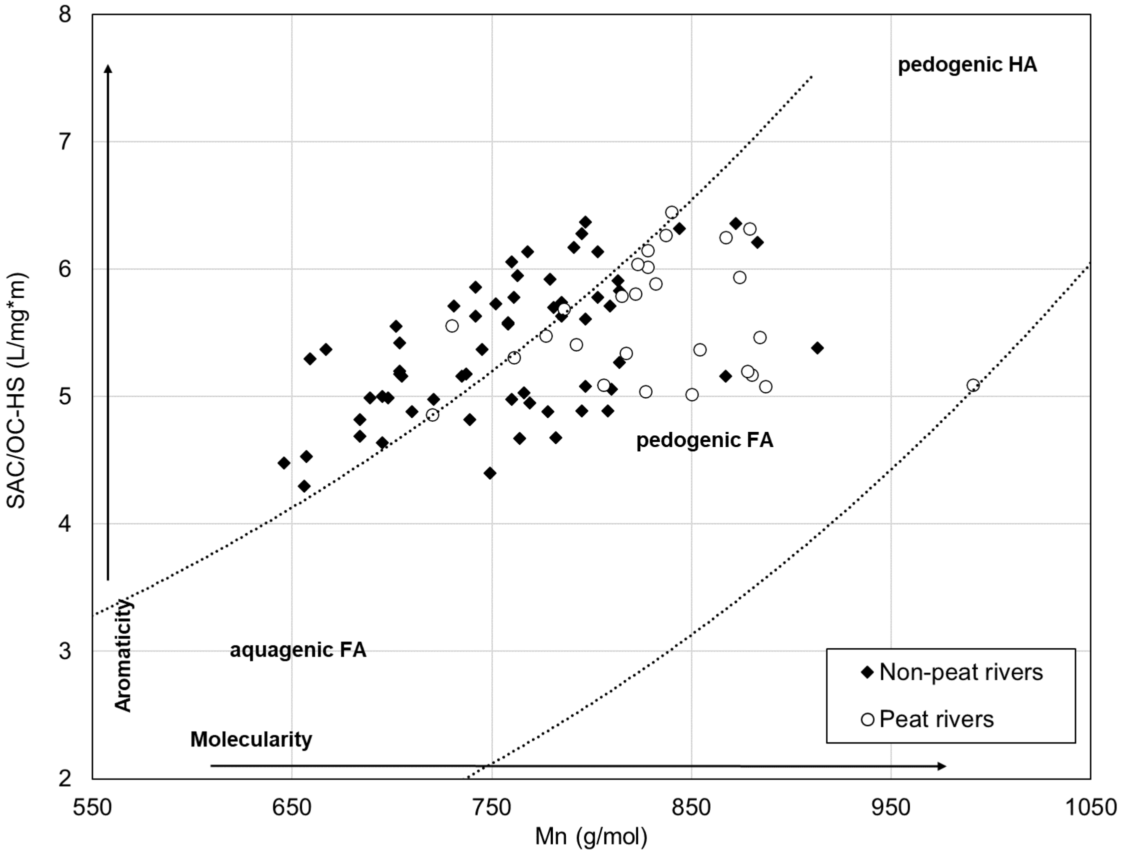

Figure 4.4: Humification pathway showing molecular weight (Mn) in gram per mole versus Aromaticity (SAC/OC) in Litre per milligrams times metre for Humic substances from peat rivers (Halladale, Thurso, Cree) in white circles, and non-peat rivers (Clyde, Conon, Deveron, Dee, Forth, Spey, Tay) in black diamonds.

Area normalised flux of DON associated with humics (DON HS) and biopolymers (DON BP) over time are displayed in Figure 4.5 for each individual river.

The three peat-dominated rivers, Cree, Thurso and Halladale transported in total 197 kg/km^2 DON BP over the 9 months of this study and 425 kg/km^2 DON HS with River Cree contributing the highest fluxes in both DON fractions (Figure 4.5). The two rivers in North Scotland, Halladale and Thurso, follow a similar up and down trend in DON fluxes, beside in September 2017 where DON BP flux increased by 14 kg/km^2/month in comparison to the 6.4 kg/km^2/month increase in River Thurso.

DON HS and DON BP follow the same trend in River Cree beside the sample from July where DON HS decreased, and DON BP increased (Figure 4.5).

The two more urbanised non-peat rivers, River Forth and Clyde, transported a total of 90 kg/km^2 of DON BP and 157 kg/km^2 of DON HS. Both rivers showed the highest DON fluxes in October 2017 and the lowest in April (Figure 4.5).

The DON fluxes in River Conon follow an exponential increase from May to November and a drop in December, as before the bulk DOC and its fractions. In total the River Conon transported 37 kg/km^2 of DON BP and 63 kg/km^2 DON HS. The other smaller non-peat river, River Deveron, had a DON HS flux of total 50 kg/km^2 and DON BP flux of 24 kg/km^2. The DON flux of these two smaller non-peat rivers reflects 54 % of the total 9 months DON BP flux and 52 % of the total DON HS flux from the seven non-peat rivers.

River Dee and Tay had a comparable trend in their DON fluxes, whereas River Spey showed a similar temporal trend as River Deveron. River Spey was the only river who had a higher DON BP flux than DON HS, measured in September 2017 (Figure 4.6). Overall, the highest DON BP and DON HS was measured in September in all three rivers (Figure 4.5). In total, the three rivers transported 49.6 kg/km^2 of DON BP and 97.2 kg/km^2 DON HS over 9 months.

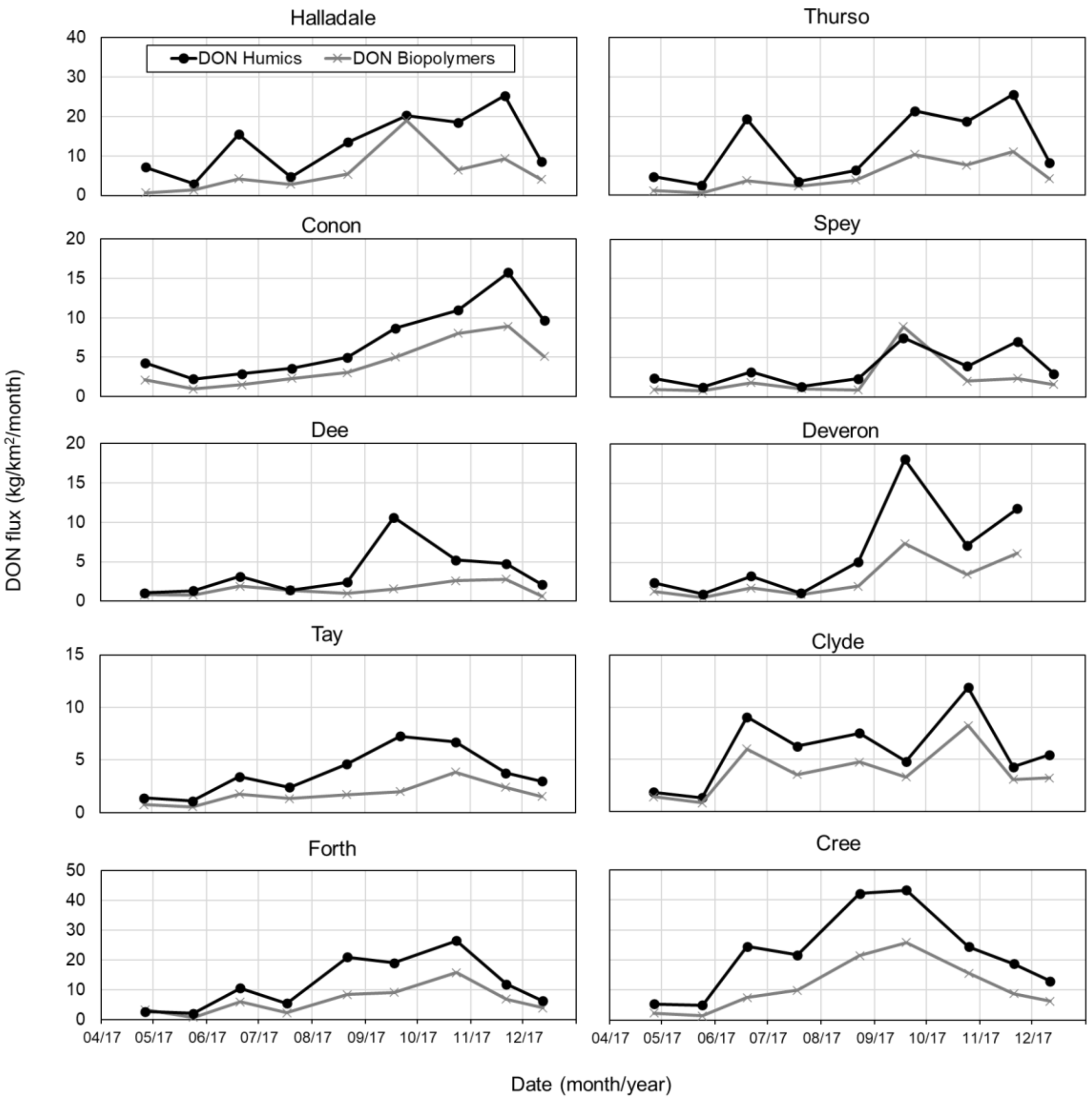

Figure 4.5: Dissolved organic nitrogen (DON) flux in kilogram per month per hectare for Humics (DON HS) and Biopolymers (DON BP) from all ten observed rivers from April to December 2017.

4.4. Discussion

4.4.1. Influence of precipitation on river discharge and DOM

Over the past century precipitation was found to be one of the main driver of change in discharge in European rivers [261]. However, soils and drainage basins temporarily store each pulse of rainfall to give a streamflow output more inhibited than that of the rainfall input [49]. This leads to a lag-time between precipitation and discharge [262–264].

In this study, precipitation and discharge showed a positive covariance with a 24-hour delay in all rivers (n = 223 - 275, $p < 0.01$, Appendix 4.9); however, the correlation coefficient was in all cases below 0.5, suggesting additional fundamental controls on the discharge and possibly DOM transport.

Though, this finding is based on total daily precipitation and mean daily discharge, which reduces the ability for a high temporally-resolved interpretation of the precipitation-discharge relationship. Nevertheless, it is noteworthy that the year 2017 was one of the wettest years in the Cree, Halladale and Thurso river catchments (Appendix 4.8) and healthy peatlands can accumulate large amounts of water [265, 266] and DOC [10, 266, 266, 267] that can get transported due to large rain events after a long dry period or due to water oversaturation in the soil [266] leading to a weaker relationship-discharge relationship.

The precipitation-discharge relationship can also be influenced by anthropogenic induced factors including river regulation, dam construction, and land cover changes [261, 268]. Dam construction could be an explanation for the weak response of river discharge to precipitation in the Conon catchment and also the overall hydrological pattern observed in the Conon river (Figure 4.1). The upper Conon hydroelectric scheme involves a large and complex system of water transfers, storage, control and power generation [269]. The scheme is likely to exert a considerable effect on the downstream river response both in terms of water and DOM quantity and composition.

Moreover, it was observed that the samples taken in winter months (October - December) may be driving the overall DOC-precipitation trend by contributing more DOC during heavy precipitation; however, the data density is too small to find a statistically significant seasonal trend in this dataset. During rainfall periods, various DOM components contribute to streamflow at different times and to different extents [270, 271], which is likely to impact the supply of DOM. Although, rainfall-runoff transports a large amount of terrestrial OM, including lignin and humic substances, to rivers [272, 273] this study found that while the rivers Conon and Spey show a good linear correlation ($n = 5 - 7$, $R^2 = 0.4 - 0.5$, Appendix 4.5) between precipitation and DOC, only the Clyde river showed a statistically significant positive correlation ($p = 0.05$, Mann Kendall correlation).

The low data resolution for precipitation, discharge and DOM is most likely the main reason why no or weak relationships were found between precipitation-discharge and precipitation-DOM, hence high-resolution time series are necessary to get a better insight in these relationships. A more detailed understanding will be especially important in peaty river catchments due to the complexity of drying and rewetting processes of peat and its potential to release DOM during these times.

4.4.2. Relationship between river discharge, DOC and DON

The biogeochemistry and environmental roles of DOM within rivers are dependent on a series of natural and anthropogenic factors within the watershed-estuary coastal continuum, such as hydrology, land cover, and in situ biogeochemical transformations [16, 251, 274–277].

In regards to the river hydrology it has been observed in previous studies that DOC concentration usually increases during high flow conditions [47, 275, 278], leading to increasing DOC fluxes, respectively [39, 52, 229]. Although most rivers in this study showed a weak linear correlation between discharge and DOC (Appendix 4.4), the statistical analysis with the Mann Kendall test revealed that seven of the ten rivers showed a statistically significant correlation between DOC and discharge ($R = 0.3 - 0.8$, $p < 0.05$). The only rivers that showed no significant correlation and the weakest linear relationship were River Clyde, Cree and Thurso (Appendix 4.4). The sampling location in the Clyde is largely affected by the city Glasgow, a highly urban area. Urban rivers are known to export large amounts of DOC [279], natural and anthropogenic sources, but urbanisation can also lead to dramatic changes in soil permeability and stream dynamics [280], hence the correlation between DOC and discharge observed in the other large non-peat rivers is disturbed in the highly urbanised Clyde catchment. The missing link between DOC and discharge in the River Cree and Thurso may be linked to the peat coverage of these catchments. Peat is known to capture large amount of water before release to the streams and rivers, suggesting a delayed response of DOC towards discharge. Furthermore, the Halladale river shows a decreasing trend between DOC and discharge, although significant it is on the edge of significance with $p = 0.05$. This suggests that rivers from peat-dominated catchment have a notably different hydrological connectivity than mineral soil dominated rivers and release DOC at different times of the hydrological cycle. However, more data points are needed to clarify the relationship between DOC and hydrology, especially temporal high-resolution sampling could create a more detailed image of the hydrological and biogeochemical behaviour of these river systems.

Another observation in River Tay and Dee was that the DON concentration and flux increased in the humics without showing an increase in DON of biopolymers in September 2017. This could suggest increased contribution of microbial derived DOM either directly or via degradation of terrestrially derived humic substances compounds.

This observation is supported by looking at the winter-summer differences in DOC and DON. The C/N ratio of biopolymers suggest that summer waters contain aquagenic DOC sources whereas during the winter, waters are more dominated by pedogenic sources. The high proportional contribution of proteins within the biopolymers in summer samples supports the previous finding of increased downstream supply of aquagenic material (e.g. algae and macrophytes).

Furthermore, the upper catchment area of River Dee and Tay is highly dominated by upland moorlands [135, 281], which were found to act as net sinks for organic and inorganic nitrogen [282]. The dominance of N-rich moorland in parts of the river catchments could be an explanation for increased delivery of DON HS during peak flows; however, this needs further investigation.

This shifts between DON HS and DON BP also suggests a fast cycling of nitrogen compounds at specific times, like September 2017, highlighting the importance of high temporally resolved investigations.

However, these shifts between DON HS and DON BP were not observed in all rivers or in more than one or two months, which makes it difficult to link this DON BP loss or extreme gain to a specific catchment characteristic or hydrological pattern. This behaviour between carbon and nitrogen within the humics and biopolymers pools needs further investigation to get a better understanding of the underlying processes that affect these two DOM pools.

The Clyde is the only river who showed a clear increase in DOC and DON concentration during peak flow in June 2017. The Clyde catchment, especially at the sampling location, may be impacted by a high degree of urbanisation from the city of Glasgow and a number of industries [118, 119], releasing potentially additional DOM during peak flow conditions or the high degree of urbanisation is enhancing overland flow [283].

The Halladale River showed a clear response between precipitation and discharge in June, September and November of 2017 (Figure 4.2), seemingly diluting the DOC and DON concentration, but overall increasing the DOC and DON flux (Figure 4.3 and 4.6) due to more water in the system. This suggests a DOM source that has a limited mobilisation capacity but as more of the Halladale river catchment becomes wetter it has the potential to deliver more carbon and nitrogen to the river water.

A similar observation with increasing DOC and DON fluxes but decreasing concentrations was observed in November in River Thurso and in August and September in River Cree.

This unique behaviour of DOC and DON in the rivers Cree, Halladale and Thurso is possibly linked to the dominant soil type in these river catchments – peat. The few studies that have been carried out in peatland/wetland areas have found a poor or weak negative correlation between stream flow and DOC concentrations [38, 46, 181]. As peat is typically permanently waterlogged, and often forms over a poorly drained impeding mineral layer, flow at both high and low flows is through an organic layer. In this study, the three peat rivers combined showed a positive correlation between discharge and DOC, but it is not statistically significant (Appendix 4.10).

Nonetheless, these peat catchments tend to export more DOC than catchments with organo-mineral soils as less DOC is fixed by mineral material [284], which was also observed in this study.

4.4.3. Connection between DOM composition and river discharge

Although, the DOC quantity is important to quantify for short-term budget calculations and long-term trend detection, the compositional changes within the DOM pool between seasons [16, 38, 285] and especially under changing hydrological conditions are important to get a better understanding of ecosystem functioning [67, 286]. However, the relationship between discharge and DOM is complex because river flow consists of several source waters such as surface, rapid and delayed subsurface, and groundwater flows which come from different sources and can therefore affect the amount and composition of DOM in the river at any given time.

$SUVA_{254}$ is a widely used index for aromaticity of the bulk DOM pool that gives an indication about the source of the DOM. In this study the $SUVA_{254}$ ranged within the typical range for freshwater systems (from 1.0 to 6.0 L/(mg*m), [287]). The highest values were measured in River Tay, Thurso, Halladale and Cree and indicate a strong terrestrial signature [67] or potentially increased influence from iron, colloids, or nitrate [97, 288]. The increasing humics and DON concentrations in River Halladale, Thurso, Cree with increasing $SUVA_{254}$ supports the conclusion of enhanced terrestrial leaching and runoff, which was known from peat-dominated river catchments [289–292].

This strong terrestrial signal could be linked to the high soil carbon and nitrogen content of peat or the overall better connectivity between the soils and river due to the smaller catchment size and shorter river length of River Cree, Halladale and Thurso. River Tay is the only river who showed an extremely high SUVA$_{254}$ value (11 L/(mg*m)) during peak flow conditions, which could be linked to an increase in hydrophobic organic carbon (HOC) or a higher contribution of proteins within the biopolymers pool in this specific sample. However, other samples had higher HOC and proteins contribution but much lower SUVA$_{254}$, hence the exact reason for this outlier, determined by box and whisker plot, is unclear.

Furthermore, the use of SUVA$_{254}$ as a DOM quality representation is critical because it is only representing the UV amenable fractions of DOC [97], which is a limitation in this study since the non-UV amenable LMW Neutrals fraction is highly abundant in the observed rivers (Appendix 4.1).

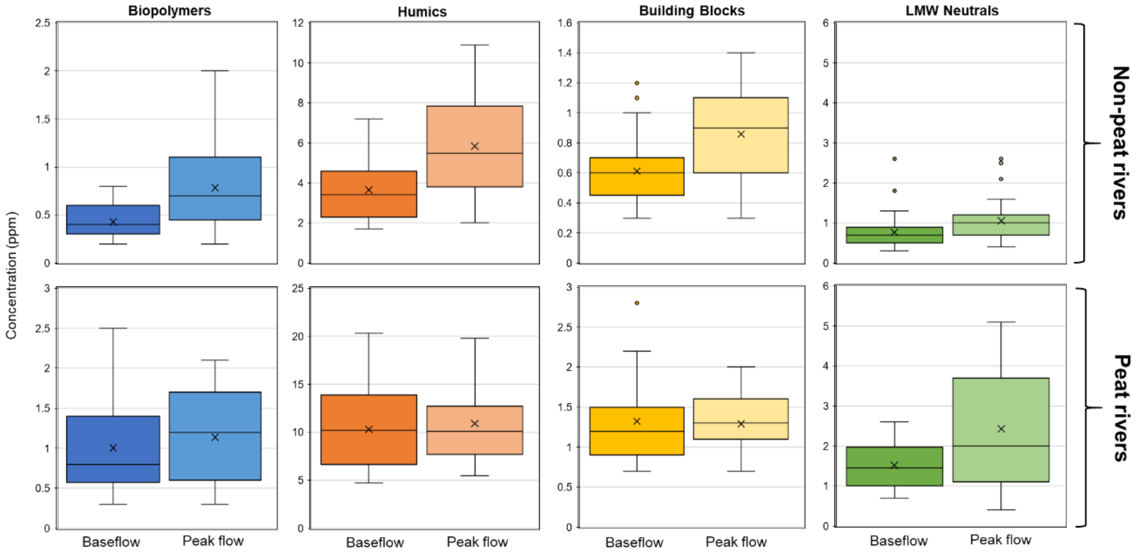

Figure 4.6: Biopolymers, humics, building blocks and LMW neutrals concentrations during baseflow and peak flow conditions for non-peat and peat rivers.

Figure 4.6 shows that in non-peat rivers all four DOM fractions (biopolymers, humics, building blocks and LMW neutrals) had on average higher concentrations during peak flow than during base flow conditions. However, the $SUVA_{254}$ does not reflect this increase in higher aromatic biopolymers and humics, which is an indicator that $SUVA_{254}$ is not always suitable to represent DOM quality.

The shift in biopolymers contribution during peak and base flow could be linked to seasonality. Biopolymers had overall higher concentrations and fluxes in winter than summer (Appendix 4.2), which could indicate that in winter an additional or different DOM source is influencing the composition. Biopolymers are produced by the cells of living organisms and the most common one is cellulose [293]; hence the increased concentration may indicate enhanced supply of plant material from overland flow due to highly water saturated soils, as previous observed in the higher C/N ratio. However, the increase of Biopolymers is unlikely to be linked to river discharge as no correlation was found between these parameters in peat and non-peat rivers (Appendix 4.10).

This study found that humics are dominating the DOM pool, which is in line with previous studies [55, 108, 156, 290]; however, in peat dominated rivers humics tend to be higher in molecular weight than non-peat rivers, which may be linked again to the dominant soil type in the river catchments. A study by Perminova et al. [294] showed that humic material from peat is generally higher in molecularity than podzolic and arable soils. However, this observation could also indicate a higher degree of degradation of HMW in non-peat rivers compared to peat-dominated rivers or a large pool of more recalcitrant compounds, predominantly humic substances, offer a substantial supplement to heterotrophic metabolism [295]. Although biologically more recalcitrant and less energy-yielding than monomers or complex polysaccharides [296], a portion of the humic substances is biologically degradable [297, 298]. However, this needs further investigation on DOM degradation in different soil and vegetation types within Scottish peatlands. In regard to river discharge and humics, the data suggests that podzol dominated rivers (see table 4.1) like Tay, Dee, Deveron, Spey and Forth experience an increasing input of humics with increasing discharge, while the peat rivers Halladale, Thurso and Cree show a much more fluctuating relationship with an overall decreasing trend between humics concentration and discharge suggesting dilution of humic sources to the river (Appendix 4.7).

A remarkable finding in this study is that LMW Neutrals are contributing up to 40 % to the total DOM pool in non-peat rivers and up to 32 % in peat-dominated rivers. LMW Neutrals are UV ‘invisible’ low molecular weight compounds that are potential degradation products of carbohydrate plant OM sources [95]. The highest concentrations of LMW Neutrals was found in the Thurso and Cree during peak flow conditions (Appendix 4.6), which supports the implication that indices like $SUVA_{254}$ underrepresenting the DOM pool. Nonetheless, it may suggest that during peak flow conditions, introduced through overland flow and/or increased precipitation, more labile DOM is introduced into peat-dominated rivers than during base flow conditions. However, this signal can be overpowered by increased humics and biopolymers transport from soil to river, which was also observed in this study. Nonetheless, the enhanced input of humics could also favour LMW neutrals by increased bacterial and microbial turnover of HMW organic compounds [32, 62].

However, the linkage between river flow and LMW neutrals concentration seems to be influenced by a seasonal component. Exports of LMW neutrals from peat rivers vary between summer and winter, showing dominantly higher export of LMW neutrals in summer than in winter, which could be linked to the drying/rewetting cycles of the peat leading to a change of DOM sources [50].

Highly saturated peats and high flow conditions in winter will contribute more allochthonous, potentially degraded, aromatic, humic DOM, which was proven in previous studies [50, 276]. On the contrary non-peat rivers show an increase in LMW neutrals with discharge during peak flow conditions (Appendix 4.6).

Although the shifts in DOM composition in large river systems is not always linked to river discharge some hydrological variables can give indications on how geology or soil types affect the catchment hydrology and potentially the mobilisation of DOM to the river. The BFI is one of the most used indices for streamflow characterisation by estimating the influence of groundwater to the streamflow (see Chapter 2 – 2.3). On average the non-peat river had a BFI of 0.58 and the peat rivers of 0.32, indicating rural non-peat rivers are more influenced by groundwater sources than the peat rivers.

Although the BFI is a fairly broad index it might indicate that LMW neutrals are not linked to groundwater resources since they occur proportionally in peat rivers just as much as in non-peat rivers.

However, the stable contribution of LMW neutrals could also be linked to a reservoir effect either induced by lakes or anthropogenic created water reservoirs [299–301] or high water storability of the soils [266, 267]. This may lead to longer residence times of water within the catchment so that DOC and its fractions remain stable.

Although the source of LMW Neutrals is unclear the results in this study imply that the abundance of LMW Neutrals is linked to the dominant soil type and seasonal hydrological changes or potentially other seasonal factors like temperature and UV radiation. It is therefore recommended that all future studies who quantify DOC through indices using 'colour' (CDOM) would need specific data calibration for soil type and a peak/base flow.

Overall, the outcomes of this study imply that current carbon budgets from rivers as well as the global carbon budget may need to be revised as carbon models and calculations are often based on CDOM measurements [98, 159, 258, 302], which are not reflecting LMW Neutrals. Although, recent carbon models included a non or low UV amenable DOM fraction [98, 172], this study shows that it will likely not be representative for all rivers since the general DOM composition and specifically the export of LMW Neutrals is strongly impacted by river flow and soil type.

Furthermore, this changing DOM composition will undoubtedly impact downstream aquatic function due to the changing supply of nutrients as conceptualised in the river continuum concept [303]. Freshwater DOM provides energy and nutrients for stream heterotrophic microbes and associated food webs [304], and the amount available for microbial uptake is dependent not only on the quantity but also on the molecular composition of the OM [180, 305, 306]. Thus, upstream changes to DOM composition can alter food webs in downstream reaches [307].

Additionally, these compositional changes can also impact safe drinking water supply as drinking water produced from sources with elevated levels of DOC can produce potentially dangerous by-products (e.g. chloroform, haloacetonitriles, chloral hydrate and trihalomethane by-products) when treated by the most common chlorination practices [308]. In particular, the phenolic/aromatic portion of some DOC profiles are believed to be reactive sites for by-product formation [309]. A previous study by Li et al. [310] showed that coagulation-sedimentation processes dominantly removed nitrogenous compounds, alcohols and aromatic hydrocarbons but increased the concentrations of halogen-containing compounds, while biological activated carbon (BAC) treatment removed particularly ketones, alcohols, halogen-containing compounds and acids.

However, Li et al. [310] noted that certain highly polar or HMW compounds not identified in their study might be released from the BAC bed. Overall, they observed that different treatment processes achieved various outcomes in removing DOM fractions, hence investigating the compositional behaviour of DOM in drinking water sources like rivers is important for the planning and implementation of treatment processes.

4.5. Conclusions

This study assessed the spatial and temporal patterns of DOM transport in relation to surrounding soil types, precipitation and discharge. It was shown that the dominant soil type of the river catchment had a visible impact on the DOM flux and composition. However, a more detailed statistical approach to differentiate the different catchments needs to be developed to get an even better understanding on the DOM-soil dynamics. Nonetheless, the soil-type signal appeared to suppress the signal of seasonality, which does not exclude that the seasonal trend is impacted by individual or extensive rain events that can influence discharge response. However, in this study precipitation was not found to be a key driver in temporal DOM variation, which is most likely linked to the data resolution and the scale of the catchments.

Furthermore, it was demonstrated that peat catchments transport more DOM per hectare than non-peat rivers but overall more DOC load came from non-peat rivers, potentially linked to urbanisation/sewers or the connectivity between soils and rivers. Furthermore, the DOM concentration is higher in peat rivers suggesting that with increasing storm events peat-dominated rivers are likely to produce even higher DOM fluxes, supporting the hypothesis that peat-dominated rivers is more affected by seasonal changes in hydrology and precipitation. Additionally, this study showed that humics are the dominant DOM fraction in peat and non-peat rivers; however, all rivers had a continuous and proportionally large supply of labile LMW neutrals. In total around 9153 tonnes of LMW neutrals were transported in the 10 large rivers over only nine months which is around 12.6 % of the total bulk DOC. As these compounds are largely “invisible” to commonly employed in-situ sensor technologies that measures carbon based on UV absorbance, the carbon budgets may be higher within the rivers worldwide as previously thought.

Translating the 12.6 % to other rivers like the Congo with DOC fluxes of 12,400,000 tonnes per year would imply that around 1,555,000 tonnes DOC was not detected, whereas in UK rivers, peaking at 1,680,000 tonnes per year DOC flux, around 210,000 tonnes per year were missed. Overall, riverine DOM and river flow are the result of the complex natural processes, which operate on a catchment scale. Conceptually, a river catchment can be perceived as a series of interlinked reservoirs, each of which has components of recharge, storage and discharge. Recharge to the whole system is largely dependent on precipitation which is often interlinked with seasonality, whereas storage and discharge are complex functions of catchment physiographic characteristics like soil type.

Chapter 5 – Insights on the DOM dynamics and composition of Scottish headwater catchments – influence of hydrology and seasonality

5.1. Introduction

Headwaters (i.e., springs and intermittent, first and second-order streams defined by Strahler [137]) are abundant and unique components of a river network. Relative to larger streams and rivers that are fed by upstream networks and affected by cumulative upstream stressors, the small drainage areas of headwater streams give these systems high levels of hydrologic independence and ecological autonomy. There is increasing evidence that the water quality, biodiversity, and ecological health of freshwater systems depend on functions provided by headwater streams. Among the functions of these streams are the maintenance of natural discharge regimes, the regulation of sediment export, the retention of nutrients, the processing of terrestrial organic matter (terrOM), and the establishment of the chemical signature for water quality in the landscape [311–313]. Headwaters not only transport material but transform it through chemical, physical, and biological reworking [314]. The origin of organic matter (OM) is of particular interest since it greatly determines its chemical and biological reactivity and hence controls specific transformation pathways [315, 316]. Elemental ratios of carbon and nitrogen (C:N ratio) and stable isotopes for source identifications and process studies in terrestrial and aquatic environments are well known. The $\delta^{13}C$ of soil organic matter (SOM) have been used successfully to identify organic carbon sources and to enhance the understanding of OM cycling [317]. The $\delta^{15}N$ isotope has been beneficial to the study of nitrogen cycling, particularly for processes such as nitrogen fixation, nitrification, and denitrification [318], and was also used as trophy indicator and for tracing the flow of N within food webs [319].

One of the most important landscapes and the biggest terrestrial carbon stores are peatlands with northern peatlands storing an estimated 4.5 Pg of carbon [10]. In the UK, peatlands account for approximately half of the total soil carbon pool [320] and are therefore of major importance in terms of the country’s overall carbon inventory. Furthermore, peatlands are vulnerable to climate and weather changes and land-use impacts [10, 13, 16, 38, 275]. The dynamic global peat and vegetation model from Müller et al. [321] explored potential drivers of carbon changes in peatlands.

The model data showed that changes of soil carbon in the northern high latitudes are mostly driven by temperature and precipitation increases in the past and likely in the future. Although the model emphasises the importance of treating and understanding peatlands as dynamic and evolving systems it only includes the terrestrial carbon stocks but does not include aqueous carbon flows. However, the role of fluvial systems in the export of carbon from peatlands has been increasingly recognised [28, 181, 248, 322, 323] with recent studies suggesting decreasing dissolved organic carbon (DOC) fluxes [246, 324] in UK rivers. However, bulk DOC is only one part of the total dissolved organic matter (DOM) pool in riverine ecosystems. DOM in the surface waters is a complex mixture of humic substances, carbohydrates, carboxylic acids, amino acids and nutrients [325]. These compounds originate from terrestrial and aquatic production and are a major energy source for the aquatic food webs [36]. As the dominance of peat DOM has a terrestrial origin [251] many previous studies used UV absorbance and fluorescence measurements allowing determine source and composition of DOM [16, 101, 258, 326]. Additionally, some studies explored the impact of seasonality [16, 38, 50, 275, 285] and hydro-meteorological conditions [275, 277, 327, 328] on the DOM pool; however, only using the restricted measurement approach of bulk DOC and UV absorbance and fluorescence, reflecting only a partial pool of DOM.

Tipping et al. [170] created a model to quantify DOM concentration, as well as indicating its quality using two different absorbance wavelengths. However, these methods only describe changes within UV-amenable aromatic DOM (humic- and protein-like components). Carter et al. [98] expanded the initial 2 component model to include a third non-UV absorbing component that is present at a low constant concentration and is a fixed value (0.8 mg/L). However, Carter’s model underestimated DOC in shallow eutrophic lakes in the Yangtze Basin, China. Thereupon, Adams et al. [172] evaluated the extinction coefficients in the UV range of algae-derived DOM that is dominant in eutrophic lake systems. They used published data on algal cultures and new data from outdoor mesocosm experiments to quantify the average extinction coefficients for algae derived DOM, which was a missing component in the Carter model. Although these models include a wide range of absorbance data from different streams and rivers from a variety of land-use types other studies observed non-UV absorbing DOM that may not be reflected by the Carter and Adams model approach as they are characterised by their molecular masses not by their ability to absorb UV light [102, 329, 330].

Advanced systems like Size-Exclusion-Chromatography are able to measure DOM pools based on their molecular size and enable characterisation between non/low-UV absorbing and UV absorbing DOM fractions. These data can help to improve the DOC model by including measured values for the non or low-UV absorbing fraction, which can be used in further models to examine the impact of climate and land-use change. Given the wide variety of sources and changing contributions of DOM over time and in different catchment systems, it is essential to better understand how DOM composition varies, to identify the underlying drivers behind this variation and consequences thereof as part of dynamic aquatic systems.

While the previous study (Chapter 4) showed that peat-dominated river catchments show a different dynamic in their DOM composition than catchments with less or no peat influence it also showed that the hydrological conditions are the main driver of the DOM dynamics in large aquatic systems. To obtain a more in-depth understanding of the riverine DOM dynamics this study focuses on two headwater catchments, as they are known as a river's source and physical, biological and chemical features of headwaters can have profound impacts on downstream waters. In this study well-established UV absorbance analysis were used together with novel Size-Exclusion-Chromatography analysis to assess the variability of DOM quantity and quality in a peatland and peat-podzol headwater. The DOM composition was investigated in regard to seasonal interactions (Summer/Winter), hydrology as well as soil type (peat vs. peat-podzol) addressing the second hypothesis of this thesis that the variability in the DOM composition of headwaters is depending on seasonal and hydrological conditions, with higher inputs of terrestrial DOM in winter during peak flow conditions than in summer with low flow conditions.

This study also takes a look into the SOM and WEOM composition to get a deeper understanding of the source of the riverine DOM and the general composition of the surrounding soils. This objective is addressing part of the third hypothesis by demonstrating the distribution of the DOM fractions in the soils and the possibility of humic rich DOM leaching from the surface rather than deeper soil horizons.

Furthermore, the study addresses the question if the Carter and Adams [98, 172] carbon model are applicable for peat-dominated headwaters to examine how carbon models can be utilised to examine DOC composition and turnover under differing scenarios from these important ecosystems.

5.2. Methods and Material

To investigate the seasonal variability of DOM concentration and composition in temperate headwaters monthly to biweekly water samples were taken from March 2017 to March 2019 in the Auchencorth Moss and Menstrie catchments. The winter (October to March) and summer (April to September) time periods are chosen based on the hydrological year [331] and visualised in the diagrams by light and dark grey boxes. In addition, another periodicity was analysed with winter being defined from December to May and summer form June to November. These two seasons are based on the hydrological regime observed in the long-term dataset from Auchencorth Moss (Appendix 5.6) with winter being dominantly rising and peak flow conditions while the summer period is characterised by low and declining flow conditions.

In addition, soil samples were collected to determine the SOM composition and to get a better understanding of the interactions between headwaters streams and their surrounding soils.

5.2.1. Field sites

The first field site of this project is the Auchencorth Moss field, located in South East Scotland (55°47’36” N, 3°14’41” W). It is an ombrotrophic peatland with an extensive fetch at an elevation of 270 m, lying 18 km South-Southwest of Edinburgh. The peat catchment is drained by the Black Burn, a small stream with a width of ~ 1 m and a depth from peat surface to channel bed of ~ 1.2 m. The stream extends for 4700 m from source to the catchment outlet. The Burn is characterised by low pH (~ 3 - 6), low temperature conditions, a dynamic hydrological response to precipitation events [25] and the water is typically highly coloured [142].

The Menstrie Burn catchment (56°09’43.7” N, 3°51’24.0” W) is a sub-catchment of River Devon, that confluence to Forth River. Menstrie Burn flows through Menstrie village and has three tributaries named: Inch 1, Inch 2 and Inch 3. The whole catchment area is approximately 12 km^2 whereby the main catchment with the three Inches is around 6 km^2. The catchment area of Inch 1 is 0.9 km^2. The main soil type of the Menstrie catchments is peaty podzol, which covers large areas in Scotland [138, 332], and although it has a peat layer it is not classified as a peatland. The river morphology is characterised by a steep slope with low deposition (mostly suspended load).

5.2.2. Precipitation and Hydrology

The discharge for the Black Burn, Auchencorth Moss was calculated by using water level data from a non-vented water level TROLL 300 instrument (manufactured by In-Situ Inc.) which recorded changes in water level, pressure, and temperature. However, the sensor derived data had to be corrected for water levels > 1.0 m using Manning's equation to capture out of channel river flows (Chapter 2, Section 2.2.2, equation 4) due to flooding of the river banks. Precipitation data for Auchencorth Moss was collected via tipping buckets run through the Auchencorth Moss Atmospheric Observatory (AMo) and downloaded from the Natural Environment Research Council's Data Repository for Atmospheric Science and Earth Observation [148].

For the Menstrie burn and its tributary Inch 1 discharge was calculated using the measured velocity and water level using a locally installed Micronics Stingray Level-Velocity Logger (5 min resolution flow measurements) and corrections were implemented following Manning's equation for out of channel river flows (Chapter 2, Section 2.2.2, equation 4).

The precipitation data from Menstrie was collected from existing Scottish Environment Protection Agency (SEPA) gauges in the lower catchment (Glenochil station) and one local rainfall station (lower Inch 1) and meteorological station in the upper catchment (lower Inch 2) installed as part of another Heriot-Watt PhD study. Additional details can be found in Chapter 2, Section 2.4.2.

5.2.3. Sampling and Analysis

Water samples were filtered in the field with 0.45 µm Polyethersulfone (PES) syringe filters as recommended by Karanfil et al. [58], collected in 60 mL high-density polyethylene (HDPE) bottles and stored in the dark under 4 °C until further analysis. All samples were analysed within 48 hours after sampling, which is within the recommended timeframe based on the results of chapter 3. All water samples were analysed in the laboratory for bulk DOC (Chapter 2, Section 2.7.1), DOM fractions including dissolved organic nitrogen (DON) (Chapter 2, Section 2.7.5) and UV absorbance and fluorescence (Chapter 2, Section 2.7.4). Furthermore, concentrations of carbon dioxide (CO_2) and methane (CH_4) in stream water were measured directly using a headspace equilibration technique [151], see Chapter 2, Section 2.5.2 and 2.7.2 for further details on the sampling and analytical method.

Additionally, soil samples were collected in 2018 and 2019 from Auchencorth Moss and 2019 from the unplanted area in Menstrie and analysed for total organic carbon and nitrogen (TOC and TN), $\delta^{13}C$ and $\delta^{15}N$, as well as extracted with MilliQ water for Size-Exclusion Chromatography analyses see chapter 2 section and Greenhouse Gases (GHG). See Chapter 2 for more details about soil sampling (Section 2.5.3), soil sample preparation (Section 2.6.2) and laboratory analysis (2.7.3). Furthermore, a detailed explanation of DOM flux calculations and statistical tests, used within this chapter, can be found in Chapter 2, Section 2.8 and 2.9.

5.2.4. 3-component carbon model

Tipping et al. [170] described a two-component model of DOM, which accounted for optical absorbance in terms of the linear sum of two components (A and B) each with its own fixed absorbance spectrum. Carter et al. [98] adopted the same approach, with one modification, which is the inclusion of a third component (C) that does not absorb light and is present at the same concentration in all water samples. Furthermore, Adams et al. [172] evaluated the extinction coefficients of DOC_C in the UV range of algae-derived DOM, a variable number not a fixed value. This study tested the Carter and Adams model with the DOC and UV absorbance data from Auchencorth Moss and Menstrie and assessed three additional model approaches to improve the relationship between measured and modelled DOC from the original Carter model. A detailed description of the model approaches can be found in Chapter 2, Section 2.10.

5.3. Results

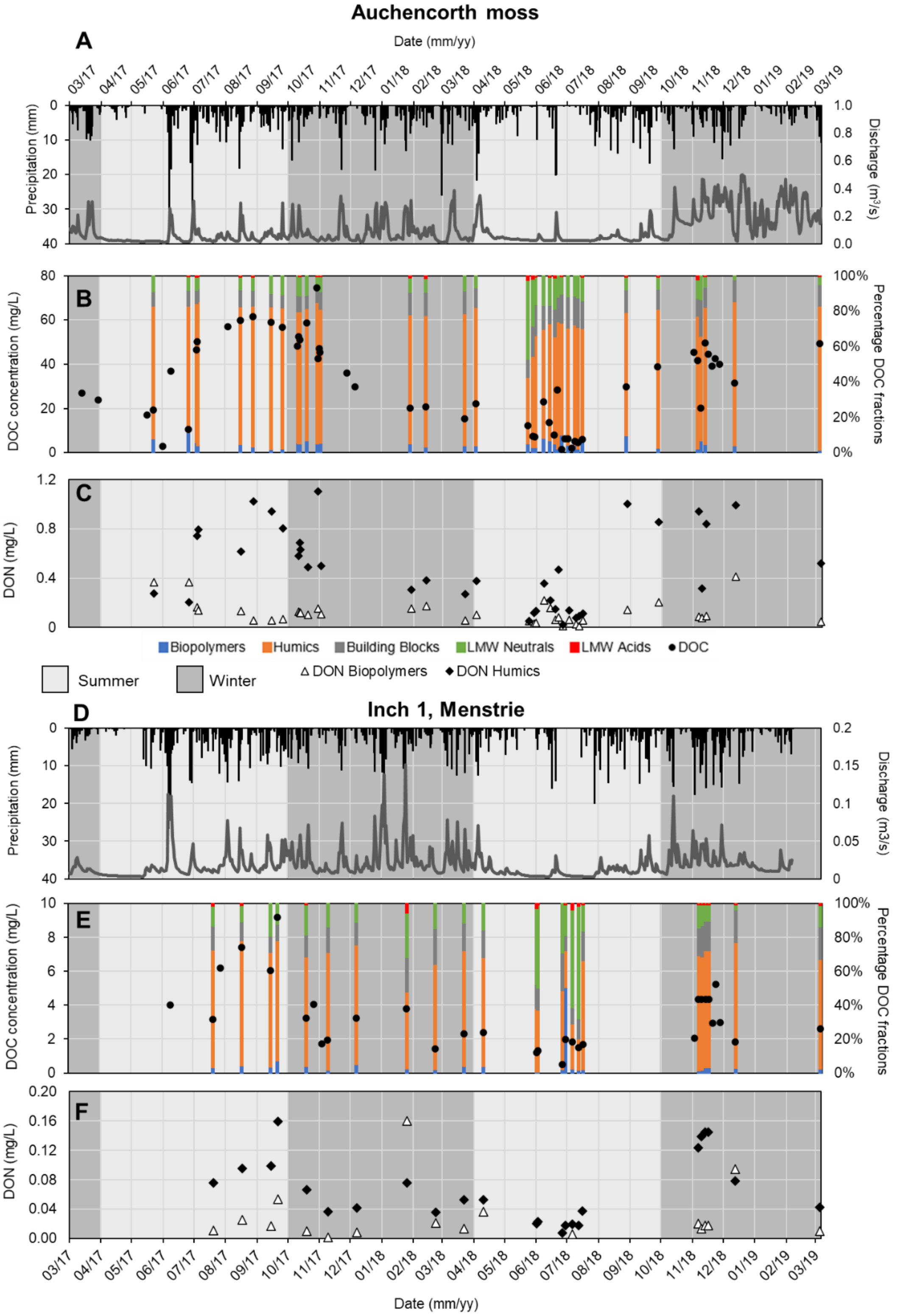

Figure 5.1: Black Burn, Auchencorth Moss time series from March 2017 to March 2019 of A) total daily precipitation in mm and average daily discharge in m^3/s, B) DOC concentrations in mg/L from derived from the LC-OCD-OND and DOC analyser (see 5.2.7) and percentage distribution of DOM fractions and C) Dissolved Organic Nitrogen concentration from Humics and Biopolymers. Menstrie Inch 1 upstream time series from March 2017 to March 2019 of D) daily precipitation in mm and average daily discharge in m^3/s, E) daily DOC concentrations in mg/L and percentage distribution of DOM fractions and F) Dissolved Organic Nitrogen concentration from Humics and Biopolymers. Summer (April-September) and winter (October-March) time periods are defined by Gordon et al (2004) and outlined by a light grey box for summer and dark grey box for winter.

5.3.1. Precipitation and Hydrology

Figure 5.1A shows the time series from March 2017 to March 2019 of daily precipitation totals and mean daily discharge from Auchencorth Moss and Figure 5.1D from Inch 1, Menstrie. In Auchencorth Moss a total of 834 mm precipitation was documented for 2017. May 2017 was the driest month with only 4 mm precipitation documented. The wettest month was June 2017 with 154 mm. In 2018 a total of 818 mm was measured at Auchencorth Moss and May 2018 was the driest month with 25 mm and November was the wettest month with 96 mm.

The Inch 1 catchment had a total of 726 mm precipitation in 2017 with April 2017 being the driest month with only 4 mm precipitation documented compared with the wettest month, June 2017, with 116 mm. A total of 817 mm of precipitation was measured for Inch 1 Menstrie catchment in 2018 with April being the driest month (24 mm) and October the wettest month with 138 mm.

Mean daily discharge ranges between 0.01 - 0.55 m^3/s for the Black Burn (Figure 5.1B) and 0.003 - 0.20 m^3/s in Inch 1 of Menstrie Burn catchment (Figure 5.1E). In Summer 2018 the Black Burn and Inch 1 had a discharge of < 0.2 m^3/s and < 0.02 m^3/s, respectively, but both rivers showed increases in discharge during a rainfall event in June 2018. The Black Burn showed high discharge variability in winter 2018 with average daily discharge between 0.07 - 0.37 m^3/s. Inch 1 showed higher average daily discharge values in winter 2017 (0.01 - 0.08 m^3/s) than 2018 (0.003 - 0.01 m^3/s).

5.3.2. DOC concentration and flux

DOC concentrations were measured with the LC-OCD-OND and DOC Analyser, shown as daily values in Figure 5.1B and 5.1E. The fluxes can be found in the supplementary material (Appendix 5.1). The seasons are classified in winter (defined as 1 October to 31 March) and summer (1 April to 30 September) [331]. For simplification, samples are not differentiated between years in the Figures but only between summer and winter.

DOC concentrations ranged from 74 mg/L in August 2017 to 1.5 mg/L in June 2018 for the Black Burn in Auchencorth Moss with an average DOC concentration of 31.1 ± 2.73 mg/L (n = 51) from March 2017 to March 2019. DOC fluxes ranged from 0.04 - 1.9 kg/ kg/km^2 per day with an average value of 0.8 ± 0.07 kg/km^2 per day. The DOC flux follows the same pattern as the concentration with increasing values in late summer towards early winter.

On average in summer 2018 transported 0.3 kg/km^2 per day less DOC than in summer 2017, whereas the winter of 2018 and 2017 had similar average DOC fluxes of 0.5 ± 0.04 and 0.6 ± 0.05 kg/km^2 per day, respectively.

The DOC concentration of Inch 1 in Menstrie was on average 3.3 ± 0.34 mg/L over the two-year period ($n = 22$). The lowest daily average DOC concentration and DOC flux was measured in June 2018 with 0.49 mg/L (Figure 5.1F) and 0.05 kg/km^2 per day, respectively. The highest DOC concentration and flux were measured in September 2017 with 9.18 mg/L (Figure 5.1F) and 0.88 kg/km^2 per day, respectively.

Samples taken during summer month show a positive correlation ($R^2 = 0.55$ for Auchencorth Moss and $R^2 = 0.62$ for Menstrie) between DOC concentration and discharge, whereas no significant correlation was reported between DOC flux or concentration with discharge in winter ($p > 0.05$). Summer and winter samples show no correlation between DOC concentration or flux and precipitation.

5.3.3. DOM composition

The timeseries (March 2017 - March 2019) of DOC concentration and the percentage distribution of the five DOC fractions as well as daily DON concentrations from humics and biopolymers are shown in Figure 5.2 for the Black Burn and Inch 1. Around 40 % of the LMW acids data was removed from the dataset as they were below limit of quantification identified within this study (see Chapter 2, Section 2.7.5).

Table 5.1 shows the mean, maximum and minimum values of DOC and its five fractions as well as DON from humics and biopolymers, $SUVA_{254}$ and $SUVA_{HS}$, and the molecular weight of the humics fraction. Also, the standard deviation and number of samples for the summer and winter samples can be found in table 5.1. Average and range of DOM concentrations of each sampling day in 2017 and 2018 can be found in Appendix 5.3.

Humics are the dominant fraction in both headwater streams and $SUVA_{254}$ is generally higher in summer than winter in Auchencorth Moss but the opposite can be observed in Menstrie samples. Additional measured UV absorbance indices were the Slope ratio (SR) which ranged from 1.9 - 2.7 (average of 2.1 ± 0.04, $n = 23$) in Auchencorth summer samples and from 2.1 to 2.3 ($n = 8$) in the winter samples whereas in Menstrie SR varies between 2.7 - 3.2 in summer (average 2.9 ± 0.08) and 2.3 - 4.6 in winter (average 3.0 ± 0.35).

The SR is higher in summer than winter for the Menstrie samples but no clear distinction between the seasons can be observed for Auchencorth Moss. The one outlier in Menstrie winter samples was from March 2018.

Table 5.1: Summer and Winter mean, maximum and minimum concentration of dissolved organic carbon (DOC), biopolymers (BP), dissolved organic nitrogen from BP fraction (DON BP), humics (HS), dissolved organic nitrogen from humics fraction, low molecular weight (LMW) neutrals and acids in mg/L from Auchencorth Moss and Menstrie water samples. Mean, maximum and minimum values from $SUVA_{HS}$ (specific UV Absorbance at 254 nm for humics), humics molecular weight (Mn) and $SUVA_{254}$ (specific UV Absorbance at 254 nm from DOC). Additionally, the Standard deviation (Std Dev) and number of sampled (count) can be seen from each of the above-mentioned indices.

	Auchencorth Moss					**Inch 1, Menstrie**				
	Mean	Max	Min	Std Dev	Count	Mean	Max	Min	Std Dev	Count
Summer										
DOC	22.9	61.5	1.6	19.8	28	3.6	9.2	0.8	2.7	13
BP	1.2	3.0	0.1	0.9	23	0.2	1.0	0.01	0.3	11
DON BP	0.1	0.4	0.01	0.1	23	0.02	0.05	0.01	0.02	5
HS	18.1	45.5	0.9	15.7	23	1.8	6.4	0.2	2.1	11
DON HS	0.4	1.0	0.02	0.3	23	0.05	0.2	0.01	0.05	11
$SUVA_{HS}$	5.8	6.9	4.9	0.5	23	5.0	5.9	3.6	0.7	11
Mn	818	1291	564	188	23	795	935	545	111	11
BB	2.4	4.9	1.0	1.5	23	0.3	0.9	0.08	0.3	11
LMW Neutrals	2.3	5.5	0.2	1.5	22	0.7	1.1	0.2	0.3	10
LMW Acids	0.08	0.24	0.01	0.06	13	0.03	0.06	0.01	0.02	6
$SUVA_{254}$	5.8	7.3	4.2	0.8	23	4.3	5.7	2.4	1.2	11
Winter										
DOC	41.1	74.6	15.4	13.4	23	3.1	5.2	1.4	1.1	18
BP	2.3	4.3	0.7	1.2	12	0.1	0.2	0.04	0.05	9
DON BP	0.1	0.2	0.05	0.03	12	0.01	0.02	0.01	0.01	9
HS	31.2	59.6	11.5	14.3	12	2.1	3.0	0.9	0.8	9
DON HS	0.6	1.1	0.3	0.3	12	0.08	0.14	0.03	0.04	9
$SUVA_{HS}$	6.1	7.6	5.3	0.8	12	5.4	6.1	4.8	0.4	9
Mn	993	1148	886	86	12	826	1095	628	175	9
BB	4.1	5.6	2.0	1.1	12	0.5	0.8	0.3	0.2	9
LMW Neutrals	3.9	6.2	1.2	1.7	12	0.5	1.0	0.2	0.2	9
LMW Acids	0.22	0.69	0.02	0.24	5	0.1	0.2	0.01	0.1	2
$SUVA_{254}$	6.0	7.7	4.5	1.0	12	5.4	6.5	3.9	0.7	9

5.3.4. Greenhouse Gases

Table 5.2 shows the partial pressure of CO_2 and CH_4 from river water (average from triplicate measurements) and atmosphere from the Auchencorth Moss and Menstrie field site of each sampling date within the field campaigns 2017 and 2019.

In Black Burn, Auchencorth Moss the average pCO_2 concentration of the river water was 1672.0 ppm in summer and 1573.5 ppm in winter, while the atmosphere had average pCO_2 concentrations of 389.2 ppm in summer and 488.7 ppm in winter. The average pCH_4 concentration of the river water was 11.7 ppm in summer and 9.3 ppm in winter, whilst the atmosphere had average pCH_4 concentrations of 4.9 ppm in summer and 4.4 ppm in winter.

The average pCO_2 concentration of the river water in the Inch 1, Menstrie was 420.4 ppm ($n = 3$) in summer and 499.8 ppm in winter ($n = 17$), while the average pCO_2 concentration of the atmosphere was 354.6 ppm in summer and 494.0 ppm in winter. The average pCH_4 of the river water was 5.6 ppm in summer and 4.5 ppm in winter, and the average pCH_4 concentration of the atmosphere was 5.8 ppm in summer and 4.2 ppm in winter.

Table 5.2: Partial pressure of carbon dioxide (pCO_2) and methane (pCH_4) in parts per million (ppm) from river water and atmosphere from Black Burn, Auchencorth Moss and Inch 1 Menstrie. Standard deviation was calculated from river water triplicate measurements, atmosphere data is one data point.

Date	Carbon Dioxide				Methane			
	River	River-Atm.	Atm.	Std. Dev.	River	River-Atm.	Atm.	Std. Dev.
Black Burn, Auchencorth Moss								
16/08/2017	757.7	397.0	360.6	310.9	9.7	2.9	6.8	1.6
28/08/2017	1818.9	1457.7	361.1	341.9	16.0	11.4	4.7	2.5
15/09/2017	1534.5	1135.9	398.5	162.3	10.7	6.3	4.4	0.9
26/09/2017	1393.1	1091.5	301.6	96.8	8.8	4.1	4.7	0.4
11/10/2017	1469.8	1061.9	407.9	86.2	8.6	4.1	4.6	0.5
12/10/2017	1198.5	818.4	380.1	80.5	7.9	3.1	4.8	0.4
20/10/2017	1650.5	1166.5	484.0	105.5	10.2	4.8	5.4	0.5
30/10/2017	2735.1	2331.7	403.4	137.7	24.6	19.9	4.7	0.7
28/11/2017	1053.5	658.9	394.6	126.1	8.5	3.4	5.1	0.5
29/01/2018	786.7	312.5	474.1	39.0	7.1	1.3	5.8	2.0
13/02/2018	1349.6	829.8	519.8	127.7	9.4	3.7	5.8	1.2
23/03/2018	1016.7	561.6	455.1	23.5	7.0	2.0	5.1	0.5
03/04/2018	1053.0	603.1	450.0	90.6	7.0	2.4	4.6	0.3
24/05/2018	3474.8	3011.3	463.5	53.0	17.9	14.0	3.9	0.6
19/07/2018	4175.0	3570.4	604.7	582.4	20.8	16.4	4.4	4.2
02/11/2018	1845.9	1253.4	592.5	151.3	9.5	5.2	4.2	0.2
06/11/2018	2509.2	2041.0	468.2	n.a.	9.7	6.0	3.7	n.a.
09/11/2018	1700.0	1191.9	508.1	52.9	9.5	5.6	3.9	0.3
13/11/2018	1371.0	876.4	494.7	74.7	7.3	3.6	3.7	0.0
16/11/2018	1833.3	1295.1	538.3	122.1	8.3	4.6	3.7	1.0
20/11/2018	2120.6	1573.7	546.9	224.0	10.4	6.7	3.8	1.4
23/11/2018	1361.0	840.5	520.5	147.1	7.0	3.3	3.7	0.1
27/11/2018	1535.6	1043.7	491.9	156.1	8.1	4.6	3.5	0.9
30/11/2018	1073.9	516.0	557.8	52.7	6.1	2.2	3.9	0.1
12/12/2018	1675.4	1133.6	541.8	26.6	9.3	5.2	4.1	0.6
24/01/2019	2286.0	1774.6	511.4	12.8	11.3	7.5	3.8	1.3
05/03/2019	1572.8	1063.3	509.5	109.8	7.1	3.3	3.9	0.7
Inch 1, Menstrie								
17/08/2017	292.3	-93.5	385.8	310.9	6.2	0.4	6.7	6.6
14/09/2017	502.5	163.2	339.2	162.3	4.7	0.0	4.7	0.9
19/10/2017	500.7	108.8	392.0	105.5	4.7	0.3	5.1	0.5
03/11/2017	441.8	42.5	399.3	12.0	4.2	0.6	4.8	0.2
09/11/2017	501.9	140.8	361.1	75.0	4.8	0.0	4.8	0.1
07/12/2017	542.2	-279.9	822.1	84.7	4.4	0.6	4.9	0.4
25/01/2018	509.5	24.7	484.8	27.1	3.9	0.0	3.9	0.0
22/02/2018	464.6	-28.6	493.3	22.5	3.9	0.0	3.9	0.0
23/03/2018	403.5	-10.4	413.9	34.6	6.7	0.5	7.1	0.7
10/04/2108	570.0	-54.6	624.6	29.7	4.0	0.1	3.9	0.1
01/06/2018	466.4	127.6	338.8	78.0	5.8	0.3	6.1	0.7
19/07/2018	502.7	102.4	400.3	22.3	6.2	0.2	6.3	0.0
02/11/2018	549.5	72.6	476.9	11.1	3.7	0.0	3.7	0.0
06/11/2018	586.3	73.8	512.5	106.6	3.6	0.1	3.6	0.0
09/11/2018	538.1	88.5	449.6	73.9	3.6	0.0	3.6	0.0
13/11/2018	537.3	109.4	427.9	130.5	3.5	0.0	3.5	0.0
16/11/2018	555.0	56.9	498.1	88.2	3.5	0.0	3.4	0.0
20/11/2018	401.1	-98.4	499.4		3.4	0.1	3.4	
23/11/2018	433.4	-79.6	513.1	39.1	3.4	0.0	3.5	0.0
27/11/2018	483.7	-119.2	602.8	42.0	3.4	0.0	3.4	0.0
12/12/2018	539.0	-2.8	541.8	198.1	9.3	5.2	4.1	0.0
23/01/2019	443.3	-68.1	511.4	59.3	11.3	7.5	3.8	0.0
05/03/2019	508.1	-1.4	509.5	47.7	7.1	3.3	3.9	0.1

5.3.5. Soil organic matter (SOM) and water-soluble SOM composition

The data from SOM analyses (Iso Analytical, UK) and water-soluble SOM composition for each soil sample from Auchencorth Moss and Menstrie can be found in Appendix 5.4.

In both catchments TOC decreased with soil depth; however, in 2018 at 45cm soil depth the TOC increased to 18.8 %w/w in Auchencorth (Figure 5.2). TN showed an overall decreasing trend with depth with values between 0.1 - 1.9 %w/w in Auchencorth in 2018 and 0.1 - 2.4 %w/w in 2019, and between 0.2 - 1.8 % in Menstrie. The C/N ratio decreased with depth in 2019 in both catchment whereas in 2018 it increased after 35 cm soil depth.

Organic δ^{13}C increased with soil depth from isotopic heavier to isotopic lighter ^{13}C in both catchments. The δ^{15}N values from Auchencorth Moss were initially increasing from topsoil to ~ 30 cm of soil depth before it decreased in deeper soil horizons, although, in 2018 δ^{15}N increased again in soil depths > 50 cm to 1.8 - 2.6 ‰. Menstrie soils showed a similar δ^{15}N depth profile with an initial increase from topsoil to 30 cm soil depth, followed by a small decrease in soil depth > 40 cm.

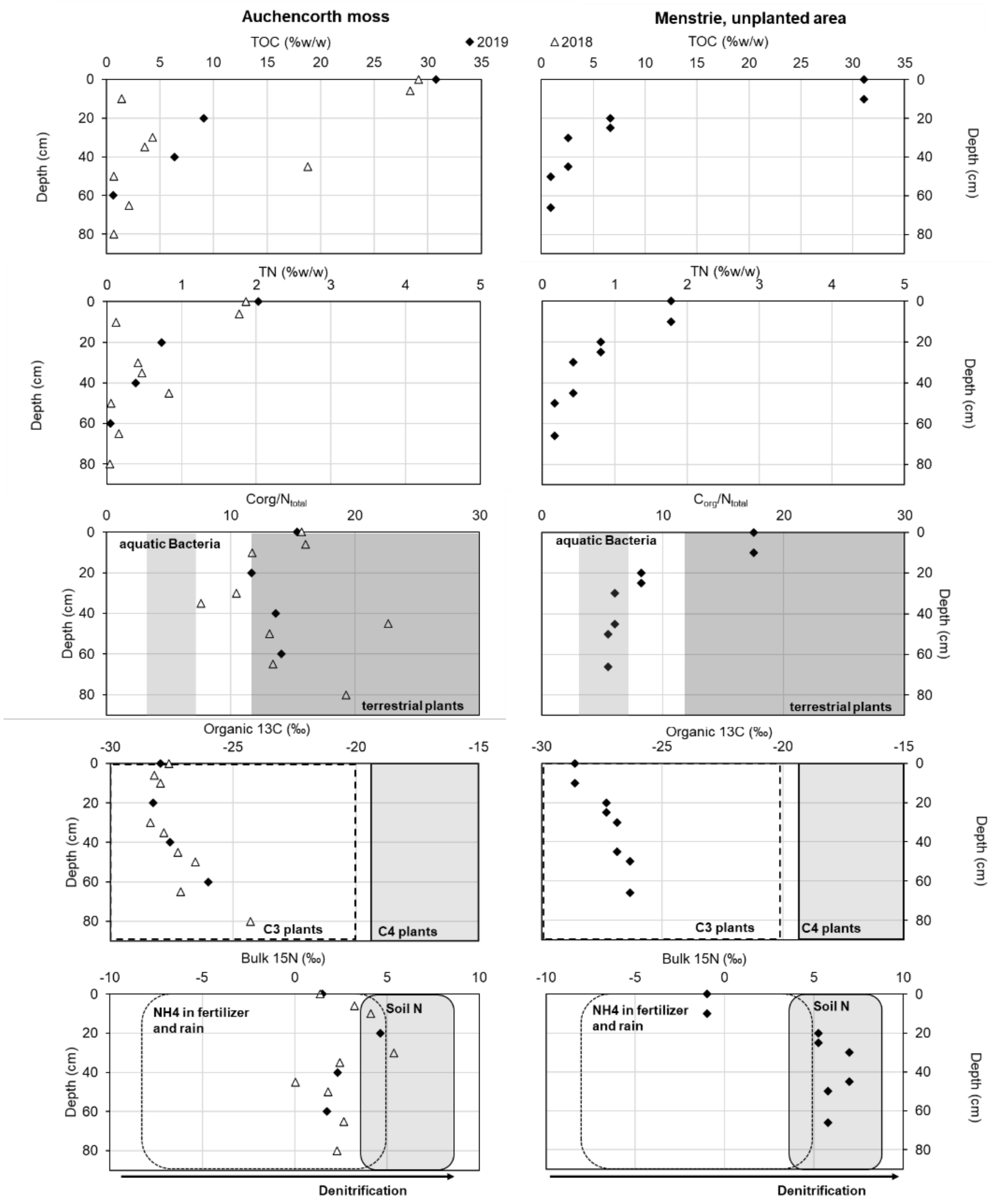

Figure 5.2: Upper diagrams show Auchencorth Moss SOM data and lower diagrams show SOM data from the unplanted area in the Menstrie catchment with a) Total organic carbon (weight %), b) Total nitrogen (weight %), c) Ratio of total organic carbon and total nitrogen (C/N), d) organic ^{13}C in per mile (‰) and e) bulk ^{15}N in per mile (‰).

The concentration of DOC and its fractions of water extractable soil organic matter (WEOM) is shown in Figure 5.3. The concentrations were corrected to the extracted soil amount (in gram) and used volume of MilliQ (in Litre) leading to the unit mg/g. All data from the soil extracts are reported as average values from duplicate measurements.

DOC concentrations of Auchencorth Moss WEOM decreased with soil depth in both catchments with the biggest decline from surface layer/topsoil to the subsoil. Auchencorth soils from 2019 had higher DOC concentrations in the first 25 cm than the soils from 2018. Biopolymers concentrations decreased from surface layer to topsoil to subsoil, similar to bulk DOC, in both catchments with the difference that 2018 samples had a higher topsoil concentration of biopolymers than 2019 Auchencorth samples.

Humics concentrations were higher in 2018 than 2019 but in both years the concentration decreases intensively from topsoil to subsoil, same as 2019 Menstrie soils. In the Auchencorth samples the humics concentration increased from 35 - 50 cm soil depth around 500 mg/g from the initially low concentration at 30 cm depth, which can also be observed in the bulk DOC and to a small extent in the biopolymers and building blocks. building blocks average concentration decreased with depth, although samples from 2019 had a higher concentration in the topsoil than 2018 samples in Auchencorth. In the topsoil from 2019 of Menstrie and Auchencorth, LMW neutrals were the most abundant DOC whereas soils of 2018 from Auchencorth show 5-times lower concentrations in the topsoil. LMW acids were only quantified in the first 30 cm of the soil profile beside on deep sample in Auchencorth Moss (80 cm), but overall decreasing concentrations were observed with soil depth (Figure 5.3).

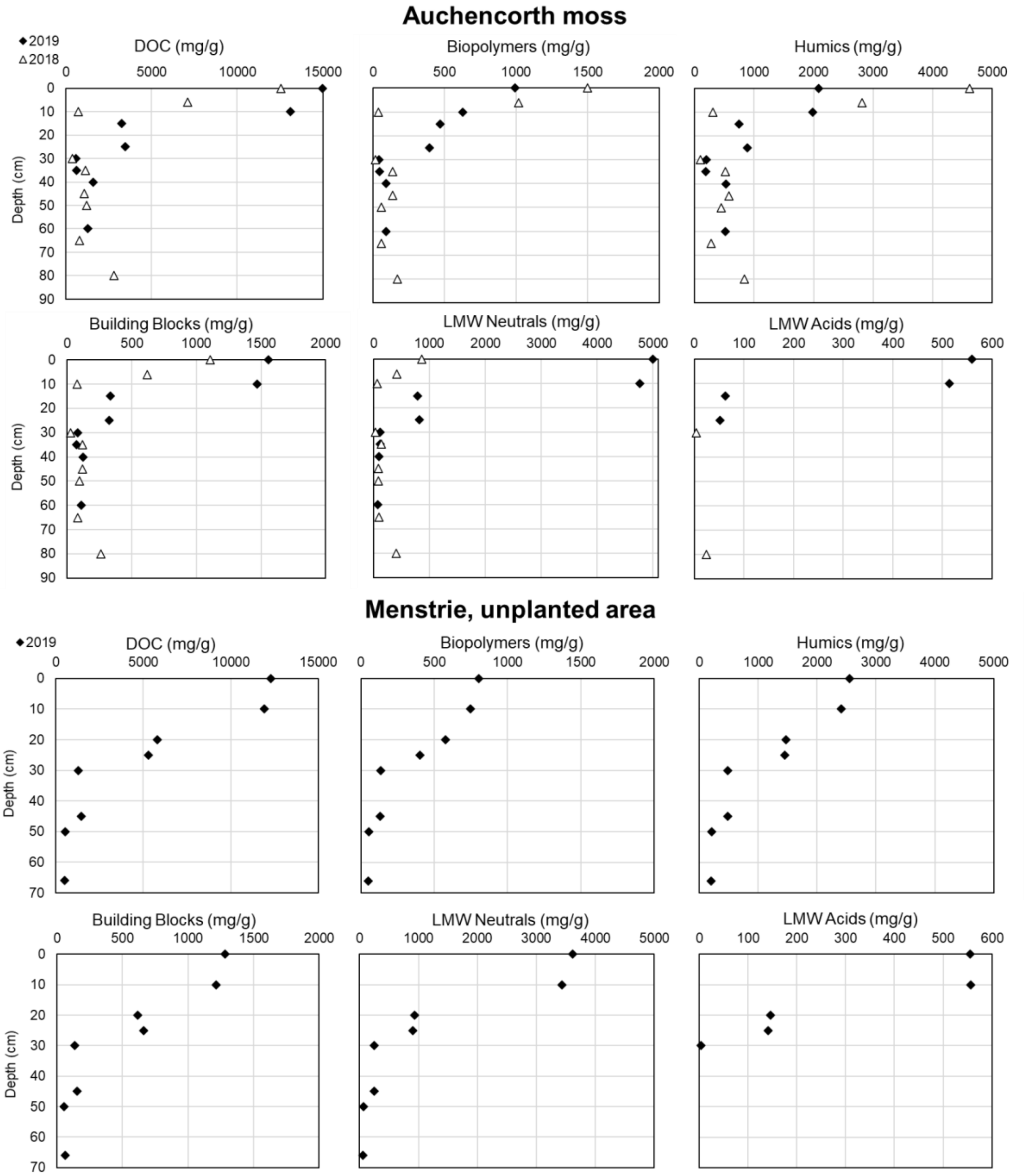

Figure 5.3: Concentration of bulk DOC and its fractions plotted against soil depth from water-extractable soil organic matter (WEOM) from Auchencorth Moss and the unplanted area in the Menstrie catchment.

5.3.6. UV Absorbance Carbon model

The 3-component UV absorbance models from Carter et al. [98] and Adams et al. [172] were tested with the DOC and UV absorbance data generated within this study of the two headwater catchments. The results of the two models and results from model improvements can be found in Appendix 5.5.

The original model approach from Carter et al. [98] showed a linear relationship between modelled versus measured DOC using wavelengths 270 and 350 nm but the model underestimates the DOC concentration. Auchencorth Moss samples showed a good linear relationship with R^2 between 0.74 and 0.93 ($p < 0.05$) whereas Menstrie samples show a weak linear correlation ($R^2 = 0.18 - 0.4$, $p > 0.05$) in either of the model approaches.

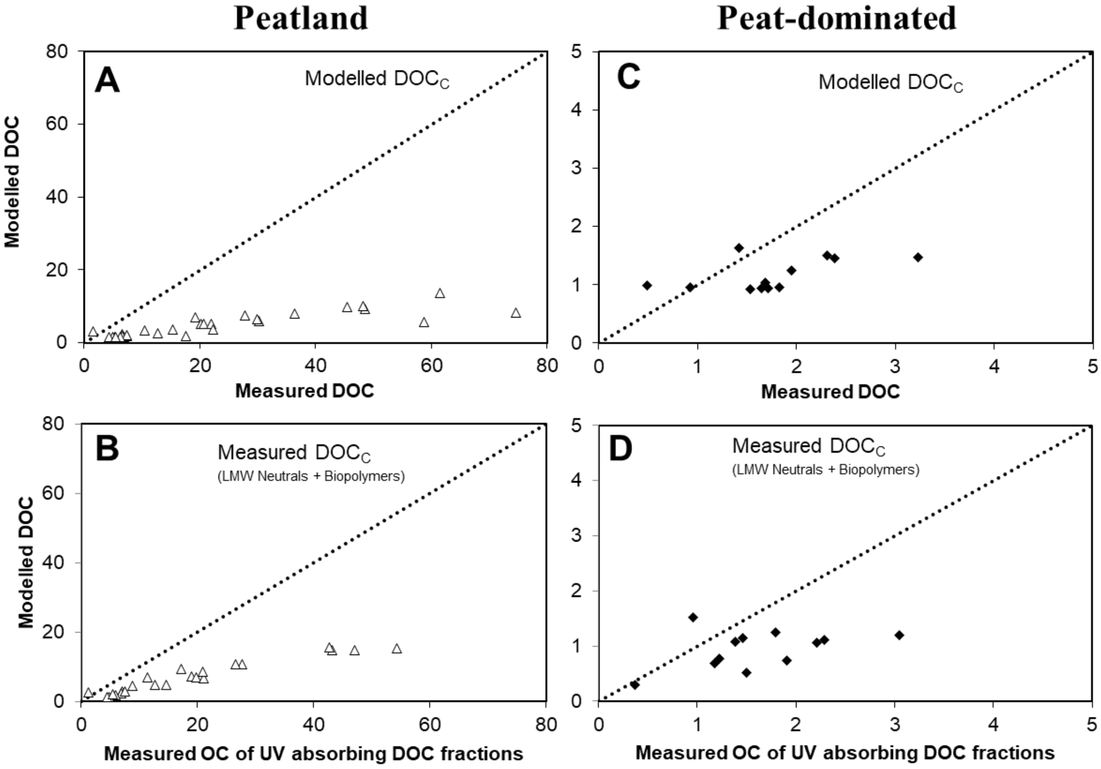

Figure 5.4: Comparison of measured DOC concentration versus modelled DOC concentration for the peatland headwater and peat-dominated headwater using A & C) the original Carter model with a fixed value for component C of 0.8 and B & D) modelled DOC, including LMW Neutrals and the non-UV absorbing part of biopolymers as DOC$_C$, versus measured OC of UV absorbing fractions. The dotted line shows the equilibrium between measured and modelled DOC.

On average the Carter et al. [98] model underestimated the DOC concentration from Auchencorth Moss by 67 % (Figure 5.4 A) and the Menstrie DOC concentrations were underestimated by 11 % (Figure 5.4 C). The Adams model underestimated the DOC concentration from Auchencorth Moss by 77 % and by 31 % for Menstrie.

The first model improvement (measured LMW Neutrals concentration, see section 2.10) improved the modelled DOC from Auchencorth Moss but the model still underestimates the DOC by 69 % on average. In Menstrie the new model underestimated the DOC concentration by 51 %, an increase of 40 % from the Carter model.

The second improved model, including LMW Neutrals and biopolymers (low-UV absorbing fraction) as non-UV absorbing component C, still underestimates the DOC concentration from Auchencorth Moss by 64 % and underestimates the Menstrie DOC concentrations by 44 %. The last model improvement, using CDOC as measured DOC value and LMW neutrals & biopolymers as component C, showed the best fit; however, still underestimates the DOC by 62 % in Auchencorth Moss (Figure 5.4 B) and 37 % in Menstrie (Figure 5.4 D).

5.4. Discussion

5.4.1. Are Auchencorth Moss and Menstrie good organic matter source representations of the UK river system?

Most aquatic carbon research in the United Kingdom (UK) was conducted on large river systems [233, 248, 250, 258], not in headwater streams. However, Auchencorth Moss is a well-established research site, with monthly measured DOC concentrations since 2006 in the framework of many different projects. DOC concentration data from three main studies were published since 2007 [142, 326, 333] which are illustrated in Appendix 5.6 together with the new time series obtained by this study. The average DOC concentration of the Black Burn of 38.0 ± 1.16 mg/L is higher than the average DOC concentrations measured across 13 different UK peatland systems, which ranged from 3.4 to 25.5 mg/L [17, 246, 334].

Menstrie Burn and its tributaries is a new research site without DOC measurements before 2017, established within this research study. Menstrie has with 3.3 ± 2.5 mg/L a low DOC concentration for a catchment that is dominated by peat and peaty podzol especially in comparison to catchments like Auchencorth Moss. Nevertheless, Dawson et al. [14] found DOC concentration between 2.13 and 43.6 mg/L in the headwaters of the Glen Dye catchment (UK), which is dominated by peats (61.7 % of the catchment) and humic podzols, similar to the Menstrie catchment although the podzols are dominating Menstrie.

Furthermore, Worrall et al. [233] compiled a comprehensive data set of DOC concentration records for UK catchments from 198 sites showing that comparable upland catchments to Menstrie show long-term mean DOC concentrations of 0.2 - 2.6 mg/L (1990 - 2001).

In another study by Worrall et al. [246] median DOC concentration of 5.5 mg/L were calculated for the period from 1974 – 2015 based on decade-long DOC concentration trends from 62 river catchments within the UK. These studies show that the Menstrie catchment, although peat-dominated, reflects the lower DOC concentrations found in many other UK upland peat catchments. Potentially implying that these peat horizons are less vulnerable to drying out hence releasing less carbon of our time period. Other examples are from US temperate wetland headwater sites that had DOC concentrations of 2 - 32 mg/L [335]. Overall, the peat-dominated Menstrie catchment is representing widespread river systems in the UK with low DOC concentrations (< 10 mg/L) and peaty podzols, while the Auchencorth Moss may have high average DOC concentrations but it is nonetheless a good representative of a UK peatland.

Table 5.3: Headwater monitoring sites and the details of their catchments that were included in this study. The DOC is an average value from 2017 and 2018 sampling campaigns. Rainfall and river flow values are the average of the 2017 and 2018 sum. Urban and organic soils percentage contribution was not quantified within the headwater catchments Auchencorth Moss and Menstrie. The other river data was taken from Worrall et al. [324]. The average DOC are the expected value from the modelling in Worrall's study for 2019. The rainfall and river flow are from the National River flow Archive (www.nrfa.ac.uk) and are the average annual values for the period 1961 – 1990. The proportion of each catchment that is under urban land use (Urban (%)) was based on the June Agricultural Census for 2004.

River	Site	DOC (mg/L)	Area (km^2)	Rainfall (mm)	River flow (mm)	Urban (%)	Organic soils (%)
Inch 1	Menstrie	3.3	0.9	788	184	n.q.	n.q.
Black Burn	Auchencorth Moss	31.1	3.4	826	221	n.q.	n.q.
Ogwen	Talybont	1.5	76	2415	1879	2.7	100
Mawddach	Ganllwyd	3.2	137	2068	1568	0.1	100
Conwy	Cwm Llanerch	2.5	348	2039	1538	0.5	99.1
Dwyryd	Maentwrog bridge	2.3	80	2187	1687	2.4	98.8
Dysynni	Pont-y-garth	0.8	71	2191	1685	0.1	98.6
West Dart	Huccaby	2.5	70	1967	1413	1.8	98.5
Elan	Glyn footbridge	2.8	199	1790	1266	0.4	98.5
Gwyrfai	Bontnewydd	1.8	46	2123	1549	1.8	97.8
Dwyfar	Dolbenmaen	2	61	1973	1396	0.6	96.7
Wnion	Dolgellau	1.8	108	2111	1608	0.3	94.4
Dee	Llandderfel	3.9	340	1775	1275	0.4	93.5
South Tyne	Warden bridge	5.8	802	1125	643	0.9	90
Plym	Plym bridge	1.8	89	1576	1012	3.6	86.5
Alwen	Glanakwen ford	5.1	201	1294	791	0.3	86.1
Findhorn	A96 road bridge	9.1	780	1065	584	0	85.3
Dart	Totnes weir	2.2	274	1729	1179	3.1	84.6
Thurso	Thurso	12.4	485	1043	563	0.2	83.3
Lune	Forge weir	3.4	992	1520	1028	1.3	82.7
Carron	A890 road bridge	3.3	241	1784	1269	0	82
Water of Luce	Airyhemming	13	171	1506	964	0.4	81.9
Cree	Newton Stewart	9.6	366	1760	1270	0.8	81.4
Eamont	Udford	1.9	407	1774	1310	2.1	80.3

5.4.2. Applicability of the two-component CDOM model on two Scottish peat-dominated headwater streams

Modelling DOC concentration of rivers from UV absorbance data with the help of carbon models is well established in previous research [98, 170, 172, 260, 302, 336]. Testing the original model from Carter et al. [98] with fixed DOC_C values on the data of this study led to an underestimation of modelled DOC by a factor of 4.5 for Auchencorth Moss samples and 1.5 for Menstrie samples. In the case of this study the model outcomes suggest that other components have to affect the absorbance measurements leading to the weak relationship with measured DOC.

Model improvements

One hypothesis in regard to the misalignment of absorbance and DOC is that LMW Neutrals are not the only DOC fraction that is UV invisible. Biopolymers can have two main sources, proteins (UV amenable) and polysaccharides (not UV amenable), hence the non-UV absorbing biopolymers fraction was included in the DOC_C. This would align with the suggestion of Adams et al. [172] that algal derived DOM is part of the weak UV absorbing fraction since algal material is likely represented in the biopolymers fraction. However, it is not likely to find algal derived DOM in the two observed headwaters.

Overall, the improved model, using measured LMW Neutrals and biopolymers concentration as non-UV amenable component DOC_C, is still underestimating the modelled DOC concentration, suggesting another carbon source is missing in the model calculation. Further analysis of the chemical composition of LMW neutrals have to be conducted to get a better idea which compounds are included in the fraction and which LMW organics are not measured and could affect the carbon model. Neither of the model improvements lead to the same results found in previous global studies [98, 172, 302] suggesting that the Carter and Adams model are not representing aquatic systems that are high in carbon and are more intimately linked to the landscape like headwaters.

Potential model interferences

In previous tested UK samples, pH and Iron (Fe) are shown to influence spectra [337] but no relationship ($R^2 < 0.3$) between pH and absorbance was observed in the Black Burn and Inch 1 samples which confirms with the study by Carter et al. [98] that found only a slight pH dependence, falling by 0.018 per pH unit. They recommended to correct the chosen wavelengths by subtraction of the absorbance at 700 nm to account for instrumental drift [277].

This was not possible in this study because absorbance measurement was only running up to 500 nm. However, in practice the correction had negligible effects [98], indicating that the UV absorbance data is most likely not effected by instrumental drift.

The use of absorbance 254 nm in comparison to 270 nm, used in the Carter model, was proven to have a negligible effect (0.08 ppm difference on average between models) on the model outcome of this study. However, $SUVA_{254}$ (UV absorbance at 254 nm divided by DOC concentration) is a widely used simple measure of DOC quality, which also provides a measure of aromaticity and hydrophobicity [97]. Since f_A is derived after factoring out the non-UV-absorbing component C, which contributes to the calculation of $SUVA_{254}$ the absence of a good relationship may be linked to high concentrations of non-UV absorbing DOC which are not included in previous iDOM contributions. However, while $SUVA_{254}$ is widely employed in DOM research, the 254 nm wavelength has two disadvantages:

1. possible interference due to nitrate in systems with high levels, because Nitrate is observed at 220 nm and high concentrations can interfere with signals at 254 nm [338] and/or
2. at high DOC concentrations (> 50 mg/L) the absorbance may be too high for reliable determination, which can be resolved by diluting the samples which was done for most of the Black Burn samples.

The interference of nitrate or other nitrogen compounds (organic and inorganic) may be a plausible reason for the poor modelled DOC. In the Black Burn samples a relationship between dissolved organic nitrogen (DON) and UV absorbance was observed, whereas Menstrie Inch 1 samples showed no correlation between DON and UV absorbance wavelengths 254, 270 or 350 nm. However, both seasons and both headwaters showed increasing DON HS concentration with increasing DOC and an overall good linear correlation ($R^2 = 0.6 - 0.8$).

These observations suggest that bulk DOC and UV absorbance is affected by DON in the Black Burn samples, whereas in Menstrie samples only DOC is affected by increased DON concentrations. This could indicate that Nitrogen is a stabilising factor in DOC preservation of the biopolymers and humics pool during riverine transport. Inorganic nitrogen like Nitrate is measured in wavelengths from 200 - 250 nm [338, 339] and the LC-OCD-OND measures nitrogen compounds at 220 nm hence it should not be reflected in the UV absorbance wavelengths 254 nm.

In Menstrie the nitrate concentrations are on average higher than the DOC concentration, which supports the idea that nitrate could have a negative effect on the UV absorbance due to the high abundance in the water samples. In the Black Burn samples nitrate is less concentrated than DOC but it still has a similar abundance as biopolymers, LMW neutrals and building blocks suggesting that it has a perceptible influence on the DOM pool.

Carter et al. [98] suggested that some water samples (e.g. lakes from China) absorb UV light extremely weakly, which is supported by Adams et al. [172] who implied that DOC_C or invisible DOM (iDOM) is not exactly invisible, it may rather been weakly absorbing, as was the case for their tested algae-derived DOM. Adams et al. [172] model approach, using the wavelengths 270 and 350 nm as well as their modelled algae values as component C, was tested but it did not improve the modelled DOC concentrations in comparison to the modelled DOC from the Carter model. Although the lake samples from the Carter model are fundamentally different to the temperate headwaters in this study it shows that improved technologies to quantify iDOM are needed to improve carbon models. Nonetheless, the Carter and Adams model could be used to identify waters that are compositionally distinctive (i.e. waters that do not fit the global trend). This study showed that certain water types or ecosystems like peatland headwaters are not suitable for a generic global characterisation and that they and maybe other zones (e.g. lake systems) need their own calibration.

5.4.3. Spatial variations in DOM concentration and composition – comparison between peatland and peat-dominated headwater catchment

The most apparent difference between the catchments was the overall DOC concentration, which is much lower in Menstrie than in Auchencorth Moss which leads to lower concentrations of the different fractions.

Furthermore, the DOC concentration in Inch 1 in the Menstrie catchment are more likely linked to river discharge ($p < 0.01$ in summer and winter) than in the Black Burn, Auchencorth Moss ($p = 0.06$ in winter and $p = 0.01$ in summer), as shown in Figure 5.5. However, this correlation was predominantly observed in winter samples suggesting that DOC concentration is either driven by seasonality or that generally higher amounts of water in the riverine system drives this correlation. In regard to the DOM fractions, only the summer data from Auchencorth Moss suggests a statistically significant correlation between discharge and the 4 main DOM fractions ($p < 0.05$; Appendix 5.8 and 5.9).

In regard to precipitation neither of the headwaters showed a correlation between precipitation and DOC and its fractions (Appendix 5.8 and 5.9), which could be linked to a lag-time between precipitation, discharge and DOC. Furthermore, a shift of periodicity for winter from September-march to November-May showed that HMW DOM fractions, especially humics and building blocks, correlate well with precipitation ($p < 0.05$, Appendix 5.11 and 5.12).

However, the data resolution of this study is too broad with one sample a month to analyse precipitation-discharge-DOC lag-times.

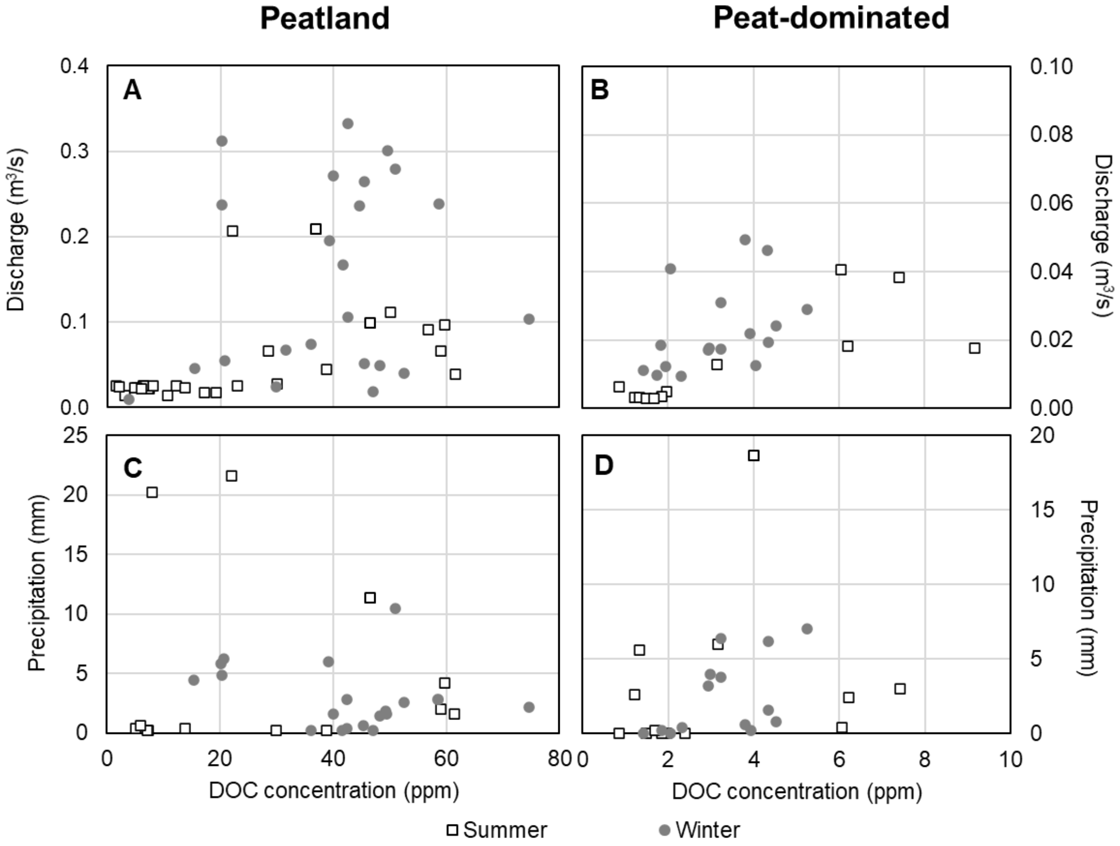

Figure 5.5: DOC concentration versus A) discharge in m^3/s from Black Burn, Auchencorth, B) discharge in m^3/s from Inch 1, Menstrie, C) precipitation in mm from Black Burn and D) precipitation in mm from Menstrie. Winter samples are represented as grey circles and summer samples as white squares with black rim.

The overall abundance of the DOC fractions is similar in both catchments with humic substances and its breakdown products (building blocks) dominating the DOC pool, contributing 50 – 90 % of the DOC pool. Biopolymers are contributed on average less than 10 % to the DOC. LMW neutrals reached higher relative percentages in Menstrie than Auchencorth Moss and this could be linked to the depth of the peat layer, which is deeper in Auchencorth, hence humics quantities are likely to overprint other fractions. LMW neutrals are the second most abundant DOM fraction of the two headwaters with contributing on average 10 – 20 % to the bulk DOC.

These findings confirm the previous observations from larger Scottish rivers (see Chapter 4) that LMW neutrals play an important role in the riverine ecosystems of peat-dominated catchment, although the relative abundance is decreasing from headwaters to larger river systems.

Furthermore, the higher relative abundance of LMW neutrals in Menstrie headwater could imply increase bioavailability and potentially higher CO_2 outgassing to the atmosphere from the peat-dominated headwater catchment. The CO_2 data of this study did not show a trend between LMW neutrals contribution and CO_2 efflux from Inch 1 in the Menstrie catchment (Appendix 5.7). Although, in the peatland headwater the pCO_2 efflux data points correlate weakly with the LMW neutrals the correlation is statistically significant ($p < 0.05$, Appendix 5.7). This observation could have large implications on the current understanding of the carbon feedback from headwaters and rivers as LMW neutrals are often neglected in carbon studies. Nonetheless, more data would be necessary to prove this hypothesis as the CO_2 - LMW neutrals trend in Auchencorth Moss is not statistically significant with only one samples showing high LMW neutrals abundances. Overall, the spatial dynamics of DOM, especially LMW neutrals, and the interaction between DOM fractions needs further investigation.

5.4.4. Interannual and seasonal compositional variations of DOM composition in a peatland and peat-dominated headwater catchment

Temporal changes in the DOM fractions was dominantly observed between years and less between seasons, although seasonal differences within a year can play a role in the DOC dynamics. Water samples from winter month showed similar interannual differences in their abundance of DOM fractions; however, winter samples in 2018 have a higher average concentration and flux of DOM than winter 2017.

One reason for the difference between the years is data density. In 2017 less samples were collected than in 2018, hence less data points are contributing to average yearly concentrations and fluxes. Furthermore, the hydrological regime between summer 2017 and 2018 was different as shown in Figure 5.1. In Summer 2018 a UK-wide dry period was observed that impacted the riverine ecosystems like decreased discharge and less precipitation over longer time periods, influencing the DOM that gets transported into the river and the fluxes downstream.

However, the results suggests that changes in DOC flux and concentration observed after the 2018 drought are the result of changes in the amount of flow and not due to the additional sources of DOC production, which was observed in a previous large UK wide study by Worrall and Burt [340]. However, the DOM fraction that had the highest decrease in flux between summer 2017 and 2018 were the humics.

This supports the finding that they are driving the bulk DOC and that the absence of precipitation led to less humics in the river water, hence less DOC being transported from the surrounding soils into the headwater. Furthermore, the humics in summer 2018 are on average less aromatic and lower in molecular weight than summer 2017 indicating higher degraded humics, hence potentially increased contribution to lower molecular weight DOM fraction like the LMW Neutrals pool. Amon and Benner [62] found that HMW DOC was utilised to a greater extent from bacteria than LMW DOC in freshwater and marine environments, meaning that HMW DOC is more bioreactive and less diagenetically altered than LMW DOC. Additionally is it known that light is a major factor for bacterial functions such as DOC composition [341] potentially enhancing the turnover of HMW to LMW weight. Pickard et al. [326] found that during 8h irradiation experiment 7 % of DOC in Auchencorth Moss is labile to photo-processing, with conversion to CO_2 as the main loss pathway. However, further investigations are necessary towards microbial availability and turnover of humics within these headwaters, but the data resolution is not high enough to get a better understanding of the dynamics within a longer dry period and the degradation processes.

In regard to the differences between the seasons within the catchments the data suggested only slightly different seasonal patterns in the DOM composition. Figure 5.6 A and B shows the molecularity based on molecular weight (g/mol) versus aromaticity based on $SUVA_{254}$ for both catchments derived from the humics fraction from the LC-OCD.

The diagram 5.6 C and D show data derived from the Aqualog examining bulk waters with the slope ratio representing the molecular weight and UV absorbance 254 nm the aromaticity of the bulk DOC pool. The Humification pathway from the LC-OCD demonstrates higher molecularity of humics in winter than summer and a shift from humic acids to fulvic acids which becomes clearer in summer for the Auchencorth Moss samples (Figure 5.6 A). A similar change can be observed within Menstrie samples, although no differentiation between the seasons can be made (Figure 5.6 B).

This shift to fulvic acids especially in summer suggests a decrease in polymerisation and an increase in exchange acidity leading to potentially more degradable DOM, as noted in the differentiation between summer 2018 and 2017. These changing characteristics within the humics may be an explanation to why the Slope ratio derived from UV absorbance measurements does not reflect the molecularity shift as well as the molecular weight (Figure 5.6 C and D), since different functional groups within the humics pool absorb UV light at different wavelengths [342].

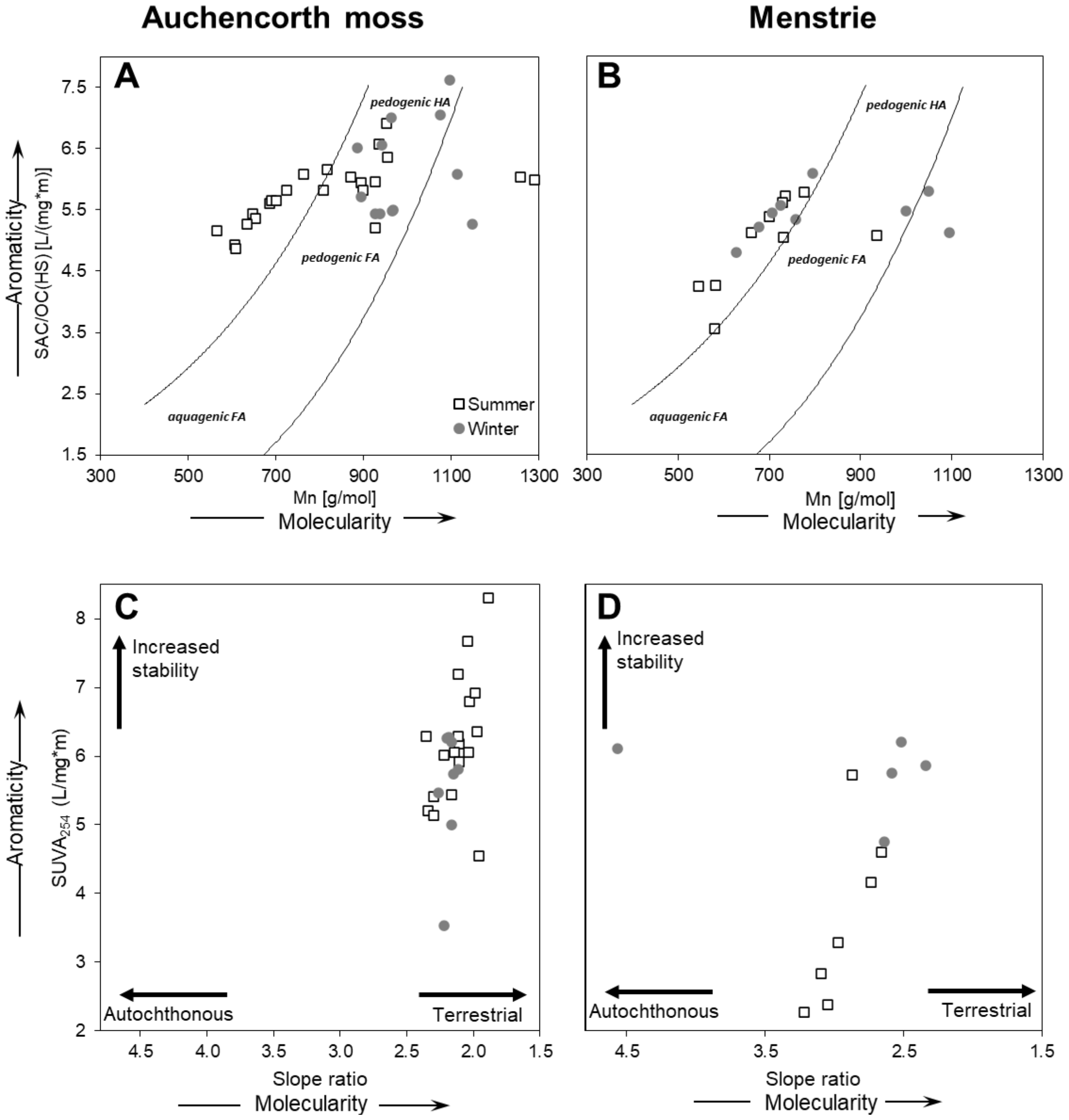

Figure 5.6: Molecularity versus Aromaticity of Black Burn water samples and Menstrie Inch 1 water samples. A) and B) show molecular weight and SUVA$_{254}$ (SAC/OC) derived from the LC-OCD-OND in from of the Humification pathway [95]. C) and D) show the Slope ratio, indication for molecular weight, versus SUVA$_{254}$ (Absorbance 254/DOC) derived from bulk samples from the Aqualog.

Building blocks showed a better fit with DOC in summer, however winter samples from Menstrie showed a steeper increase of building blocks with increasing DOC compared to summer samples (Figure 5.7). Degradation from higher molecular weight humics to lower molecular weight building blocks in winter cannot be ruled out since the Humification pathway indicates a change in molecularity and aromaticity within the winter samples, especially in Menstrie. The C/N ratio from humics is on average higher in winter than summer in both catchments. These values are comparatively high in contrast to C/N values from soil derived humic and fluvic acid fractions [343]; however, soil C/N ratios are not fully translatable to aquatic C/N ratios.

Though, it is known that lower C/N ratio stimulates microbial activity and decomposition [344, 345] suggesting that humics were more likely degraded in summer months either in-situ or more degraded OM was transported from the soils.

As previous mentioned LMW neutrals are abundant in both seasons and both catchments, nonetheless they show only in Auchencorth Moss a good correlation with DOC in both seasons suggesting a continuous supply of LMW neutrals independent from seasonal factors like snow cover in winter or high temperatures in summer. Winter samples from Menstrie showed a great correlation between DOC and LMW neutrals, although the one outlier from January 2018 that snow melt introduced more LMW neutrals into the headwater than heavy precipitation. The summer samples from Menstrie supports the previous assessment that extreme dry periods have no effect on the LMW neutrals abundance, hence hydrological patterns do not play a role in the mobilisation of LMW neutrals. Overall, these findings suggest that LMW neutrals are mobilised from the upper soil horizons or even the vegetation itself as the soils do not have to be hydrological connected to the headwater to mobilise LMW neutrals.

This study also shows that LMW neutrals and biopolymers are more dominant during low flow conditions, while humic substances were predominantly transported during hydrological peak periods (Figure 5.2). This is similar with observations from other studies who found DOM quality is closely linked to hydrologic conditions [275, 327, 328, 346]. However, a statistically significant correlation between LMW neutrals/biopolymers/humics and discharge was only observed in the summer data of Auchencorth Moss (Appendix 5.8). A study by Broder et al. [16] found that during snowmelt and spring events, near-surface protein-like DOM pools were exported whereas a microbial DOM fraction originating from groundwater and deep peat layers was increasing during drought.

Therefore, it can be assumed that the riverine DOC composition is strongly linked to the water storability of the surrounding soils, allowing transport of OM from different soil horizons at different times of the year. The increase in humic substances during high flow conditions could also be caused by increased precipitation or even precipitation events; however, no link between DOC and precipitation was observed (Appendix 5.8 and 5.9). As peat-covered catchments are known to act like aquifers (or a “sponge”), storing precipitation up and releasing it gradually during dry periods [347], the relationship between precipitation, hydrology and DOM composition can be difficult to identify.

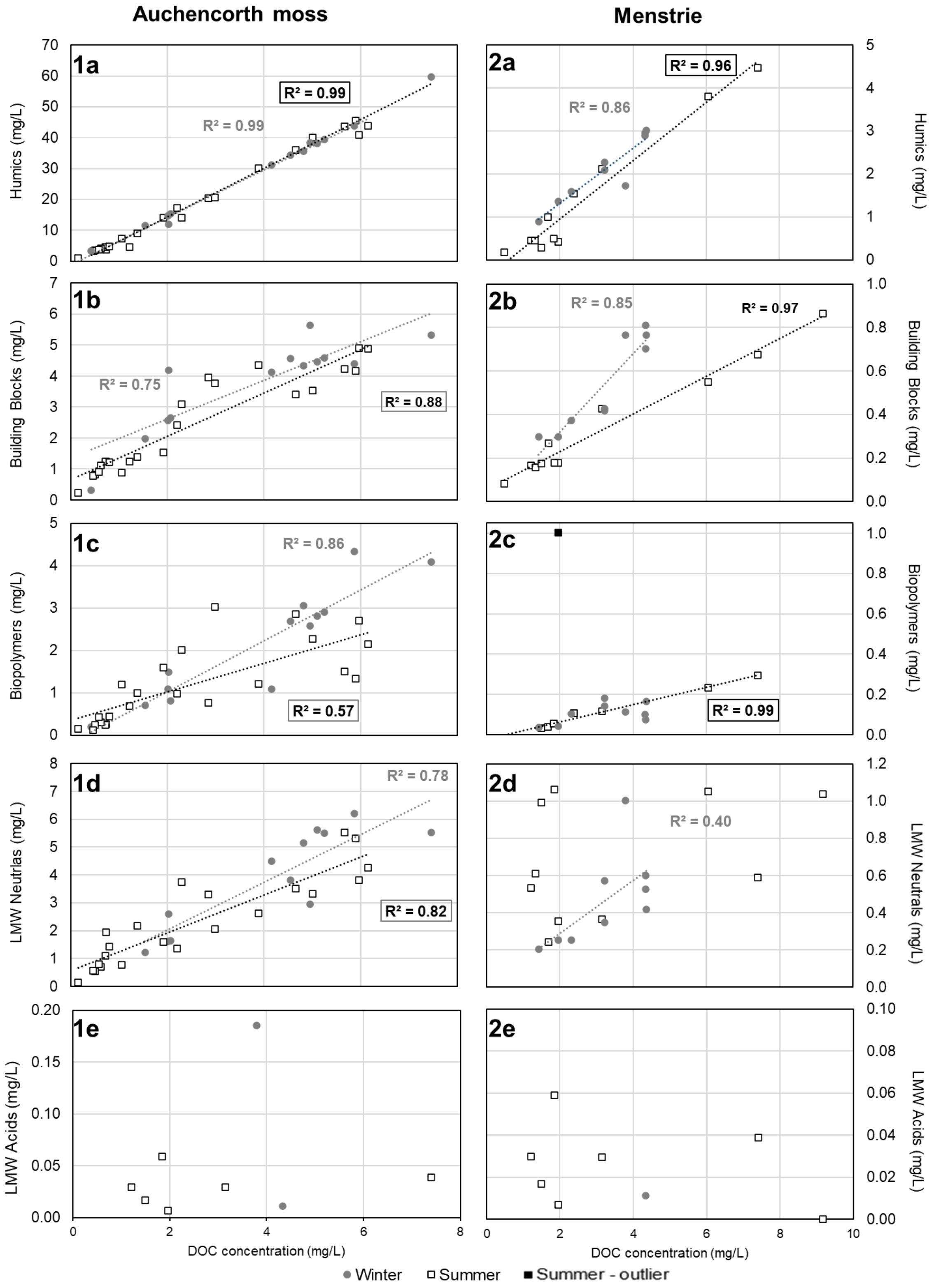

Figure 5.7: LC-OCD-OND derived DOC concentration in milligram per litre versus a) humics, b) building blocks, c) biopolymers, d) LMW neutrals and e) LMW acids for 1) Auchencorth Moss and 2) Menstrie Burn. Winter is shown as grey circles (October to March) and summer as white squares with black rim (April to September) from 2017 to 2019. Black square represents the summer sample in Menstrie that is excluded from the linear relationship. Statistically relevant ($p<0.05$) linear relationships are shown with their respective R^2.

5.4.5. Spatial variation in peat soil composition and assessment of terrestrial-aquatic connectivity

The hydraulic properties of peat strongly depend on the peatland vegetation and the degree of decomposition of the plant debris [266]. Additionally, unsaturated and saturated peat show completely different pathways for the stored water and the activation of these pools depend on the fractal nature of the pore distribution patterns within the peat layer [266]. However, Menstrie is not officially classified as a peatland due to its peat thickness of < 30 cm [267] although the topsoil had TOC contents similar to Auchencorth Moss. The TN content is also similar in both catchment soils which suggests that peaty podzol catchments like Menstrie are important carbon storages similar to peatlands, hence they should not be excluded in the discussion about peat preservation and restoration.

The C/N ratio from the soils indicate bacterial sources of the DOM in the deeper soil horizons whereas the $\delta^{13}C$ values show a clear C_3 plant signal over the whole soil profile in both catchments. This supports the previous observation that nitrogen in any form affects the DOM pool. The $\delta^{15}N$ values indicate mainly C_3 plants but they suggest an impact of ammonium (NH_4) from precipitation or fertilizer [348, 349]. Ammonia (NH_3) in solution (water) forms NH_4 which could explain shift from soil N to NH_4 in deeper and water saturated soil horizons of Auchencorth Moss. As the soils in the Menstrie catchment are from the unplanted, less polluted, area of the catchment the NH_4 should not arrive from fertilizer. Therefore, the low $\delta^{15}N$ values of the topsoil in Menstrie suggest that precipitation is the main source of nitrogen to the upper soil horizons.

The water extractable organic matter (WEOM) results give indications about the composition of the different soil horizons and suggest where specific DOC fractions are originating from within the soil profile. The WEOM concentration from 2019 soils confirmed that Menstrie as a peat-dominated catchment not only stores high quantities of organic carbon as peatlands, like Auchencorth Moss, but could potentially supply headwaters with similar DOC concentrations. However, these soils and its WEOM reflect only a small area of the catchment with diverse soil depth profiles. In regard to the composition of the WEOM, LMW Neutrals were the most abundant fraction in the topsoil in 2019. Knowing that LMW Neutrals are specifically abundant in the topsoil suggests that increased surface flow can increase LMW Neutrals concentration in the headwaters.

This assumption could explain the good linear fit of LMW Neutrals and DOC in winter months since the deeper soils are highly saturated with water due to continuous precipitation in early winter leading to increasing surface flow or even surface runoff. This implies that in winter soils and headwaters are better hydrologically connected in line with the River Continuum Concept, which is based on the idea that a watercourse is an open ecosystem that is in constant interaction with the bank and moving from source to mouth [350], but less in summer.

In the summer months of 2018 surface flow was restricted due to lower soil saturation caused by longer dry periods. This leads to the assumption that LMW Neutrals are not just transported into the river through surface flow from soils but potentially produced in the headwaters by photochemical or microbial degradation of HMW compounds. The soil isotope ^{13}C coupled with the C/N ratio implies that Menstrie soils are highly impacted by bacterial/algal/microbial sources (Appendix 5.2), hence LMW Neutrals could be linked to increased bacterial or microbial activity in headwaters.

Overall, both catchments showed similar exponential declines in the DOM fractions with soil depths. This is especially important when one considers the potential implications of disturbance with respect to carbon losses from soils; where disturbance is likely to be higher in Menstrie where surface peat layers are also thinner and more susceptible to disruption, when compared to Auchencorth Moss.

It is noteworthy that the riverine DOC concentrations from Menstrie are not reflecting the WEOM concentrations, although the soil water storability is potentially lower in Menstrie than Auchencorth Moss due to the thinner peat layer. Moreover, the water extractions are using MilliQ water, which is not buffered in anyway, hence DOC concentration leaching potential is likely overestimated. A potential factor to this low DOC concentrations in the Menstrie headwater could be the topography. The headwater in Menstrie is located on a hill enabling potentially more rapid DOC export downstream. However, to get a better understanding of the DOC dynamics within a catchment high-resolution and downstream measurements would be necessary. Single point measurements are only offering a small picture of the DOC composition and dynamics between soils and the headwaters without factoring in DOC alteration caused by downstream transport or DOC input by additional headwaters.

5.5. Conclusions

The Auchencorth Moss and Menstrie catchment were found to be good representatives as first order streams for UK river systems as they are covering a wide range of DOC concentration and physical parameters that are widespread in Scotland like soil type, topography, hydrology and land-use.

The following conclusions were drawn from this study:

i. Humics dominate the DOM pool and its pattern between peatland and peat-dominated landscapes are similar. This is also apparent in soil data despite traditional soil classifications.
ii. The mobilisation of LMW Neutrals from soils to headwater is seemingly independent of soil type (peat depth), seasons, hydrological pattern and precipitation, and their origin is possibly the upper soil horizons (plants and plant roots).
iii. Carbon models based on UV absorbance (CDOM) are inadequate at characterising DOM concentration leading to underestimations of DOC concentrations in headwaters, the upland component of the UK river systems, as they not include all non-UV absorbing fractions, and so potentially underestimate DOC flux
iv. DON and nitrate may be a key factor in influencing the CDOM models, as they can chemically alter the DOM fractions; however, further work is needed to get a better understanding of the interaction between Nitrogen components and CDOM.

This study highlighted the importance of considering all DOM fractions and various physical controls (e.g. precipitation, discharge, soil type, land cover) as well as the importance of higher temporal and spatial data resolution.

Chapter 6 – Effect of extreme precipitation events on the dissolved organic matter composition in peat-dominated headwaters streams

6.1. Introduction

High-latitude regions are expected to experience an increase in extreme weather conditions like summer droughts and heavy rainfall events in the coming decades in response to climate change [7]. Single heavy rainfall events may have more pronounced effects than long-term shifts of the water table in peatlands [351]. As peat catchments tend to export more DOC than catchments with organo-mineral soils [284], as shown in chapter 4, this study focuses on the impact of precipitation events on the DOM pool in a Scottish peatland and peat-dominated headwater catchment. These headwater catchments were chosen as they are both good representatives in for temperate river systems (see Chapter 5) and are small in size, allowing easier analysis of the relationship between precipitation and the DOM pool.

A previous study by Muller et al [249] indicated that the surface water chemistry of peatland headwaters is often a reflection of the chemistry of individual precipitation events, yet the compositional behaviour of the DOM within the aquatic environment is less certain. Most studies have used spectrophotometric methods to understand fluctuations in the DOM composition under hydrological extremes [275–277, 277, 328, 352], showing that storm events (high discharge conditions associated with heavy precipitation; [353]) in temperate peatland catchments lead to increased contribution of humic-like material [326, 354]. Although this information has been derived from UV absorbance analysis overlooking the presence of optically ‘invisible’ DOM [102], the results from Size-Exclusion Chromatography in the previous chapter (Chapter 5) confirmed that peak flow conditions dominantly mobilise humics. However, a link between precipitation, discharge and DOM was not observed in the previous chapter emphasising the importance of temporal high-resolution sampling campaigns during different meteorological periods to unravel the dynamics between DOM and precipitation.

Therefore, this study was designed to provide a new insight on the impact of precipitation events on riverine DOM composition in a Scottish peatland and peat-dominated headwater catchment. Chromatographic DOM fractionation with UV absorbance indices were combined to determine (i) whether the DOM pool from a peat-dominated headwater responds differently to precipitation events than a peatland headwater; (ii) whether precipitation events enhance the transport of optically invisible DOM relative to UV amenable DOM; and (iii) to predict the implications of precipitation events for long-term trends in DOM exports from peat-dominated and peatland headwaters. These objectives address the first part of the third hypothesis that precipitation events will favour the input of more recalcitrant, higher molecular weight DOM into the water column over more labile and lower molecular weight DOM. Overall, this chapter is giving an even deeper insight into the temporal dynamics of DOM in headwater systems, while linking it to a specific environmental process, which is beside demonstrating spatial changes the main aim of this thesis.

6.2. Methods

The Auchencorth Moss, a lowland peatland, and the Inch 1, Menstrie catchment, an upland peat-dominated catchment, are the two focus catchments of this chapter as before in Chapter 5. Detailed field site descriptions can be found in Chapter 2, section 2.1.2.

6.2.1. Sampling and analysis

Both catchments were sampled using autosamplers in summer (24th May 2018 to 19th July 2018) and winter (1st to 30th November 2018) of 2018 to capture DOM concentration and composition changes in a high time resolution. All autosamplers were equipped with 24 Polypropylene bottles (ISCO = 1 L, EPIC = 750 mL), which were cleaned 2 times with MilliQ water and twice rinsed with river water before usage.

All water samples were collected in pre-cleaned (12 hours in 10% Hydrochloric acid solution and 24 hours in MilliQ) 60 mL high-density polyethylene (HDPE) bottles and brought to the laboratory at the *Lyell Centre*, Heriot-Watt University for storage at 4 °C and analysed within 24 hours of collection from the autosampler. Additional information’s about the sampling procedure can be found in Chapter 2, section 2.5.1. Water samples were filtered in the laboratory with 0.7 µm glass microfiber filters (GF-F filters by Whatman) to retain the particulate organic matter of the river water.

The water samples were analysed in the laboratory for DOC and DOM fractions including humics, biopolymers, building blocks, low molecular weight (LMW) acids and neutrals as well as dissolved organic nitrogen (DON). Flux calculations of DOC and DOM fractions can be found in section 2.8 and for a full description of the analytical methods used see section 2.7 of Chapter 2.

6.2.2. Water level, discharge and precipitation

Water level and precipitation data were collected from each catchment for the sampling period, see sections 2.2.2 and 2.4.2 for more details on the data collection and calculations.

Rainfall characteristics vary depending on how the events are delimited [355], but identifying rainfall events is complex. Often four main contributory factors are contributing to the identification of rainfall events: (i) intensity and (ii) duration of precipitation, (iii) the wetness of the ground and (iv) the response of the rainfall catchment [356]. The key items considered in this study were the first two, which are the main meteorological criteria. Based on rainfall intensity and duration analysis rainfall events were defined as ≥ 3 mm within 4 hours for the Menstrie catchment, whereas in Auchencorth Moss a rain event was defined as ≥ 2 mm within 4 hours.

6.2.3. Statistical analyses

To provide a complete and comparable DOC dataset the total least squares (TLS) regression from Cantrell [169] was used to get a better linear fit ($y = mx + b$) between DOC data from the GE Analyser and the LC-OCD-OND. For the Black Burn, Auchencorth Moss samples the standard error were $m = 0.025$ and $b = 0.59$, whereas Inch 1, Menstrie samples had standard errors of 0.025 for the slope (m) and 0.056 for b (y-intersect). A detailed explanation of the method can be found in section 2.9 of Chapter 2.

6.3. Results

The following sections report river discharge and precipitation data as well as the results of DOC, DON, $SUVA_{254}$ and DOM fractions (including humics aromaticity and molecular weight). Table 6.1 summarise the identified summer and winter precipitation events in both headwater catchments. The high-resolution timeseries of river discharge, precipitation, DOC and DOM fractions are illustrated in Figure 6.1, while $SUVA_{254}$ data is illustrated in Figure 6.2 and DON data can be found in Appendix 6.1.

6.3.1. Hydrology and Precipitation

Figure 6.1A and 6.1D show the precipitation and discharge records from Black Burn, Auchencorth Moss and Inch 1, Menstrie in a 30 min resolution.

The rainfall in Auchencorth Moss in May to July was unevenly distributed with only five precipitation events. In total 69.4 mm precipitation was measured in Auchencorth Moss between 24th May and 19th July. The base flow in the observed summer months was 0.03 m^3/s on average, increasing to a maximum of 0.31 m^3/s on the 20th June (Figure 6.1A) following the precipitation event on the 19th June. The total precipitation in November 2018 in Auchencorth Moss was 95.8 mm. The winter base flow was around 0.28 m^3/s with the highest discharge on the 21st and 30th November 2018 of 0.55 m^3/s (Figure 6.1A) and increasing discharge during every recorded rainfall event.

The Inch 1 in Menstrie received only 70.6 mm in total in June and July, whereas in November a total of 224 mm precipitation was measured. The discharge was on average 0.004 m^3/s in the observed summer months with a maximum discharge of 0.04 m^3/s on 16th June 2018. The driest period in November was observed from 16th November to 20th November. The base flow from Inch 1 was around 0.03 m^3/s and the largest increase in discharge was measured on the 29th November 2018 with 0.15 m^3/s after 7 hours of continuous rainfall (in total 32 mm) (Figure 6.1D).

Table 6.1: Identified summer and winter precipitation events from Auchencorth Moss and Inch 1, Menstrie. (*) – precipitation continued after the initial precipitation event.

Auchencorth Moss				Menstrie			
Summer							
Date	Time	Amount (mm)	Duration (hours)	Date	Time	Amount (mm)	Duration (hours)
01/06/2018	12:30-13:30	9.67	1	02/06/2018	16:00	5.2	0.5
13/06/2018	19:30-23:00	3.0	3.5	13/06/2018	20:00-00:00	7.2	4*
16/06/2018	08:30-10:30	3.64	2*	16/06/2018	10:00-14:00	14.8	4
19/06-20/06/2018	21:30-01:30	26.7	4*	19/06-20/06/2018	23:00-03:00	9.8	4*
13/07/2018	13:00-15:30	2.02	2.5	16/07/2018	17:00-21:00	5.4	4
Winter							
01/11/2018	00:30-05:30	8.87	5	03/11/2018	07:00-15:00	11.68	4*
07/11/2018	16:00	2.22	0.5	04/11/2018	00:30-03:30	4.57	3
08/11/2018	17:30-20:30	3.03	3	05/11/2018	04:00-08:30	9.91	4.5
09/11/2018	19:30-23:00	4.85	3.5	07/11/2018	15:30-16:30	3.04	1
10/11/2018	17:00-20:00	4.03	3	08/11/2018	16:30-20:30	10.2	4*
14/11/2018	06:30-11:30	3.62	5	09/11-10/11/2018	19:30-23:30	4.1	4*
20/11/2018	13:30-17:30	2.6	4	14/11/2018	10:00-14:00	3.56	4*
21/11/2018	02:00-06:00	5.43	4*	20/11/2018	15:00-19:00	4.57	4*
	09:30-13:30	4.03	4*	21/11/2018	03:30-07:30	25.15	4*
24/11/2018	15:30-19:30	3.44	4	22/11-23/11/2018	21:00-01:00	3.3	4*
28/11/2018	13:30-20:30	4.82	7	25/11/2018	16:00-20:00	3.3	4
29/11/2018	06:00-13:30	10.7	7.5	27/11/2018	16:30-20:00	6.86	3.5
	19:00-21:30	4.24	4	28/11/2018	14:30-18:30	16.76	4*
30/11/2018	14:30-18:30	5.44	4	29/11/2018	09:30-13:30	23.1	4*

6.3.2. Concentration and flux of DOC and DON

Figure 6.1B and 6.1E show the bulk DOC concentration of Black Burn, Auchencorth Moss and Inch 1, Menstrie over the whole sampling period during summer and winter, whereas Figure 6.1C and 6.1F show the DOC concentrations before and during a chosen rainfall event. DON concentrations over time can be found in the supplementary material, Appendix 6.1. The average, maximum and minimum concentration of DOC and DON as well as their standard deviation and the number of data points (n) can be found in table 6.2, while the DOC and DON fluxes are described in the following section.

Black Burn, Auchencorth Moss

A total of 4457 kg/km^2 was exported over 57 days during the summer campaign, whereas in November 2018 a total of 4608 kg/km^2 was exported over 30 days. In summer, the DOC concentrations increased by 31.3 mg/L over a period of 24 hours after the first rain event on 1st June and again after a second large rain event between 19th and 20th June by 28 mg/L (Figure 6.1B). This is an increase of 337% during the rainfall event on the 19th June and 480% increase after the 1st June rainfall event. These two rainfall events exported around 10% of the total DOC exported during the observed summer months. In winter, DOC concentration showed an increasing trend from 7th to 15th November with averaged daily values of around 37.5 mg/L to 51.5 mg/L, respectively. After this increase DOC concentration dropped to an average of 38.4 mg/L. The highest DOC concentration (67 mg/L) and DOC flux (0.24 kg/h) was measured on the 2nd November, 40 hours after heavy precipitation. Other rainfall events like the one on the 14th November (Figure 6.1C) transported 26 kg DOC over 24 hours, the two rainfall events on 9th and 10th November transported around 93 kg DOC in 48 hours.

In total 33.8 kg/km^2 DON BP and 69.2 kg/km^2 DON HS were exported in summer, while only 2.7 kg/km^2 DON BP and 20.5 kg/km^2 DON HS are mobilised in winter. The precipitation event on the 19th of June transported around 7 % of the DON HS and 5 % of the DON BP from the total DON export. The rainfall event on the 14th of November mobilised around 14 % of the total DON BP export and 24 % of the total DON HS export.

Inch 1, Menstrie

A total DOC of 568 kg/km^2 was exported over 48 days in the summer period. In the summer months DOC concentration reached a maximum of 3.7 mg/L on 9th July, during a dry period (Figure 6.1E). Two of the three summer rainfall events exported a total of 120 kg/km^2 DOC which is around 21 % of the total DOC export in summer. In November 2018, a total of 2257 kg/km^2 was exported over 30 days. The first rainfall event in November increased the DOC concentration by 15.2 mg/L over 24 hours, resulting in a DOC export of 9.75 kg/km^2 per day. Other large DOC concentration rises were observed on the 8th of November with an increase of 5.9 mg/L over 11 hours, on 10th November with an increase of 7 mg/L over only 3 hours, 6 mg/L on the 14th November over 8 hours and the second biggest increase of 9.5 mg/L from 20th to 21st November, all linked to heavy rainfall and discharge events. A total of around 18.65 kg/km^2 DOC ($n = 47$) was transported during the five previous mentioned precipitation events, representing around 24.8 % of the total DOC export in November.

In summer, a total of 0.9 kg/km^2 DON BP and 7.4 kg/km^2 DON HS were exported in summer, while in winter 1.0 kg/km^2 DON BP and 6.3 kg/km^2 DON HS were mobilised. The precipitation event on the 2nd June transported around 14 % of the DON HS and 15 % of the DON BP from the total DON export. The winter rainfall event on the 14th November mobilised around 48 % of the total DON BP export and 45 % of the total DON HS export.

6.3.3. DOM composition

Figure 6.1B and 6.1E show the percentage distribution of the DOM fractions from Black Burn, Auchencorth Moss and Inch 1, Menstrie. Figure 6.1C and 6.1F show the concentration of the five DOM fractions during a chosen precipitation event. The average, maximum and minimum concentration of the DOM fractions as well as their standard deviation and the number of data points (n) can be found in table 6.2, while the fluxes and percentage distribution are described in the following section.

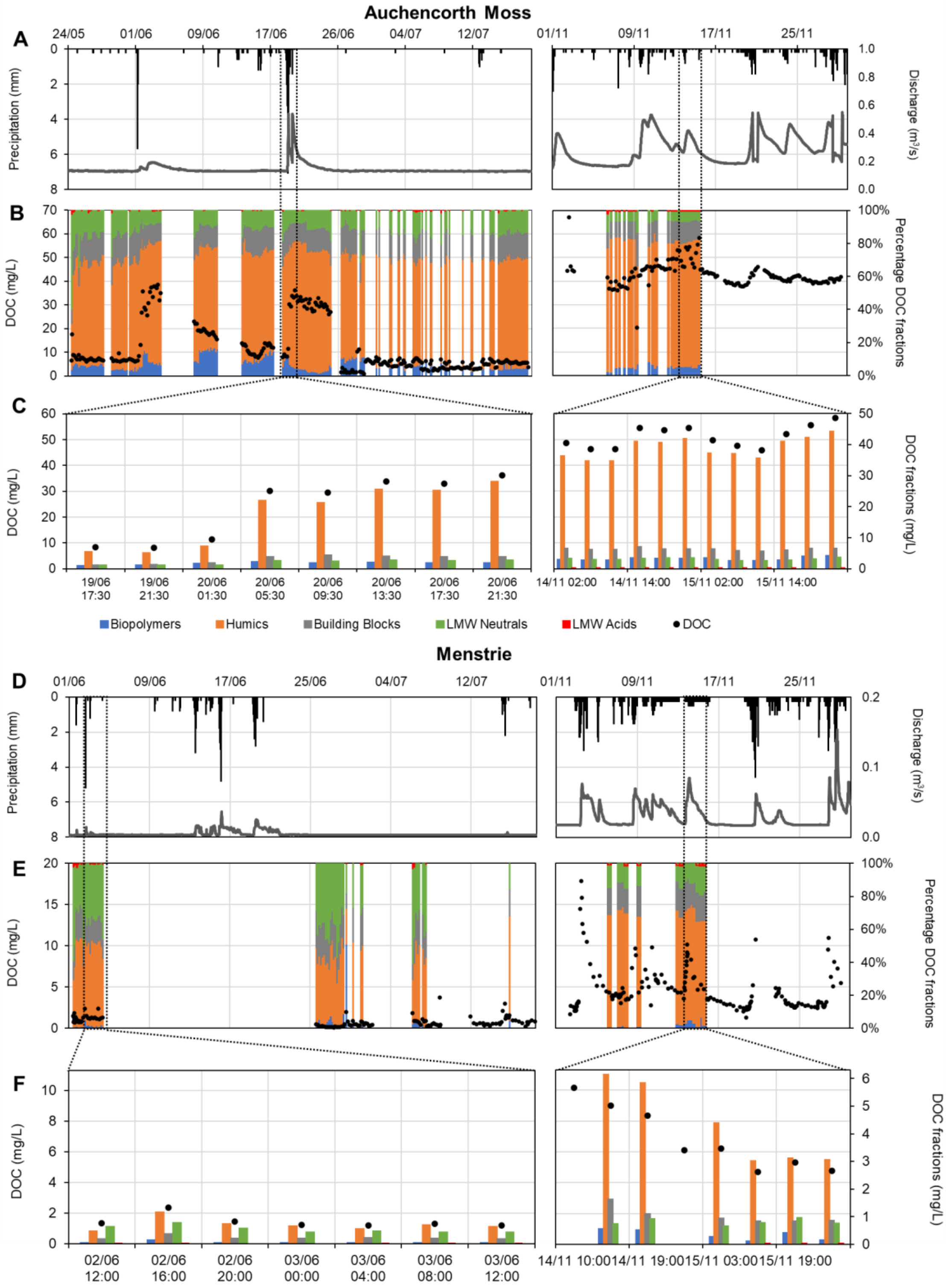

Figure 6.1: High-resolution time series for Summer 2018 (June-July)and winter 2018 (November) from Black Burn, Auchencorth Moss with (A) half-hourly total precipitation data and average discharge, (B) 4 hourly dissolved organic carbon (DOC) concentration and percentage distribution of DOC fractions and (C) concentration from bulk DOC and its fractions during a chosen rainfall event. High-resolution time series for Summer 2018 (June-July)and winter 2018 (November) from Inch 1, Menstrie with (D) half-hourly total precipitation data and average discharge, (E) 30 minutes to 8 hourly DOC concentration and percentage distribution of DOC fractions and (F) concentration from bulk DOC and its fractions during a chosen rainfall event.

DOM fractions (Black Burn, Auchencorth Moss)

Biopolymers contributed on average 7.2 and 5.9 % to the total DOM pool, respectively. A total of 33.8 kg/km^2 biopolymers were exported over 57 days in summer and 2.71 kg/km^2 in the thirty November days.

In summer, the lowest percentage contribution of biopolymers (1.5 %) was measured after a heavy rainfall event in June, whereas in winter the lowest proportional contribution was during a dry period on 6th November. The highest percentage contribution in summer of 16 % is during a dry period during decreasing DOC concentration (Figure 6.1B) and the highest concentrations and proportional contribution in winter is during the storm events on 9th and 10th November.

Humics contributed on average 64.1 % to the DOM pool in summer and 65.3 % in winter. A total of 2748 kg/km^2 was exported in summer and 863 kg/km^2 in winter. The highest humics contribution in summer with 77 % was measured after a heavy precipitation event on 21st June. The lowest contribution of humics of 27.8 % was observed on the 24th May 2018 during a dry period. The two rainfall events exported around 10 % of the total DOC exported during the 57 summer days. In winter, the maximum humics concentration was 44.2 mg/L measured on the 15th November, whereas the highest percentage contribution of 82.2 % was measured on the 11th November 2018 (Figure 6.1B). The three precipitation events on the 10th, 11th and 14th November contributed around 32 % to the total humics export in November.

In summer months building blocks contributed between 8 - 19 % in the total DOM pool. The two rainfall events in summer mobilised around 10 % of the total building blocks export of 540 kg/km^2. In winter, the highest measured concentration of building blocks was 7 mg/L on the 14th November during a precipitation event (Figure 6.1C), which exported 21 % of the total building blocks export of 135 kg/km^2. The lowest building blocks concentration and export was measured 24 hours after the rainfall event on the 10th November.

LMW neutrals are the 3rd most abundant DOM fraction after humics and building blocks in the Black Burn with an average contribution of 11.4 %. A total of 440 kg/km^2 LMW neutrals was exported in summer from the Black Burn. The concentration and flux of LMW neutrals were lowest during the two precipitation events (Figure 6.1B and 6.1C). The highest percentage contribution (35 %) and flux (0.01 kg/km^2 per hour) of LMW neutrals was observed during a dry period at the beginning of the sampling period.

The proportional distribution of LMW neutrals in the Black Burn winter samples was stable at around 5.7 % (Figure 6.1 B), with two samples throughout dry periods showing a slightly higher contribution of 10.8 and 12.9 %, respectively. A total of 68 kg/km^2 LMW neutrals were exported in November with 21 % mobilised during the rainfall event on the 14th November (Figure 6.1 C).

LMW acids show the lowest concentrations, hence contribute less to the total DOM pool than other fractions. LMW acids contributed only 2.5 % to the total DOM pool in summer and 1.7 % in winter (Figure 6.1 B).

DOM fractions (Inch 1, Menstrie)

Biopolymers contributed between 0.4 - 51 % to the DOM pool in summer and between 1.3 - 7.8 % in winter. During the summer precipitation events on the 2nd June and 15th July biopolymers contributed 2 - 5 % to the total DOM pool and around 13 % of the total biopolymers export of 11.6 kg/km^2 got mobilised. In November, the biopolymers export was 8.4 kg/km^2 with the highest concentration and flux after the precipitation event on 14th November (Figure 6.1F).

Humics contributed on average 46 % to the DOM pool in the summer months and 68 % in winter. In summer, a total of 158 kg/km^2 was exported with the lowest humics concentration and flux observed on the 28th June during a dry period, contributing only 39.5 % to the total DOM pool. In winter, the highest percentage contribution was 72.2 %, observed on the 7th November, and the highest concentration and flux was measured on the 14th November, all during precipitation events (Figure 6.1F). The continuous precipitation from 14th to 16th November mobilised 53 % of the total humics export of 142 kg/km^2 from this month.

Building blocks represented on average 17% in both seasons. Both time periods show the highest concentration of building blocks during rainfall events with 0.35 mg/L on 2nd June and 1.6 mg/L on 14th November (Figure 6.1F). The summer rainfall event transported around 12 % of the total building blocks export of 55.4 kg/km^2, whereas the rainfall event on the 14th November mobilised 44 % of the total export of 34.4 kg/km^2.

LMW neutrals were the 2nd most abundant fraction with proportional contributions of 6 - 58 % in the summer and 8 - 18 % in winter. In summer, the highest proportional contribution was during a dry period and the lowest contribution after the first rainfall, similar to the Black Burn. The rainfall event beginning of June transported around 15 % of the total summer LMW neutrals export of 101 kg/km^2.

In the winter samples the highest percentage contribution of 17.8 % (0.9 mg/L) was on the 15th November after continuous heavy rainfall (Figure 6.1F). This heavy precipitation mid-November transported 48 % of the total 25.2 kg/km^2 LMW neutrals export.

LMW acids concentrations were between 0.01 - 0.06 mg/L in summer and 0.01 - 0.03 mg/L in winter, contributing a maximum of 3.2 % in summer DOM pool and 0.5 % in winter (Figure 6.1).

Table 6.2: Mean, maximum and minimum concentration from dissolved organic carbon (DOC), biopolymers (BP), dissolved organic nitrogen from biopolymers (DON BP), humics (HS), dissolved organic nitrogen from humic (DON HS), building blocks (BB), low molecular weight (LMW) neutrals and acids in milligram per litre (mg/L). Mean, minimum and maximum values from the specific UV absorbance from humics ($SUVA_{HS}$) in L/(mg*m) and molecular weight in g/mol. Standard deviations and number of data points (count) is also displayed for each of the previous mentioned indices.

Auchencorth Moss, Black Burn										
	DOC	**BP**	**DON BP**	**HS**	**DON HS**	**SUVA HS**	**Mol-Weight**	**BB**	**LMW neutr.**	**LMW acids**
Summer										
Mean	11.0	1.0	0.1	9.1	0.2	5.7	715	1.8	1.5	0.04
Max	38.6	2.6	0.5	29.0	0.7	7.6	924	4.8	10.1	0.4
Min	0.8	0.1	0.01	0.5	0.02	4.9	533	0.1	0.1	0.01
Std Dev	10.1	0.9	0.1	7.9	0.2	0.4	81	1.2	1.1	0.06
Count	279	208	208	208	208	208	208	208	208	64
Winter										
Mean	42.9	2.6	0.1	35.4	0.8	4.6	1165	5.5	2.8	0.09
Max	67.0	4.1	0.2	44.2	1.2	6.5	1299	6.9	4.5	0.7
Min	20.2	0.9	0.03	12.0	0.3	4.7	926	3.6	2.2	0.01
Std Dev	5.0	0.7	0.06	5.9	0.2	0.5	91	1.0	0.5	0.1
Count	141	32	32	32	32	32	32	32	32	25
Menstrie, Inch 1										
Date	**DOC**	**BP**	**DON BP**	**HS**	**DON HS**	**SUVA HS**	**Mol-Weight**	**BB**	**LMW neutr.**	**LMW acids**
Summer										
Mean	0.8	0.03	0.01	0.04	0.02	5.1	692	0.1	0.3	0.01
Max	3.7	1.0	0.02	1.1	0.06	7.1	935	0.4	1.1	0.06
Min	0.08	0.01	0.01	0.03	0.01	3.6	471	0.02	0.03	0.01
Std Dev	0.5	0.1	<0.01	0.2	0.01	0.5	104	0.06	0.2	0.01
Count	149	85	40	85	85	85	85	85	85	24
Winter										
Mean	4.7	0.2	0.02	3.5	0.2	5.2	1091	0.9	0.6	0.02
Max	17.9	0.6	0.06	6.1	0.3	6.0	1169	1.6	0.9	0.03
Min	1.3	0.05	0.01	2.4	0.1	4.6	1001	0.5	0.4	0.01
Std Dev	2.7	0.2	0.01	1.1	0.04	0.5	49	0.2	0.2	0.01
Count	166	14	14	14	14	14	14	14	14	6

UV Absorbance indices – SUVA$_{254}$

The Black Burn summer samples had SUVA$_{254}$ values between 2.4 and 8.4 L/(mg*m), whereas the winter samples vary between 4.7 and 6.4 L/(mg*m), see table 6.2. The lowest SUVA$_{254}$ value was measured in the first water sample on 24th May, whereas the samples with low DOC and high SUVA$_{254}$ in summer were all sampled during the long dry period (26th to 30th June 2018). Winter samples indicate a slight decrease with increasing DOC concentration and the lowest SUVA$_{254}$ of 4.7 L/(mg*m) was measured during the heavy rainfall event on 10th November; however, no relationship between precipitation and SUVA$_{254}$ was found. In Inch 1 summer samples SUVA was between 1.7 and 8.1 L/(mg*m), with the highest values on 28th June corresponding to the highest proportional contribution of LMW neutrals and the lowest SUVA$_{254}$ value on the 4th June, 24 hours after a short rainfall period (2 mm).

Humic substances characteristics

In Figure 6.2 molecular weight (Mn) is plotted against SUVA$_{HS}$, indicator for humics aromaticity, from the Black Burn, Auchencorth Moss and Inch 1, Menstrie samples for winter and summer. The samples are grouped in (i) no rainfall, (ii) medium rainfall (0.2 - 1.9 mm) and (iii) heavy rainfall (≥ 2 mm), corresponding to the amount of rainfall that has been measured during or up to 4 hours before sampling. The average, maximum and minimum values of the molecular weight and SUVA$_{HS}$ can be found in table 6.2. The summer samples taken during heavy precipitation show a trend to lower molecular weight than samples taken during dry periods. The three winter samples with the lowest molecular weight were collected on the 9th, 10th and 11th November: before, during and after a heavy rain event.

Inch 1, Menstrie, samples had SUVA$_{HS}$ values from 3.6 - 7.1 L/(mg*m) in summer and 4.6 - 6.0 L/(mg*m) in winter. The samples with the lowest SUVA$_{HS}$ values were collected on the 27th and 29th of June as well as 7th of July, all during low flow conditions and no precipitation. The highest SUVA$_{HS}$ was also measured during a dry period in the sample with the lowest DOC concentration (0.08 mg/L). The molecular weight varied between 471 - 935 g/mol in the Menstrie summer samples and 1000 - 1170 g/mol in the winter samples.

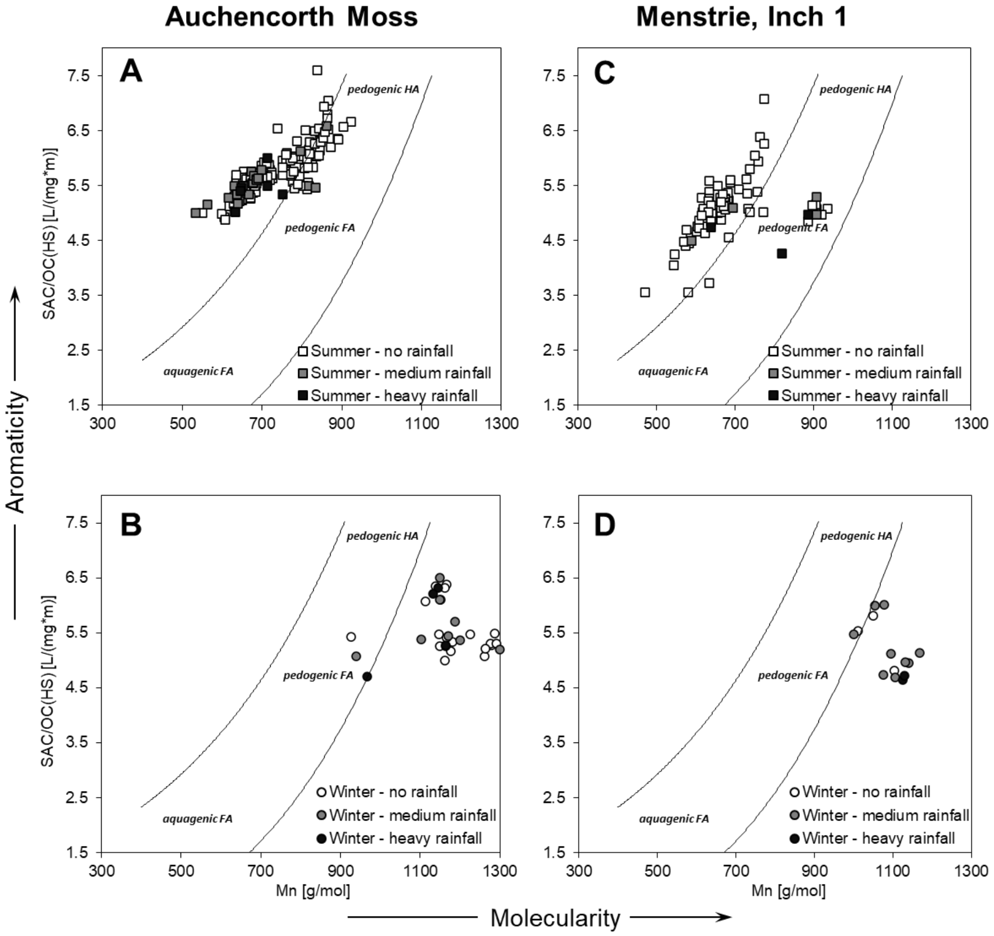

Figure 6.2: Humification pathway – molecular weight (Mn) in gram per mole (g/mol) against specific UV absorbance at 254 nm from the Humics fraction ($SUVA_{HS}$, SAC/OC) in litre per milligram times meter (L/(mg*m)). All samples are grouped in 3 groups depending on amount of precipitation measured within 4 hours of water sampling showing A) Auchencorth Moss summer samples, B) Auchencorth Moss winter samples, C) Menstrie Inch 1 summer samples and D) Menstrie Inch 1 winter samples.

6.4. Discussion

6.4.1. The impact of precipitation effects on hydrology and DOC & DON mobilisation – a comparison between peat-dominated and peatland headwater

The difference in topography and soils can play an important role in the hydrological response of headwaters during precipitation extremes and their DOC delivery [357]. The Black Burn flows through the Auchencorth Moss, a lowland ombrotrophic peatland, whereas the Inch 1 flows through an upland peat-podzol dominated catchment. Moreover, Inch 1 is located within the Ochil Hills with an elevation of 300-500 m and hill slopes of 5 - 15 degrees [358]. The Black Burn is located on the south east of the large Pentland Hills with an elevation of 249 - 300 m [13] and a low slope of around two degrees.

However, both headwater show the most significant rainfall-discharge correlation with a lag-time of around 2 to 4 hours ($p < 0.01$ for both catchments, Appendix 6.5) indicating that both headwaters have a flashy hydrological response to rainfall, which is already known for the Black Burn at Auchencorth Moss [13, 23].

During the dry summer both headwaters show a rise in discharge as well as DOC and DON concentration and flux during heavy precipitation events, but almost no effect on the river discharge and a much smaller impact on the DOC and DON flux during short and low-intensity precipitation periods. In winter, low-intensity but continuous rainfall appears to affect the discharge just as much as high-intensity rainfall events. In the Inch 1 catchment the DOC and DON concentrations show a clear increase during high-intensity precipitation events and also during continuous low-intensity precipitation. In the Black Burn the winter DOC data implies that the peat soils are oversaturated since precipitation events show increase the DOC and DON fluxes in a much smaller scale than in summer. Furthermore, the constant low-intensity precipitation in winter is keeping the DOC flux and concentration on a steady level in comparison to the summer period when rainfall events are more sporadic and of higher magnitude. These observations suggests that the impact of precipitation events on DOC and DON is dependent on the antecedent hydrological conditions (pre-event base flow), the magnitude of pre-event rainfall and the magnitude of the actual rainfall event, supporting the findings by Broder et al. [327] and Guarch-Ribot et al. [276].

The difference in DOC and DON response in both catchments to precipitation events could be linked to the surface topography, because it is a key control on how precipitation affects baseflow [359] and the topographic gradients control the rate at which rain and soil water moves downslope [360]. The steeper slope in the Menstrie catchment could enhance a quicker transport of DOM and POM from the surrounding landscape to the headwater during wet and dry soil conditions, than in the flatter Black Burn catchment. However, in headwaters with short lag time pattern the source area of runoff water is usually limited to the near-spring area with saturated overland flow being the dominant flow path [361]. Nonetheless, the DOC results imply that under low flow conditions the DOC concentration are higher over longer time periods in the Black Burn than in the Inch 1, supporting the suggestion that peatlands are longer water saturated than peaty podzols.

This might indicate that the DOM in the Black Burn becomes more available for microbial degradation and potentially contributes to CO_2 outgassing even without photochemical facilitation due to the high colouration of the river water ("brown-water stream", [32]).

However, the water retention capacity of the soil and the soil moisture before the precipitation event can also play an important role in the hydrological response [362] and OM mobilisation from the soils [363]. An oversaturated (wet) peatland is more likely to release stored soil water [266], then a fairly dry peatland, increasing the discharge and transporting DOC into the stream without a rainfall event. Figure 6.3 shows a conceptual model describing potential DOC transport processes in relation to precipitation and antecedent hydrological conditions. However, this conceptual model is simplified to demonstrate the general transport processes of water/OM in the river catchment, but not all soil types will translate to this model depending on their physical properties (e.g. pore volume). Heavy precipitation events during the dry summer possibly transported DOC into the headwater from lateral subsurface flow [364] and/or overland flow, which may differ depending on the surface topography and the soil water storage capacity. Broder et al. [16] found that storm events during dry periods export DOM predominantly form a deeper peat layer while events with wet preconditions activate additional near-surface DOM pools due to increasing water levels in the catchment. This could suggest that precipitation events mobilise compositionally different DOM pools between catchments and potentially between seasons. This hypothesis will be discussed in the next section of this study.

Walczak et al. [365] found that the physical properties (bulk density, total porosity, water retention, differential water capacity) of peat depend to a large extent on the relation between the organic and mineral parts, showing that an increase in the OM content resulted in an increase in water retention. Although, in the previous chapter it was observed that both catchments had similar soil organic carbon concentrations the Auchencorth Moss is completely covered by a thick peat layer, while Menstrie has peat, peaty-podzol and mineral soils. Hence, Auchencorth Moss soils may store more carbon in deeper soil horizons that can be released during precipitation events with wet pre-conditions.

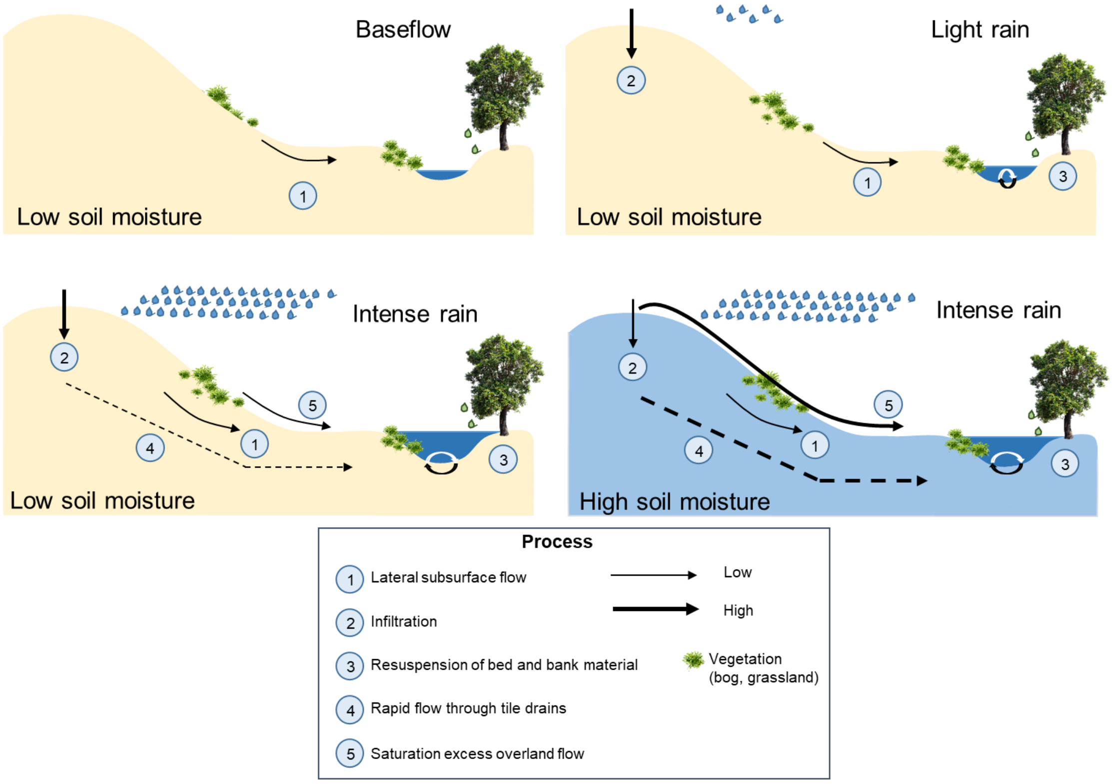

Figure 6.3: Conceptual model highlighting the key processes that control stream DOC export under different hydro-meteorological conditions (modified, original from Blaen et al. [364]).

Overall, the findings of this study in DOC and DON mobilisation suggest that the intensity and the duration of rainfall events are important factors in the DOC mobilisation, but the intensity of the impact depends on antecedent conditions. Given that precipitation regimes are expected to shift to higher frequencies in the near future [366, 367], it is important to assess the potential consequences on water quality parameters. Furthermore, these findings strengthen the importance of high-resolution time series since no relationship was observed between precipitation events and DOC and DON in the previous chapter of this thesis.

6.4.2. Coloured or invisible DOM? – the role of precipitation events in mobilising particular DOM fractions

Humics dominate the DOM pool in both headwaters which is consistent with the observations from the previous chapter (Chapter 5). However, the high-resolution sampling revealed that the DOM composition between peatland and peat-dominated landscapes differs with a higher contribution of LMW neutrals and building blocks in the peat podzol headwater suggesting a different source material or potentially increased degradation of HMW DOM in-situ.

Statistically, the precipitation data only correlates significantly with building blocks in summer in both catchments ($p < 0.05$, Appendix 6.6 and 6.7) and LMW neutrals show a significant correlation with precipitation in the Menstrie winter ($p = 0.01$, Appendix 6.7).

Both catchments experienced a number of rainfall events in winter and summer of 2018. One specific rainfall event in summer and winter was chosen to examine the DOM dynamics during and after a rainfall event.

In the Black Burn humics and building blocks showed the largest increase within 4 hours after the largest summer rainfall event, outweighing the contribution of LMW neutrals and biopolymers, which increased in concentration but not as much as the other two fractions. Furthermore, the humics concentration increased beyond the initial rise leading to an increased DOC concentration while the discharge decreased following a precipitation event. This may indicate a supply of humics from further upstream leading to an enrichment of humics within the water column or a larger input from the riparian zone than at Inch 1.

Pickard et al. [326] also found that phenolic composition changes following a winter rainfall event, suggesting mobilisation of material from higher up the catchment in Auchencorth Moss. Furthermore, rainfall events introduced a compositionally different humics fraction in the Black Burn, Auchencorth Moss. The molecular weight and aromaticity of the humics, as well as the DON concentration, increased during and after rainfall events relative to the humics during dry periods. This observation implies that precipitation events during low flow conditions mobilise not only more humics but chemically heavier and more aromatic humic substances. One possible explanation for the change in humics composition could be that rainfall events transport younger, less degraded OM from the surrounding landscape and topsoil, which potentially gets degraded to molecular lighter and less aromatic humics during low flow conditions after the rainfall. The increased humics C/N ratio after the summer rainfall event support the hypothesis that rainfall events introduce DOM from fresh plant material.

Although the actual source of this compositional different humics is unclear, a previous study by Collins et al. [368] found that drier initial soil conditions are more likely to enhance the contribution of ‘new’ water to the runoff. However, this addition of ‘new’ water is closely linked to the antecedent moisture conditions of the soils, because it is also likely that the contribution of old water is significant and prolonged, despite the hydrologically responsive nature of the catchment [368].

Independent of the source of the humics the increasing supply of these compounds will lead to more processing cost, while varied humic composition increases the complexity of treatments causing additional disturbance for the processing.

The data from Inch 1 showed increasing concentrations of all fractions during the summer rainfall event; however, the increase was instant and not with a 4 hours' time delay, as observed in the Black Burn (Appendix 6.2). The increase in LMW DOM during rainfall events with low baseflow conditions was observed in a previous study by Broder et al. [16], who also found that during drought periods DOM shows a more microbial signature, originating from long DOM residence times in the soil and peat. The C/N ratios from biopolymers and humics in Inch 1 suggests that the DOM during dry periods has a more microbial source than during high precipitation (Appendix 6.4) or is more degraded. This observation was not made in the Black Burn, Auchencorth Moss, which may be related to the dominance of peat with a prominent terrestrial signal.

Nonetheless, the rain event increased selectively the humics fraction, as observed in the Black Burn, shifting the dominance from LMW neutrals DOM to a humics controlled DOM. It has been noted that less aromatic but higher molecular weight humic substances get mobilised during precipitation events compared to the humic fraction before the rain event. This is opposite to the observations made in the Black Burn in Auchencorth Moss. A possible explanation for this shift in the humics pool could be a contribution of inorganic compounds or increased nitrogen contribution. DON concentrations, measured with the LC-OCD-OND, were indeed higher during and after the rainfall event than before, which supports the concept that nitrogen compounds may affect the humics composition.

Vázquez et al. [50] found that during rewetting, when stream water recharges the surrounding riparian ground water, the water flow across the interface facilitates retention of the heavier DOC fractions and increased DON concentration from these HMW fractions. However, an enhanced mobilisation of clay particles from deeper soil horizons could also increase the molecular weight of humics without increasing their aromaticity.

The daily measurements in Chapter 5 show no difference in the fluxes of any DOM fractions in Inch 1, Menstrie between the drier 1st and the wetter 2nd June whereas when assessed using 30 min to 8 hours frequency data the rainfall event on the 2nd June actually doubled the humics and building blocks flux and increased the LMW neutrals flux as well by about 20 %.

Furthermore, in the previous chapter of this thesis the Black Burn show higher humics, biopolymers and LMW neutrals fluxes on the dry 14th June compared to the much wetter 19th June which was not observed in the high-resolution data.

Rain events in winter seem to have a smaller impact on the DOM composition than summer rain events, which may be linked to the soil moisture, hence much wetter antecedent soil conditions before a precipitation event. Both headwaters show less variability between the DOM fractions during the wetter winter, demonstrating a clear dominance of humics (> 50 % contribution) and their building blocks. Winter rain events dominantly mobilise humic substances in the Black Burn (Appendix 6.1) from the surrounding peat soils; however, it appears that continuous rainfall is driving the rising humics concentration while single rain events are the drivers in the summer. A similar observation was made in Inch 1 of the Menstrie catchment, but the data from the 14th November rainfall event show decreasing concentrations, indicating a potential dilution of the DOM fractions and bulk DOC. However, the DOM fluxes reveal that only the higher molecular weight fractions were diluted, while more LMW neutrals were exported after the winter rainfall events compared to humics and building blocks. This is contrary to the DOM fluxes after a summer rainfall event, as shown above.

These findings suggest that precipitation events with wet and dry antecedent conditions mobilise a compositionally different DOM pool e.g. mobilisation from different soil horizons and/or a potential alteration of DOM during transport. This implies that the antecedent soil moisture conditions within the catchments play an important role in the rainfall-DOM relationship, which was observed in previous studies [276, 327, 369].

Furthermore, the intensity and frequency of the rainfall events may also be important to consider in the DOM mobilisation. The summer rainfall events captured in this study can be characterised with high intensity in a short time span, while winter rainfall events had a lower intensity but occurred more frequently. The frequent rainfall in winter enables DOM mobilisation at a consistent level from wetted soils, while the first heavy rain event mobilises less and compositional different DOM due to the preceding dry conditions. Both seasonal datasets show that a single high intensity rainfall event (≥ 2 mm in 30 min to 1 hour) will mobilise less DOM than a longer rain event (≥ 2 mm in ≥ 3 hours), but all rain events will dominantly transport humic substances. Therefore, the observed rainfall-DOM composition relationships suggest that the rainfall amount plays a secondary role while the rainfall duration and frequency of the rainfall events have a larger impact on the DOM mobilisation.

As global warming is predicted to make peatlands more vulnerable to degradation through drying [370], it was also projected that extreme precipitation events increase in frequency and duration [7] which could enhance carbon export from peatlands as suggested in this study. However, this study together with the conclusions of the previous chapters show that peat-dominated rivers show similar compositional DOM dynamics under changing hydro-meteorological conditions as peatland rivers. This is important to acknowledge because catchments like Menstrie are not defined as peatlands due to the thickness of the peat. However, this thinner peat layer could be even more affected by climate changes in the future than peatlands, as it dries out quicker during droughts and captures less water than peatlands, potentially releasing more carbon to the rivers.

Particularly, the potential of microbial availability of LMW neutrals needs to be further investigated, as they could well provide another substantial component of riverine CO_2 outgassing due to their lability. At this point, the scale and importance of the LMW neutrals observations for global carbon models and future carbon simulations is unclear.

6.5. Conclusions

This study confirms that high temporally resolved sampling (30 min to 8 hours) of headwater rivers is required to capture the relationship between precipitation events and DOM composition changes.

This study found that precipitation events dominantly transported humic substances likely independent of seasonality, which is in line with previous studies [16, 275]. The humic substances transported during precipitation events in the peat-dominated (peaty podzol) catchments are less aromatic than humics mobilised during dry periods and the C/N ratio suggests that the peatland headwater transported more degraded humics than the peat-dominated headwater. This increased humics contribution with lower aromatic substances during and after rainfall events can lead to increased downstream transport of aliphatic and nitrogen-rich compounds which can have large implications on the drinking water treatment due to enhanced browning of the water and humics are well known as strong disinfection by-product precursors [174, 371].

During longer dry periods 30 - 60 % of the DOM were UV “invisible” LMW neutrals, and notably, over the complete sampling period on average 12 % of the DOM that gets exported from these headwaters to larger river systems downstream are “invisible” LMW neutrals.

This is comparable to the average contribution observed in large Scottish river systems (12.6 %) in Chapter 4 of this thesis suggesting that LMW neutrals are either consistently mobilised downstream from the surrounding topsoil (see Chapter 5), or being produced in-situ by microbial or photochemical degradation of HMW DOM. The high ‘invisible’ LMW neutrals contribution can have implications on the carbon feedback from headwaters and larger river systems, indicating a highly labile DOM component could be remineralised within the water column. Nonetheless, the potentially higher vulnerability of peat-dominated catchments to climate change and their higher abundance of LMW neutrals makes them more likely to release carbon to the atmosphere.

Chapter 7 – Research summary, future work and final remarks

The nature, distribution, and reactivity of organic matter (OM) in a given river systems is determined, to a large extent, by the strength and nature of environmental processes, like precipitation and river discharge. In the last decades, increased emphasis has been placed on understanding the geochemical and ecological roles of dissolved organic matter (DOM) in aquatic ecosystems as it has distinctive chemical characteristics associated with its source materials and these characteristics may pose a challenge to water quality, ecosystem functioning and Greenhouse gases (GHG) degassing. Understanding DOM transfer between the terrestrial and aquatic ecosystems and processes within the water column are important considering the unprecedented anthropogenic environmental changes we are experiencing in the biosphere, lithosphere, atmosphere, and hydrosphere. In particular the reactivity of DOM during reprocessing, supplying nutrients into river systems (productivity) and GHG outgassing to the atmosphere.

7.1. Research summary

The motivation for this research was to characterise and quantify DOM from Scottish rivers and headwaters over a two-year period from 2017 - 2018. This research project shows that the long-term sampling and processing of water samples is key to identify internal variability of DOM under changing environmental conditions within the seasonal cycle. The primary aim of this study was to determine the compositional behaviour of DOM in temperate Scottish rivers and headwaters under changing geochemical, physical and environmental conditions. This aim led to three scientific hypotheses to be tested in this study:

i. *DOM flux and composition will vary between river catchments as a function of the dominant soil type with higher export of high-molecular weight humic-like DOM from peat-dominated catchments compared to overall higher low-molecular weight DOM fluxes from non-peat catchments.*

ii. *DOM composition is variable in a headwater stream related to seasonal and hydrological conditions, with higher inputs of terrestrial DOM in winter during peak flow conditions than in summer with low flow conditions.*

iii. *Precipitation events will favour the input of more recalcitrant, higher molecular weight DOM into the water column over more labile and lower molecular weight DOM by mobilising OM from the surface and the upper humic rich soil horizons rather than infiltrating deeper soil horizons.*

The following sections focus on the three hypothesis and main objectives of this study. Each of the subchapter summarises important findings and their potential implications.

7.1.1. Influence of soil type on the DOM pool

This study showed that the soil type plays a key role in the compositional behaviours of DOM in larger Scottish rivers. In the headwater catchments of our study, the DOM dynamics were not observed to be greatly impacted by their soil type, as peat and peat-podzol headwaters showed many similar DOM patterns (e.g. increased humics in winter, increased LMW neutrals contribution in summer), although the overall DOC concentrations and DOM fractions identified were observed in different abundances. Notably, the assumption that peat-dominated catchments export predominantly high-molecular weight humic-like DOM while non-peat catchments export lower-molecular weight DOM is only partially true as the greater changes in DOM composition were observed between seasons in response to changes in hydrology and precipitation.

Overall, the topsoil from the headwater catchments, unrelated if peatland or peat-podzol, was found to be dominated by LMW neutrals suggesting that the LMW DOM in the headwater's origins from fresh plant material and/or plant roots. Moreover, all rivers (large scale and headwaters) showed a continuous and at times proportionally large supply of UV 'invisible' LMW neutrals. However, the concentration of LMW neutrals in the soils is much higher than in the headwaters, which offers the potential to increase leaching of LMW DOM from soils into the aquatic environment in the future through disturbed soils (e.g. peat extraction, deforestation). Although the exact origin of LMW neutrals and its role in the aquatic carbon cycle needs further investigation the results of this study suggest that the future increased supply of labile LMW DOM may promote instream microbial growth and thereby act as a nutrient source for aquatic plants. This can have large implications on the ecosystem functioning of these streams as increased biofilms can affect the light penetration depth of the rivers, changing the biological and chemical composition within the water column.

The few data of SOM composition showed that peatland and peat-dominated (peat-podzol) landscapes are storing a similar amount of carbon, independent of peat thickness and soil classification. While the soil data is limited to one location within the catchments and only one specific sampling date, this interesting observation may imply that peat podzol catchments could release much more carbon to the aquatic environment than currently observed. This could have large implications on the carbon feedback of these environments and add potentially even more CO_2 to the atmosphere as more carbon may be remineralised in the streams of peat-podzol catchments.

7.1.2. Seasonal and hydrological controls on dissolved organic matter mobilisation and composition

This PhD provides evidence to support the second hypothesis of this study where higher inputs of terrestrial DOM can be observed in winter than in summer due to increase stream flow was found to be correct. However, additional environmental factors including soil type and precipitation can affect the relationship between seasonality, hydrology and DOM.

It was found that temperate Scottish rivers and headwaters show a seasonality in their DOM mobilisation with enhanced exports of DOC and humics during winter months compared to the summer season. This increase of humics in winter compared to summer, assuming peatland DOM sources are in abundance and do not become depleted over time, will have large implications on the drinking water treatment industry as they have to constantly adjust their treatment method depending on season and hydrological condition. After all, humics are known to be precursors for potentially harmful disinfection by-products through specific treatment methods like chlorination.

On the other hand, LMW neutrals were continuously supplied or produced within the water column, independent of river hydrology and season. This high contribution of LMW DOM will affect the river biota, as it is likely to be preferred as food source by microbes and bacteria, consequently increasing the abundance of biota which will impact the light penetration depth as well as the oxygen content in the streams.

Furthermore, this PhD study has demonstrated that DOC fluxes may be under quantified when optical methods are used which could be linked to the high DOC concentration (> 30 mg/L) in peatland streams that is not getting fully recognised in the model or other impacts on the DOM like nitrogen and iron. Underestimating DOC quantities in temperate headwaters may lead to a misrepresentation of the importance of headwaters in the global carbon cycle as well as leading to an underestimation of the global inland water carbon budget, locally and potentially globally.

7.1.3. The role of precipitation events on riverine DOM composition

This study has shown that compositional changes in the DOM pool due to precipitation events occur within 4 hours and therefore cannot be observed in monthly sampling campaigns, as these campaigns only reflect the DOM composition of one specific time and date and not changes within a day, week or month.

This demonstrates that high-resolution (half-hourly to 8 hours) sampling campaigns are necessary to prove assumptions like the third hypothesis of this study. The results of this study clearly indicate that precipitation events preferentially mobilise humics from the surrounding soils while LMW neutrals fluxes appear not to be affected by precipitation. As heavy precipitation leads to increased transport from allochthonous sources the increase in terrestrial originated humics is expected. However, LMW neutrals are present in high abundances of topsoil's and may also be produced in-stream by degradation of higher molecular weight DOM, suggesting two main sources of LMW neutrals that could alternate between each other and leading to a constant transport of LMW neutrals downstream. Future work needs to establish the direct sources of LMW neutral compounds and establish the major degradation pathways of higher molecular weight substances.

Additionally, this temporal high-resolution data implies that the relationship between DOM and precipitation events is not linked to the intensity of the event but more likely to antecedent soil moisture conditions of the catchment and the event duration/frequency. As global warming is predicted to increase the frequency and duration of precipitation events [7] the mobilisation of humic substances from soils to headwaters is also expected to increase. This may enhance downstream transport of aliphatic and nitrogen-rich compounds which again can have large implications on the drinking water treatment due to enhanced browning of the water, as well as the health of the river system due to potential changes in the food webs.

7.1.4. Stability and reactivity of DOM over time

In general, most field-based geochemical studies may be biased in the need to conduct analyses that are ex-situ. The first research chapter of this PhD study aims to establish confidence in the other environmental datasets about the temporal stability of DOC and DOM compound groups in the river water.

This study shows that minimal preservation methods keep the overall DOM composition unchanged for up to 4 days, providing confidence in our sampling and analytical approach to investigate temporal and spatial dynamics of DOM using automatic water samplers.

Additionally, it was found that freezing water samples enhanced the preservation time of humic substances but led to decreasing contribution of the remaining compound groups. Pasteurisation was found to degrade humic substances during the heating process but appears to preserve LMW neutrals and biopolymers. Overall, no preservation method can be recommended to preserve DOM as each of the tested method showed clear deficiencies, so in general it is recommended to consider the best preservation method from study to study depending on which aspects of DOM were analysed.

While this study is helpful to ensure high standards for the sampling of DOM, it is intriguing to consider that these preservation artefacts may allow investigation into the fate of DOM in the environment.

7.2. Future work

The evidence presented in this study clearly shows that the large heterogeneity observed within the soils and waters of the river catchments requires a higher spatial and temporal resolution to adequately characterise the changing sources and supply of OM.

Based on the observations made in this study a main focus of future research needs to be on spatial variances in the DOM, including but not solely focusing on upstream-downstream dynamics of a river system and covering different soil classifications. Future research will also determine whether the patterns observed in Scottish rivers and headwaters are representative of other temperate catchments. If so, this will likely have significant implications on the overall carbon balance of temperate rivers and possibly the global carbon balance. Specifically the role of peat-podzol catchments in the carbon cycle needs further investigation as the soil data in this study indicated that these catchments have a large potential to release more carbon in the future [17].

This study was the first to extract soil organic matter from peatland and peat podzol catchment to qualify the extractable organic matter from soils with the LC-OCD. As the findings in this study give a new insight in the origin of the DOM fractions it would be recommended to directly link soil organic matter with leachable organic matter to improve our understanding about the transfer between soil organic matter and riverine dissolved organic matter.

While this project led to a number of interesting and new findings several challenges could not be solved, bringing up a number of questions that can be followed up in future studies:

i. *Which chemical compounds contribute to the different DOM groups and what physical and biological processes are impacting transformations within a group?*
ii. *To what extent are the DOM compound groups interlinked, and how do biotic and abiotic transformations connect these OM pools?*
iii. *Does the vegetation type play an important role (e.g., sphagnum mosses, grasses) in the compositional differences of DOM in rivers, in addition to soil type, and what is their resilience to climatic changes?*
iv. *What is the potential for LMW DOM to be converted to* CO_2 *during transport and what are the primary drivers (photochemical vs microbial)?*
v. *How important are LMW neutrals and other LMW molecules for the carbon balance of temperate river systems and the riverine ecosystems?*

To address the questions outlined, it is essential to use a comprehensive geochemical, microbiological and hydrological approach that utilises a complex suite of field measurements like rainfall, evapotranspiration, groundwater and surface water flow, in-situ analytic measurements like pH, salinity, electrical conductivity and coloured DOM together with advanced geochemical characterisation of the various OM pools and a hydrological model approach. In regard to the impact of environmental factors seasonal patterns in the geochemical and environmental data needs further investigation with a longer term (multi-annual) record of river discharge, DOC and DOM composition allowing for a more robust analysis of seasonal patterns.

Methodologically compound group-specific analyses are important to provide a better quantification of OM pools and new insights into the contribution of LMW compounds. Fourier transform ion cyclotron mass spectrometry (FT-ICR-MS) is one method that will be essential for advanced molecular characterisation of OM as it resolves the whole range of individual molecules from complex organic mixtures and provides molecular formulas for most of the resolved ions. Furthermore, the various soil OM fractions can differently immobilise organic carbon (OC) through the formation of organo-mineral complexes [372]. Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy can be utilised to identify the minerals and their changes among the different soil fractions and their role to alter the DOM transported to the rivers.

Additionally, laboratory work should focus on identifying appropriate end member signatures for useful biomarker proxies such as lignin phenols to determine the products of DOM degradations and the effect of microbial oxidation on the DOM. Furthermore, tailored incubation experiments are a practical way to investigate microbial uptake and turnover of LMW DOM under controlled laboratory conditions to better identify their role in the carbon cycling.

Moreover, improving the understanding of complex OM cycles and their interactions across ecosystem boundaries represents significant interdisciplinary challenges. While high-resolution monitoring can be invaluable in improving estimates of carbon fluxes and exports, there remains a need for greater cross-disciplinarily across the different facets of aquatic research. For examples, DOM dynamics in soils, although fundamentally different from those in aquatic ecosystems, previous studies have shown that transformations of labile carbon into refractory DOM also occur in soils receiving labile carbon sources such as through fall or root exudates.

Furthermore, recent studies provided interesting insights into the role of labile carbon sources in DOM production, potentially seeing a priming effect. Hence, more interaction is needed between aquatic and terrestrial biogeochemists in order to share techniques, results, and insights gained in the two environments. This interaction will also facilitate a better understanding of controls on DOM production, consumption, and export across whole landscapes and biomes.

Observational surveys have been and will continue to be essential, especially when they can describe important thresholds and non-linear relationships. Increasingly, researchers should use replicated experiments and models that can isolate key mechanisms and integrate complex and interacting effects. By integrating impact-oriented research with better predictions of future DOM loads, aquatic scientists will be able to provide policy-relevant science to help maintain valuable ecosystem services in the face of large-scale change in a major ecosystem driver.

In the end, this study is the foundation to prove that combining different research fields (hydrology, geochemistry, microbiology) and methods (field based, analytical, experimental, modelling) will be essential to fully understand the dynamics of OM in temperate river systems and their contribution to the global carbon cycle.

7.3. Final remarks

The results presented in this research offer an insight into the processes controlling the DOM dynamics in Scottish rivers and headwaters, and the impact of these processes on the DOM composition. The results of this study hopefully helped to emphasise the importance for future studies to consider riverine DOM dynamics in a smaller spatial and higher resolution temporal scale. In addition, this research may help to consider using not only traditional bulk and spectrophotometric measurements of DOM, as it was shown that they are not sufficient enough to improve our process understanding of DOM mobilisation in fluvial systems. Generally, this research showed that a combination of scientific methods and different research fields (hydrology, geochemistry, microbiology, soil science) has to be utilised to make informed decisions at local, regional and global scales about the carbon cycling of freshwater ecosystems. Overall, the scientific community will have to continue collecting field-based data from different ecosystems and utilise multiple disciplines.

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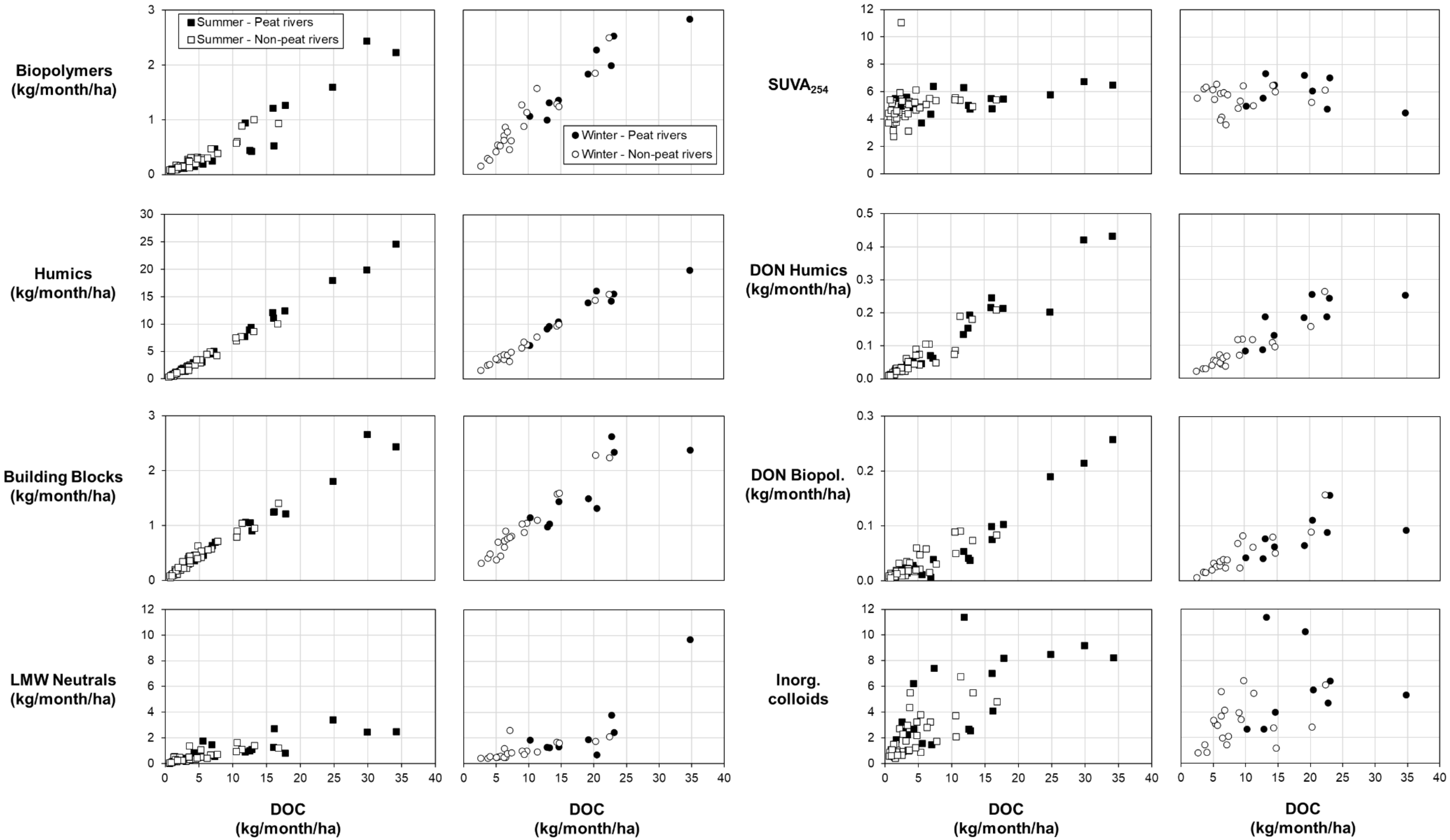
Supplementary material

Appendix 3.1: Minimum and Maximum concentration of DOC, biopolymers, humic substances, building blocks and LMW neutrals as well as DON from humics and biopolymers in parts per billion (ppb) from unpasteurised, pasteurised, unfrozen and frozen river water. Minimum and maximum SUVA values from the Bypass (SUVA254), hydrophilic DOC (SUVA CDOC), humic substances (SUVA HS) and building blocks (SUVA BB) in L/(mg*m). Deviation between untreated and treated river water samples in parts per billion (ppb) with negative values demonstrating a loss and positive values representing a gain after pasteurisation, -20 °C freezing or fast freezing.

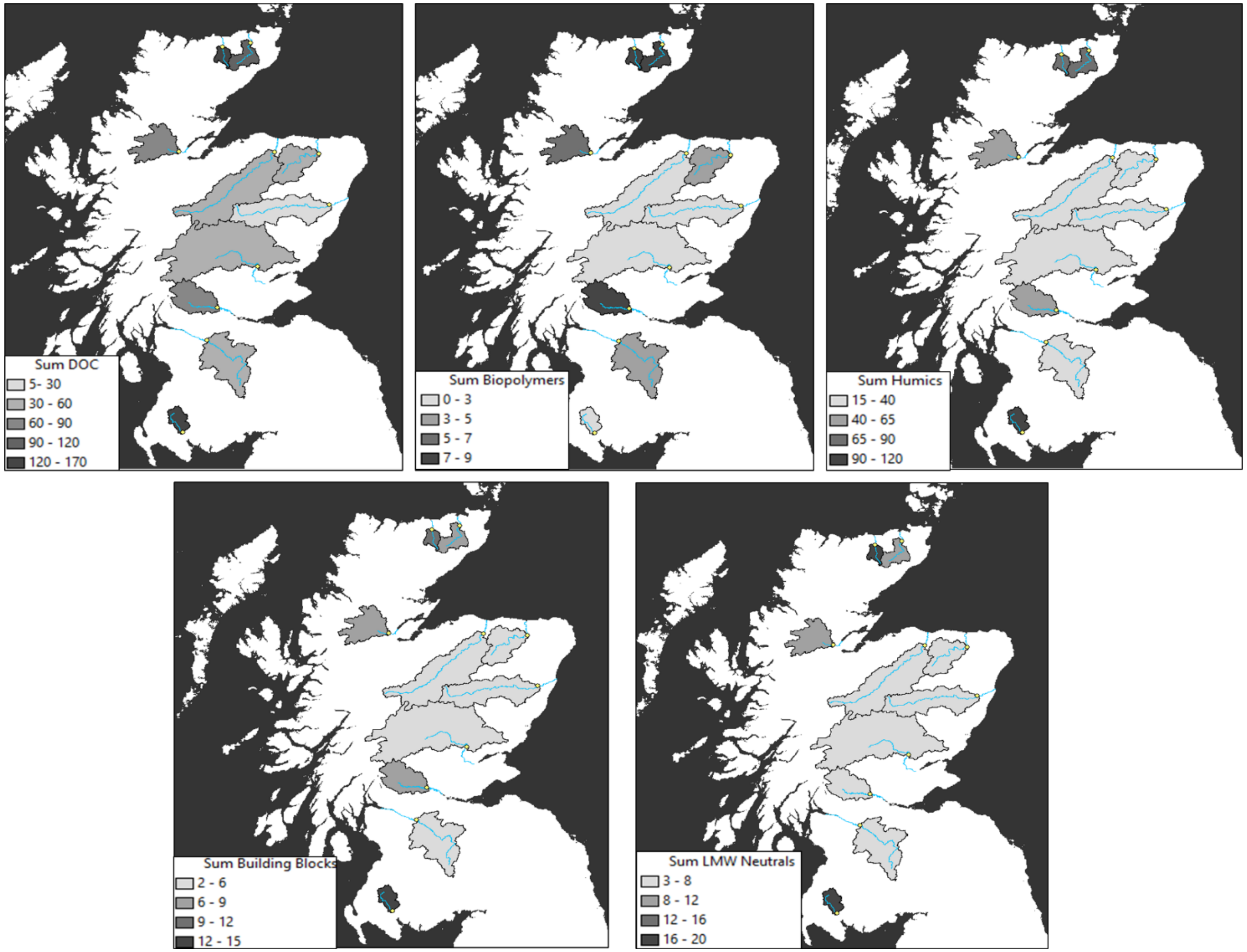
	Days	DOC	SUVA 254	SUVA CDOC	bio-polymers (BP)	DON BP	humic subst. (HS)	DON HS	SUVA HS	Mn	building blocks (BB)	SUVA BB	LMW neutrals	Nitrate
Unpasteurised														
Max	0	2786	6.5	5.2	37	28	2195	76	6.0	913	560	4.2	165	550
Min	2	2235	5.0	4.6	1	10	1712	62	5.2	575	468	3.1	92	465
Pasteurised														
Max	8	2749	5.9	5.7	48	30	2089	82	6.3	904	540	4.3	161	536
Min	32	2229	4.9	4.4	22	10	1661	57	5.2	565	483	3.3	100	478
Unfrozen														
Max	8	2670	5.4	4.9	109	16	2054	70	5.7	1022	557	4.3	216	459
Min	0	2558	4.4	4.1	74	9	1865	59	5.2	612	491	3.3	76	337
-20 °C frozen														
Max	8	2768	5.6	5.3	103	12	2063	74	5.8	1034	586	4.1	233	487
Min	2	2680	4.7	4.3	62	6	1904	58	4.8	615	516	2.8	177	401
Fast-frozen														
Max	8	3327	5.7	5.5	162	18	2263	74	5.7	1039	614	4.4	434	526
Min	32	2682	4.7	4.4	58	6	1887	60	5.0	611	501	3.0	221	405

Appendix 4.1: Maximum, minimum and average proportional distribution of LMW neutrals, humics and all UV amenable fractions (humics, building blocks and LMW acids)

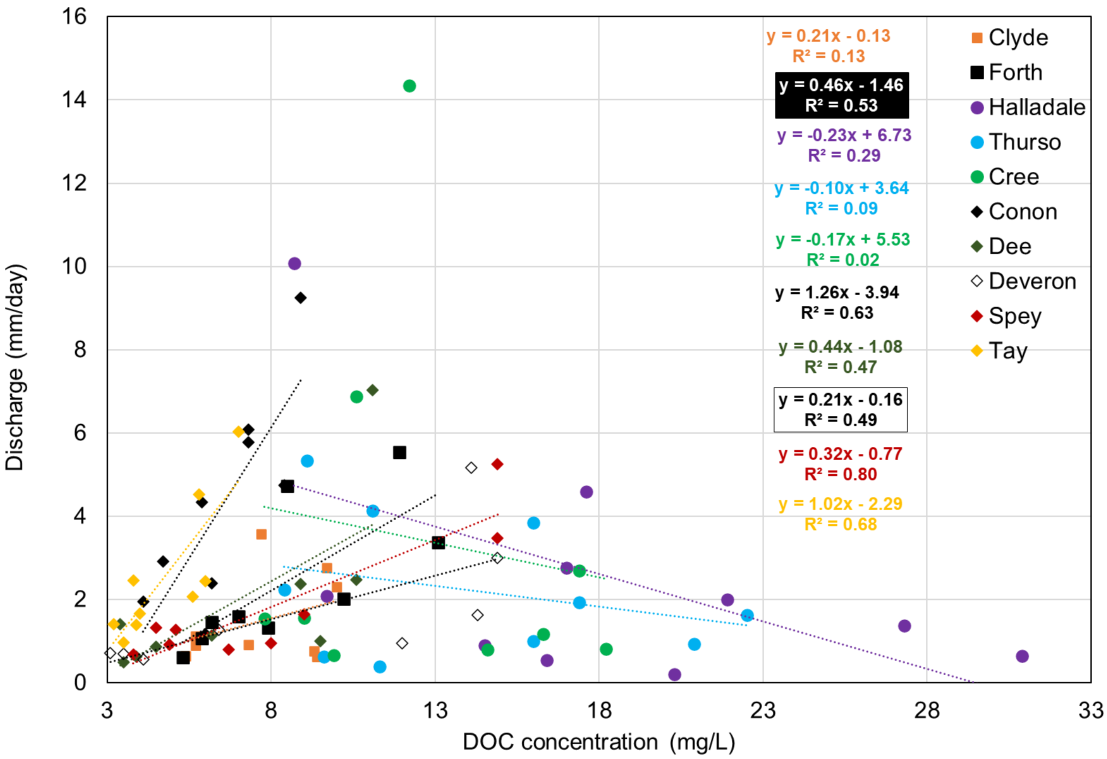
Rivers		%LMW neutrals	%humics	%UV amenable fractions
Clyde	Max	19.2	67.7	80.3
	Min	8	56	66.5
	Mean	11.2	63.2	75.2
Conon	Max	20.3	70.9	82.3
	Min	8.3	55.7	65.4
	Mean	11.9	65.5	75.6
Cree	Max	19.8	75.8	83.9
	Min	7.2	62.9	74.5
	Mean	11.8	69.3	78.4
Dee	Max	40.8	74	82.6
	Min	6.2	38.5	47.1
	Mean	18.3	55.9	66.4
Deveron	Max	15.7	71.6	81.7
	Min	6.5	51.8	59.6
	Mean	10.1	62.1	72
Forth	Max	22.8	72.3	81.6
	Min	7	60	68.8
	Mean	11.9	65.5	75.7
Halladale	Max	27.9	72.8	80.6
	Min	7.7	54.3	63.9
	Mean	14.9	67.3	76.2
Spey	Max	38.5	73.4	82.6
	Min	7.9	43	53
	Mean	13.6	65.9	75.2
Tay	Max	36.6	73.6	82
	Min	7.9	45.5	56.6
	Mean	15.4	61.8	73.1
Thurso	Max	31.9	79	85.5
	Min	3.4	56.2	64.5
	Mean	11.1	69.6	78.6



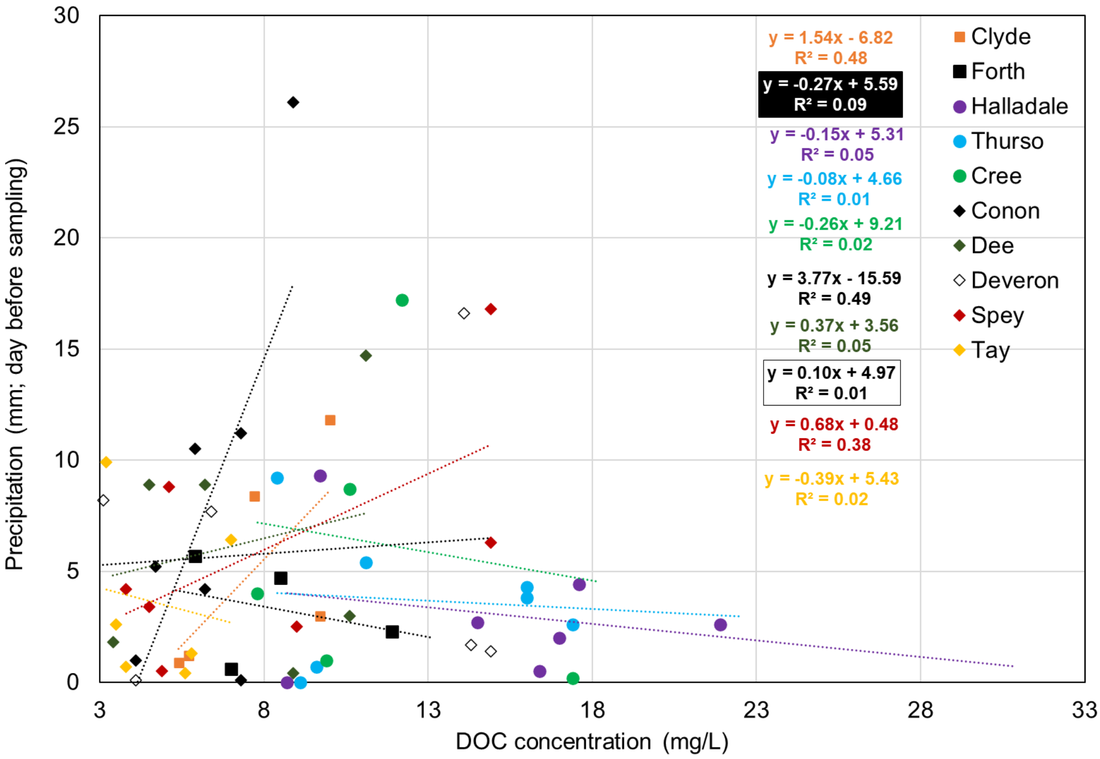
Appendix 4.2: DOC concentration versus biopolymers (BP), humics (HS), building blocks (BB), LMW neutrals (LMWN), SUVA$_{254}$, Dissolved organic nitrogen from humics (DON HS), Dissolved organic nitrogen from biopolymers (DON BP) and Inorganic colloids split in summer and winter data from peat and non-peat river group



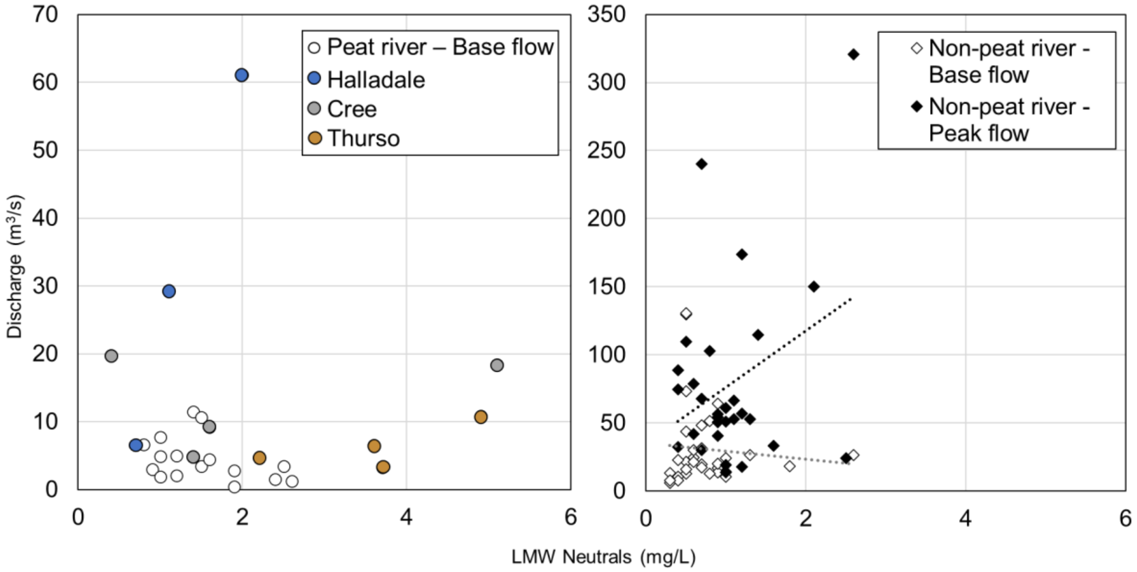
Appendix 4.3: Total kilogram per hectare of DOC, biopolymers, humics, building blocks and LMW neutrals measured in the ten Scottish rivers from April to December 2017.



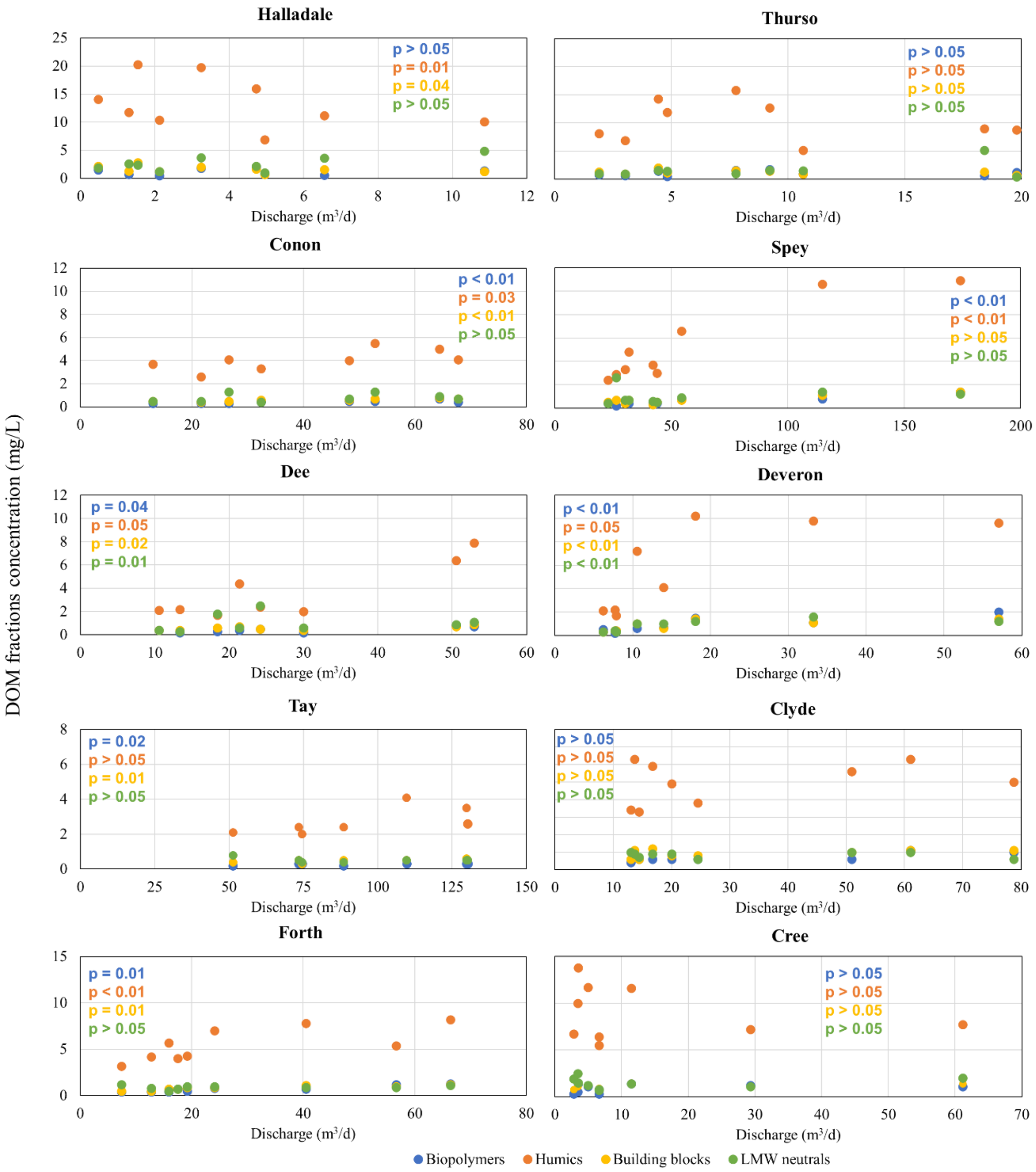
Appendix 4.4: Area normalised daily average discharge in millimetre per day (mm/day) versus bulk DOC concentration in milligram per litre (mg/L) of all 10 observed rivers with their respective linear correlation formula and R^2.



Appendix 4.5: Total daily precipitation 24 hours before sampling day versus DOC concentration in milligram per litre of all 10 observed rivers with their respective linear correlation formula and R^2.



Appendix 4.6: LMW Neutrals concentration in milligram per litre (mg/L) plotted against river discharge in cubic meter per seconds (m^3/s) from peat river and non-peat river during base flow and peak flow conditions, based on Baseflow Index analysis.



Appendix 4.7: Average daily discharge of sampling day versus humics, biopolymers, building blocks and LMW neutrals concentration from all ten observed Scottish rivers.

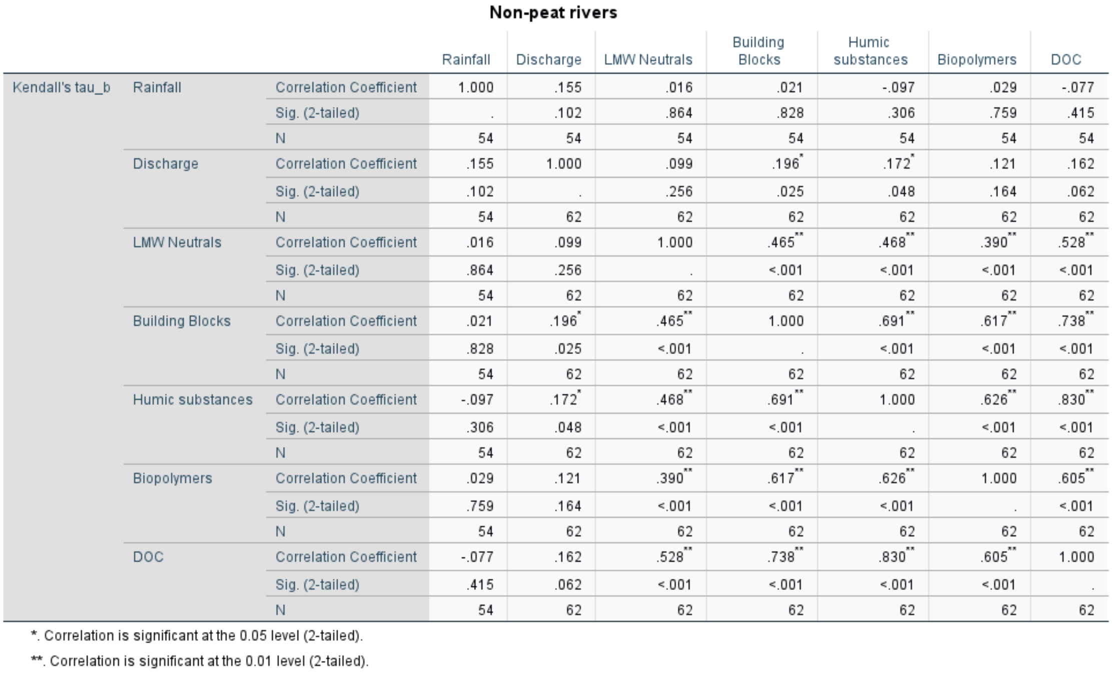
Appendix 4.8: Total precipitation from the 10 observed river catchments from 1961 to 2017, red fields show years with higher total precipitation than in year 2017.

Year	Clyde	Conon	Cree	Dee	Deveron	Forth	Halladale	Spey	Tay	Thurso
1961	1073	1596	1629	951	842	1846	971	1045	1477	888
1962	1183	1791	1690	981	1071	1581	1089	1133	1286	1037
1963	1064	1198	1565	1077	984	1506	913	1017	1232	931
1964	970	1489	1359	787	755	1460	936	864	1132	908
1965	1147	1526	1536	1004	1010	1606	1069	1132	1237	1054
1966	1128	1732	1685	1128	1094	1612	1121	1137	1391	1152
1967	1178	1854	1671	1047	992	1717	1296	1072	1451	1216
1968	1062	1308	1417	997	931	1480	883	1011	1174	907
1969	859	1439	1237	977	937	1297	947	859	1099	1046
1970	1065	1918	1615	1038	1118	1708	1170	1192	1281	1165
1971	837	1499	1180	818	683	1443	779	843	1096	737
1972	887	1155	1556	997	662	1515	737	849	1267	685
1973	776	1747	1287	835	852	1367	1065	917	1100	993
1974	1064	1719	1621	1218	960	1742	1016	1117	1521	1007
1975	881	1465	1398	921	824	1377	829	1020	1048	760
1976	940	1421	1491	1141	866	1682	871	898	1326	898
1977	1198	1623	1775	1205	1005	1641	1057	1023	1296	998
1978	970	1440	1528	1220	1045	1588	1035	1048	1405	1031
1979	1142	1716	1590	1169	995	1672	1194	1201	1293	1146
1980	1076	1773	1839	1127	1090	1621	1290	1157	1258	1128
1981	1098	1833	1880	1063	914	1681	1224	1067	1244	1146
1982	1227	1864	1964	1308	928	1864	1022	1227	1598	1045
1983	1057	1833	1618	1047	838	1690	1053	1065	1403	929
1984	1081	1586	1517	1163	1087	1601	1143	1137	1439	1147
1985	1156	1515	1534	1141	1168	1806	1158	1170	1404	1155
1986	1254	1889	1765	1075	829	2008	1110	1117	1566	1005
1987	1023	1387	1781	876	946	1486	1021	952	1153	992
1988	1184	1708	1979	1115	1005	1893	1076	1145	1516	1063
1989	1011	1844	1449	763	617	1711	871	991	1409	810
1990	1393	2297	1956	1087	938	2090	1261	1479	1724	1118
1991	1110	1661	1710	1042	788	1696	947	1072	1430	906
1992	1252	2146	1906	980	897	2036	1131	1102	1531	1091
1993	1112	1525	1684	1163	926	1746	999	1066	1509	998
1994	1285	1870	1758	1012	792	2042	1072	1063	1516	1015
1995	1072	1750	1591	1134	1116	1688	1149	1217	1382	1059
1996	884	1193	1631	839	847	1458	840	860	1113	895
1997	1072	1594	1547	1033	920	1652	1062	979	1401	983
1998	1293	1904	2002	1166	1119	1922	1359	1242	1597	1313
1999	1340	2133	1760	1105	1027	2056	1269	1273	1630	1149
2000	1324	1789	2100	1334	1155	1964	1199	1353	1598	1184
2001	973	1510	1517	1019	992	1343	1029	1048	1189	1044
2002	1288	1418	1977	1465	1119	1967	1059	1204	1669	1016
2003	891	1433	1423	692	701	1347	945	823	1044	858
2004	1263	2049	1799	1121	1089	1812	1157	1214	1530	1144
2005	1049	1897	1603	1029	1012	1743	1164	1181	1407	1171
2006	1261	1951	1838	1063	789	2005	1139	1154	1593	1203
2007	1133	1960	1637	1074	1033	1679	1092	1175	1441	1038
2008	1296	2035	2014	1093	1015	1904	1101	1279	1613	1045
2009	1220	1700	2154	1225	1155	1848	1176	1253	1541	1242
2010	868	1209	1556	1093	1153	1376	1004	1073	1132	1018
2011	1468	1999	2210	1151	882	2244	1078	1269	1866	1123
2012	1280	1635	2001	1087	1012	1820	1082	1060	1430	1059
2013	1123	1589	1747	977	752	1641	1046	944	1307	899
2014	1168	1823	1932	1379	1080	1956	1107	1214	1639	1089
2015	1353	1934	1888	1115	768	2370	1111	1273	1820	1074
2016	1105	1506	1511	1185	1001	1575	1122	991	1327	1060
2017	**1077**	**1568**	**1916**	**876**	**997**	**1528**	**1246**	**1012**	**1214**	**1223**

Appendix 4.9: Table showing the results from the Mann Kendall test for discharge and rainfall correlation for each of the ten rivers. Correlation coefficient indicating a positive or negative correlation, Sig. (2-tailed) representing the p-value with <0.01 being statistically significant and N being the number of data points.

Kendall tau_b		**Discharge**			**Rainfall**		
		Correlation Coefficient	Sig. (2-tailed)	N	Correlation Coefficient	Sig. (2-tailed)	N
Clyde	Discharge	1	.	275	.315**	<.001	223
	Rainfall	.315**	<.001	223	1	.	223
Conon	Discharge	1	.	275	.226**	<.001	238
	Rainfall	.226**	<.001	238	1	.	238
Cree	Discharge	1	.	275	.492**	<.001	232
	Rainfall	.492**	<.001	232	1	.	232
Dee	Discharge	1	.	275	.178**	<.001	226
	Rainfall	.178**	<.001	226	1	.	226
Deveron	Discharge	1	.	275	.263**	<.001	223
	Rainfall	.263**	<.001	223	1	.	223
Forth	Discharge	1	.	275	.332**	<.001	218
	Rainfall	.332**	<.001	218	1	.	218
Halladale	Discharge	1	.	275	.425**	<.001	227
	Rainfall	.425**	<.001	227	1	.	227
Spey	Discharge	1	.	275	.216**	<.001	243
	Rainfall	.216**	<.001	243	1	.	243
Tay	Discharge	1	.	275	.220**	<.001	238
	Rainfall	.220**	<.001	238	1	.	238
Thurso	Discharge	1	.	275	.338**	<.001	231
	Rainfall	.338**	<.001	231	1	.	231

Appendix 4.10: Results from the Mann Kendall (Kendall's tau_b) test for rainfall, discharge, as well as DOC, biopolymers, humics, building blocks and LMW neutrals concentration for peat rivers and non-peat rivers.

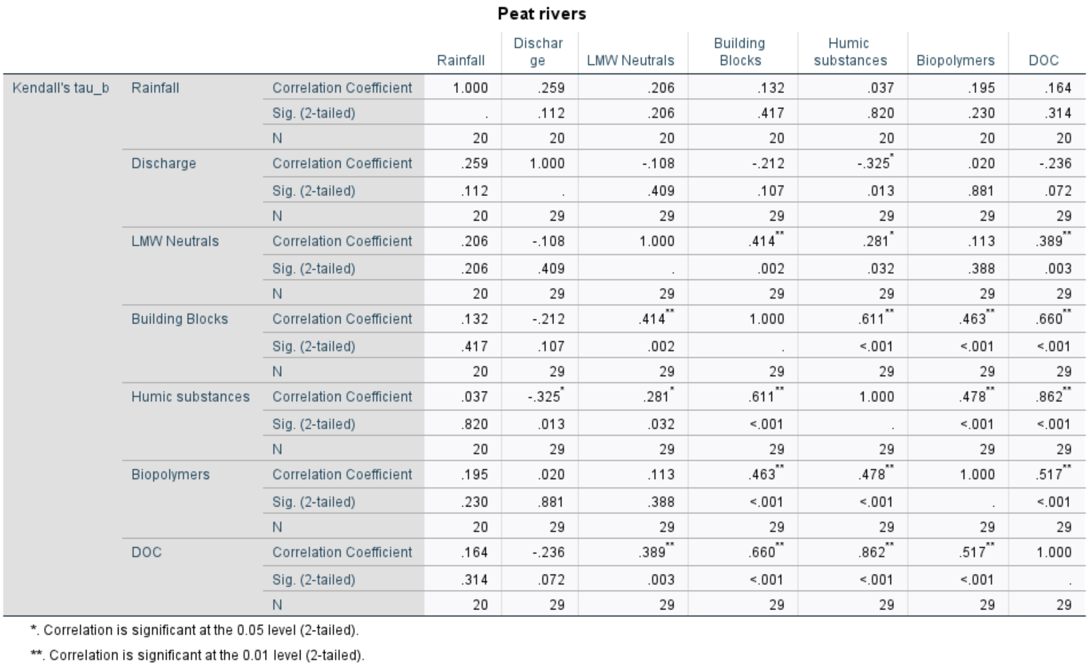


Non-peat rivers

			Rainfall	Discharge	LMW Neutrals	Building Blocks	Humic substances	Biopolymers	DOC
Kendall's tau_b	Rainfall	Correlation Coefficient	1.000	.155	.016	.021	-.097	.029	-.077
		Sig. (2-tailed)	.	.102	.864	.828	.306	.759	.415
		N	54	54	54	54	54	54	54
	Discharge	Correlation Coefficient	.155	1.000	.099	.196*	.172*	.121	.162
		Sig. (2-tailed)	.102	.	.256	.025	.048	.164	.062
		N	54	62	62	62	62	62	62
	LMW Neutrals	Correlation Coefficient	.016	.099	1.000	.465**	.468**	.390**	.528**
		Sig. (2-tailed)	.864	.256	.	<.001	<.001	<.001	<.001
		N	54	62	62	62	62	62	62
	Building Blocks	Correlation Coefficient	.021	.196*	.465**	1.000	.691**	.617**	.738**
		Sig. (2-tailed)	.828	.025	<.001	.	<.001	<.001	<.001
		N	54	62	62	62	62	62	62
	Humic substances	Correlation Coefficient	-.097	.172*	.468**	.691**	1.000	.626**	.830**
		Sig. (2-tailed)	.306	.048	<.001	<.001	.	<.001	<.001
		N	54	62	62	62	62	62	62
	Biopolymers	Correlation Coefficient	.029	.121	.390**	.617**	.626**	1.000	.605**
		Sig. (2-tailed)	.759	.164	<.001	<.001	<.001	.	<.001
		N	54	62	62	62	62	62	62
	DOC	Correlation Coefficient	-.077	.162	.528**	.738**	.830**	.605**	1.000
		Sig. (2-tailed)	.415	.062	<.001	<.001	<.001	<.001	.
		N	54	62	62	62	62	62	62

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).



Peat rivers

			Rainfall	Discharge	LMW Neutrals	Building Blocks	Humic substances	Biopolymers	DOC
Kendall's tau_b	Rainfall	Correlation Coefficient	1.000	.259	.206	.132	.037	.195	.164
		Sig. (2-tailed)	.	.112	.206	.417	.820	.230	.314
		N	20	20	20	20	20	20	20
	Discharge	Correlation Coefficient	.259	1.000	-.108	-.212	-.325*	.020	-.236
		Sig. (2-tailed)	.112	.	.409	.107	.013	.881	.072
		N	20	29	29	29	29	29	29
	LMW Neutrals	Correlation Coefficient	.206	-.108	1.000	.414**	.281*	.113	.389**
		Sig. (2-tailed)	.206	.409	.	.002	.032	.388	.003
		N	20	29	29	29	29	29	29
	Building Blocks	Correlation Coefficient	.132	-.212	.414**	1.000	.611**	.463**	.660**
		Sig. (2-tailed)	.417	.107	.002	.	<.001	<.001	<.001
		N	20	29	29	29	29	29	29
	Humic substances	Correlation Coefficient	.037	-.325*	.281*	.611**	1.000	.478**	.862**
		Sig. (2-tailed)	.820	.013	.032	<.001	.	<.001	<.001
		N	20	29	29	29	29	29	29
	Biopolymers	Correlation Coefficient	.195	.020	.113	.463**	.478**	1.000	.517**
		Sig. (2-tailed)	.230	.881	.388	<.001	<.001	.	<.001
		N	20	29	29	29	29	29	29
	DOC	Correlation Coefficient	.164	-.236	.389**	.660**	.862**	.517**	1.000
		Sig. (2-tailed)	.314	.072	.003	<.001	<.001	<.001	.
		N	20	29	29	29	29	29	29

*. Correlation is significant at the 0.05 level (2-tailed).

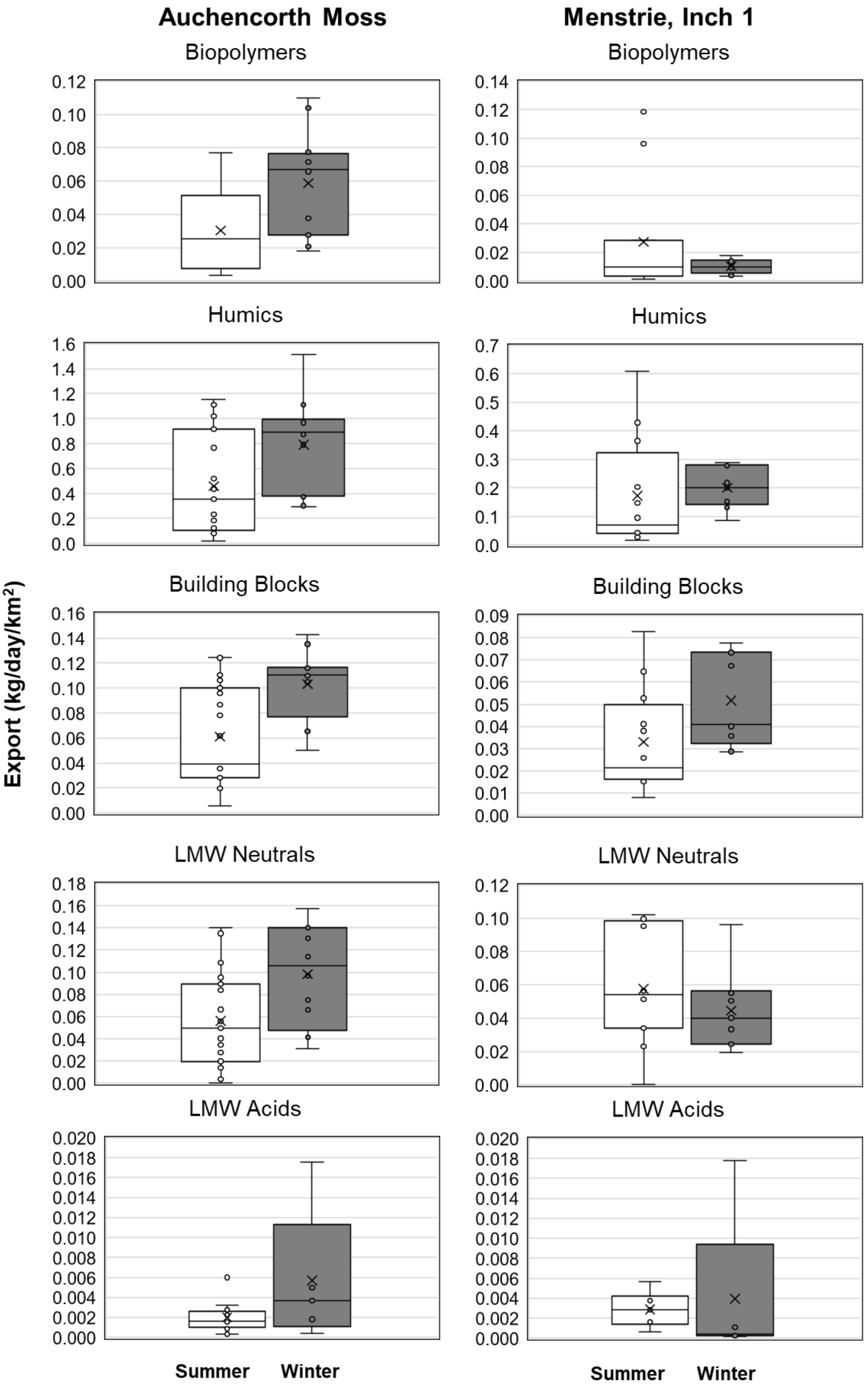
**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 4.11: Data table with concentrations (ppm) and fluxes (tonnes/month) for DOC, biopolymers, DON biopolymers, humics, DON humics, building blocks and LMW neutrals, as well as C/N ratios from humics and biopolymers, Inorganic colloids and specific UV absorbance at 254 nm ($SUVA_{254}$) from all ten rivers from April to December.

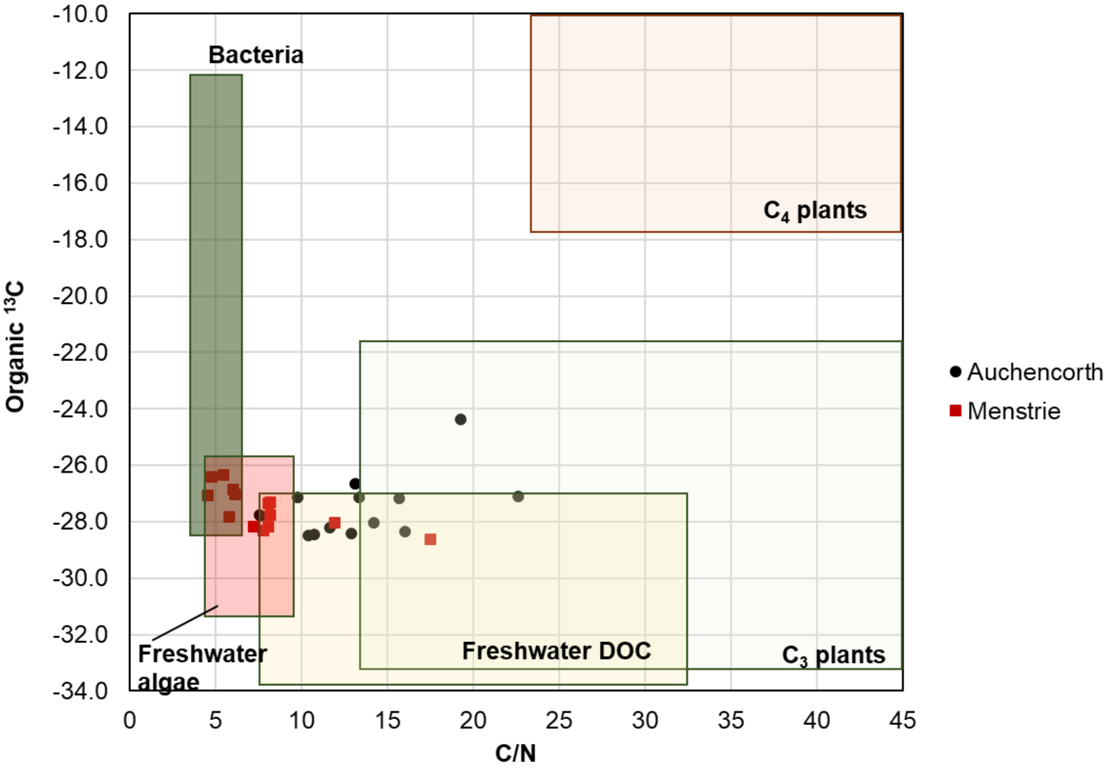
	DOC		biopolymers		DON biopolymers		C/N	humics		DON humics		C/N	building blocks		LMW neutrals		Inorg. Colloids	$SUVA_{254}$
	ppm	t/month	ppm	t/month	ppm	t/month	ug/ug	ppm	t/month	ppm	t/month	ug/ug	ppm	t/month	ppm	t/month	(m-1)	L/(mg*m)
River Clyde																		
24/04/2017	5.43	267.05	0.38	18.69	0.05	2.66	6.97	3.39	166.72	0.07	3.50	47.65	0.58	28.52	1.04	51.15	1.21	3.69
22/05/2017	5.74	196.88	0.58	19.89	0.05	1.55	12.75	3.27	112.16	0.07	2.53	44.36	0.55	18.86	0.66	22.64	0.81	4.35
19/06/2017	9.33	904.59	0.63	61.08	0.12	11.37	5.39	5.89	571.07	0.18	17.21	33.19	1.23	119.26	0.93	90.17	3.26	4.68
18/07/2017	9.38	632.46	0.80	53.94	0.10	6.74	8.00	6.31	425.46	0.18	11.89	35.81	1.13	76.19	0.95	64.06	3.00	5.07
23/08/2017	9.99	1006.36	0.57	57.42	0.09	9.00	6.43	5.60	564.13	0.14	14.28	39.50	1.02	102.75	0.98	98.72	3.81	4.90
19/09/2017	7.29	711.93	0.61	59.57	0.06	6.32	9.45	4.94	482.43	0.09	9.09	53.01	0.78	76.17	0.86	83.99	5.50	5.29
25/10/2017	9.71	1842.15	1.14	216.28	0.08	15.68	13.77	6.28	1191.42	0.12	22.58	52.72	1.05	199.20	1.00	189.72	6.46	6.45
20/11/2017	7.70	1218.18	1.04	164.53	0.04	5.84	28.27	4.96	784.70	0.05	8.13	96.60	1.08	170.86	0.61	96.51	1.97	4.16
11/12/2017	5.70	1003.12	0.59	103.83	0.03	6.13	16.99	3.77	663.47	0.06	10.37	64.06	0.76	133.75	0.57	100.31	3.11	5.47
River Conon																		
26/04/2017	6.24	509.00	0.32	26.10	0.02	2.04	12.80	4.14	337.70	0.05	4.13	81.64	0.50	40.79	1.27	103.59	0.87	4.83
24/05/2017	4.13	227.65	0.27	14.88	0.02	0.92	16.03	2.64	145.52	0.04	2.13	68.07	0.38	20.95	0.52	28.66	0.96	5.95
21/06/2017	5.97	254.40	0.28	11.93	0.03	1.45	8.32	3.74	159.37	0.06	2.77	57.56	0.52	22.16	0.50	21.31	0.95	4.35
20/07/2017	4.69	329.24	0.35	24.57	0.03	2.22	11.22	3.26	228.85	0.05	3.44	66.61	0.60	42.12	0.45	31.59	1.04	5.19
21/08/2017	7.28	743.68	0.37	37.80	0.03	2.93	12.83	4.05	413.72	0.05	4.79	86.48	0.67	68.44	0.68	69.46	1.74	5.35
18/09/2017	8.40	1022.23	0.48	58.41	0.04	4.83	12.04	5.48	666.89	0.07	8.34	79.96	0.71	86.40	1.29	156.99	2.09	5.59
24/10/2017	7.33	1375.86	0.66	123.88	0.04	7.73	15.91	4.95	929.13	0.06	10.54	88.24	0.81	152.04	0.87	163.30	2.77	6.47
22/11/2017	8.94	1947.65	0.82	178.64	0.04	8.59	20.88	6.34	1381.22	0.07	15.14	91.17	1.01	220.04	0.77	167.75	2.85	5.26
13/12/2017	5.90	1412.12	0.50	119.67	0.02	4.91	24.13	4.02	962.15	0.04	9.28	103.73	0.64	153.18	0.65	155.57	1.18	6.01
River Cree																		
24/04/2017	9.86	156.92	0.34	5.41	0.05	0.86	6.28	6.73	107.11	0.13	1.99	53.83	0.83	13.21	1.95	31.03	2.68	4.87
22/05/2017	7.79	122.89	0.34	5.36	0.03	0.52	10.42	5.52	87.08	0.11	1.81	47.99	0.70	11.04	0.73	11.52	2.26	5.62
19/06/2017	14.55	589.84	0.48	19.46	0.07	2.76	7.00	10.04	407.01	0.22	9.00	45.20	1.13	45.81	2.46	99.73	4.10	4.77
18/07/2017	18.23	585.44	1.38	44.32	0.11	3.63	12.23	13.82	443.82	0.25	7.94	55.90	1.42	45.60	1.45	46.57	7.02	5.53
21/08/2017	17.41	1094.43	1.42	89.26	0.13	7.88	11.29	11.58	727.94	0.25	15.44	47.14	1.55	97.44	1.44	90.52	9.20	6.76
19/09/2017	16.26	1252.16	1.06	81.63	0.12	9.45	8.65	11.72	902.54	0.21	15.86	56.87	1.16	89.33	1.18	90.87	8.25	6.49
25/10/2017	10.64	844.40	1.17	92.85	0.07	5.74	16.16	7.17	569.02	0.11	8.94	63.67	1.08	85.71	1.13	89.68	6.41	7.03
20/11/2017	12.18	830.31	1.07	72.94	0.05	3.25	22.49	7.66	522.18	0.10	6.88	75.87	1.41	96.12	2.03	138.38	4.72	4.76
11/12/2017	8.98	533.47	0.84	49.90	0.04	2.30	21.77	6.43	381.99	0.08	4.77	80.11	0.89	52.87	0.82	48.71	3.98	6.50
River Dee																		
25/04/2017	4.51	254.26	0.30	16.91	0.03	1.46	11.44	1.74	98.09	0.03	1.91	51.20	0.64	36.08	1.84	103.73	0.52	2.75
23/05/2017	6.25	239.05	0.48	18.36	0.04	1.44	12.75	2.43	92.94	0.06	2.41	38.45	0.51	19.51	2.51	96.00	0.93	3.16
20/06/2017	3.90	319.84	0.24	19.68	0.04	3.51	5.65	2.19	179.60	0.07	5.72	31.40	0.39	31.98	0.35	28.70	1.06	3.78
19/07/2017	3.50	162.74	0.41	19.06	0.05	2.55	7.40	2.11	98.11	0.05	2.54	38.55	0.42	19.53	0.43	19.99	1.07	3.99
21/08/2017	9.51	565.98	0.40	23.81	0.03	1.79	13.26	4.36	259.48	0.07	4.40	58.95	0.69	41.06	0.59	35.11	1.76	4.18
17/09/2017	10.62	1235.69	0.75	87.27	0.02	2.83	30.77	7.86	914.55	0.17	19.45	47.03	0.91	105.88	1.10	127.99	3.26	5.53

	DOC		**biopolymers**		**DON biopolymers**		**C/N**	**humics**		**DON humics**		**C/N**	**building blocks**		**LMW neutrals**		**Inorg. Colloids**	**SUVA$_{254}$**
	ppm	**t/month**	**ppm**	**t/month**	**ppm**	**t/month**	**ug/ug**	**ppm**	**t/month**	**ppm**	**t/month**	**ug/ug**	**ppm**	**t/month**	**ppm**	**t/month**	**(m-1)**	**L/(mg*m)**
23/10/2017	8.90	1030.98	0.83	96.15	0.04	4.74	20.26	6.38	739.06	0.08	9.53	77.46	0.71	82.25	0.95	110.05	2.96	6.57
21/11/2017	11.06	1142.40	1.27	131.18	0.05	5.09	25.70	6.41	662.10	0.08	8.68	76.30	1.29	133.25	2.08	214.85	3.70	3.95
12/12/2017	3.39	482.95	0.20	28.49	0.01	1.11	25.95	2.03	289.20	0.03	3.84	75.50	0.41	58.41	0.55	78.36	0.83	5.55
River Deveron																		
26/04/2017	6.41	150.70	0.70	16.46	0.05	1.26	12.95	4.13	97.09	0.10	2.32	41.78	0.57	13.40	1.01	23.74	1.54	4.02
24/05/2017	4.07	63.34	0.48	7.47	0.03	0.53	14.02	2.11	32.83	0.06	0.91	35.88	0.32	4.98	0.27	4.20	0.87	4.45
21/06/2017	3.48	174.81	0.21	10.55	0.03	1.66	6.49	2.17	109.01	0.06	3.13	34.85	0.44	22.10	0.42	21.10	0.71	4.11
20/07/2017	3.14	60.55	0.43	8.29	0.05	0.89	9.38	1.65	31.82	0.05	1.03	31.02	0.40	7.71	0.33	6.36	0.58	3.74
21/08/2017	11.99	349.08	0.60	17.47	0.07	1.90	9.26	7.21	209.91	0.17	4.85	43.29	1.04	30.28	1.03	29.99	4.35	5.09
18/09/2017	14.93	1266.60	1.14	96.71	0.08	7.09	13.63	9.81	832.24	0.20	17.38	47.86	1.08	91.62	1.61	136.59	5.53	4.94
24/10/2017	14.27	596.24	1.45	60.59	0.08	3.36	18.05	10.22	427.02	0.16	6.87	62.19	1.39	58.08	1.17	48.89	5.59	5.91
22/11/2017	14.12	1082.23	1.98	151.76	0.08	5.92	25.69	9.60	735.80	0.15	11.35	64.83	1.38	105.77	1.15	88.14	5.46	5.01
River Forth																		
25/04/2017	5.33	222.57	0.41	17.12	0.08	3.31	5.14	3.22	134.46	0.06	2.70	49.83	0.47	19.63	1.21	50.53	2.73	4.50
23/05/2017	5.91	131.54	0.45	10.02	0.03	0.76	13.01	4.16	92.59	0.09	2.03	45.47	0.54	12.02	0.77	17.14	1.51	5.34
20/06/2017	7.87	641.96	0.38	31.00	0.07	6.02	5.21	5.69	464.13	0.13	10.85	42.77	0.71	57.91	0.55	44.86	2.81	5.07
17/07/2017	7.04	365.81	0.48	24.94	0.04	2.28	10.98	4.26	221.35	0.11	5.49	40.29	0.88	45.73	1.03	53.52	1.00	4.41
21/08/2017	13.08	1723.65	0.73	96.20	0.07	8.63	11.14	7.85	1034.45	0.16	21.47	48.16	1.10	144.96	0.95	125.19	4.82	5.42
17/09/2017	10.20	1164.35	0.80	91.32	0.08	9.36	9.76	6.99	797.92	0.17	19.50	40.92	0.94	107.30	1.02	116.43	6.78	5.38
23/10/2017	11.86	2297.30	1.33	257.62	0.08	16.18	15.88	8.20	1588.36	0.14	27.20	58.35	1.19	230.51	1.12	216.95	6.13	6.13
21/11/2017	8.50	922.04	1.21	131.25	0.06	7.04	18.58	5.37	582.51	0.11	12.14	48.00	0.98	106.31	0.95	103.05	3.95	4.81
12/12/2017	6.16	690.32	0.72	80.69	0.04	4.02	20.16	4.01	449.38	0.06	6.37	70.53	0.70	78.44	0.73	81.81	4.15	5.96
River Halladale																		
26/04/2017	16.96	134.08	0.61	4.82	0.02	0.13	36.51	11.18	88.38	0.17	1.38	63.93	1.57	12.41	3.60	28.46	1.49	4.37
24/05/2017	16.38	53.09	0.70	2.27	0.08	0.26	8.87	11.79	38.21	0.17	0.57	67.51	1.30	4.21	2.59	8.39	2.53	5.16
19/06/2017	14.49	243.00	0.51	8.55	0.05	0.81	10.49	10.36	173.74	0.18	2.99	58.03	1.22	20.46	1.17	19.62	2.68	5.01
19/07/2017	20.28	82.02	1.50	6.07	0.14	0.55	11.02	14.12	57.11	0.22	0.91	62.82	2.19	8.86	1.92	7.77	6.24	5.14
21/08/2017	30.93	229.47	2.46	18.25	0.14	1.03	17.62	20.27	150.39	0.35	2.61	57.67	2.77	20.55	2.37	17.58	11.39	6.31
24/09/2017	27.31	480.65	1.76	30.98	0.21	3.69	8.41	19.81	348.65	0.22	3.93	88.64	1.99	35.02	3.75	66.00	8.50	5.79
24/10/2017	21.95	370.97	2.11	35.66	0.07	1.25	28.57	15.97	269.90	0.21	3.58	75.36	1.71	28.90	2.16	36.51	10.26	7.22
20/11/2017	17.63	673.12	1.44	54.98	0.05	1.80	30.50	10.06	384.09	0.13	4.90	78.44	1.21	46.20	4.93	188.23	5.35	4.47
11/12/2017	9.66	248.13	0.75	19.26	0.03	0.79	24.47	6.89	176.98	0.07	1.67	105.69	0.74	19.01	0.96	24.66	2.66	5.56
River Spey																		
26/04/2017	5.10	487.26	0.39	37.26	0.03	2.67	13.81	3.72	355.41	0.07	6.75	52.75	0.33	31.53	0.65	62.10	0.92	4.50
24/05/2017	4.86	315.92	0.43	27.95	0.04	2.35	12.05	3.31	215.17	0.05	3.50	61.42	0.43	27.95	0.68	44.20	1.02	5.19
21/06/2017	6.74	1017.81	0.24	36.24	0.03	5.15	7.10	2.90	437.93	0.06	9.06	48.27	0.68	102.69	2.60	392.63	1.05	3.15
20/07/2017	3.80	278.88	0.38	27.89	0.04	2.95	9.55	2.42	177.60	0.05	3.71	47.93	0.48	35.23	0.39	28.62	1.07	4.65
21/08/2017	7.98	747.65	0.44	41.22	0.03	2.54	16.16	4.82	451.59	0.07	6.65	67.94	0.68	63.71	0.68	63.71	2.31	5.34
17/09/2017	14.88	3006.06	0.81	163.64	0.13	25.46	6.39	10.55	2131.32	0.11	21.41	99.59	1.12	226.26	1.36	274.75	3.75	5.43
24/10/2017	9.00	1427.29	0.76	120.53	0.04	5.75	21.03	6.60	1046.68	0.07	11.11	94.22	0.68	107.84	0.91	144.32	3.35	6.18

	DOC		biopolymers		DON biopolymers		C/N	humics		DON humics		C/N	building blocks		LMW neutrals		Inorg. Colloids	SUVA$_{254}$
	ppm	t/month	ppm	t/month	ppm	t/month	ug/ug	ppm	t/month	ppm	t/month	ug/ug	ppm	t/month	ppm	t/month	(m-1)	L/(mg*m)
22/11/2017	14.87	2639.43	1.42	252.05	0.04	6.71	37.61	10.86	1927.65	0.11	20.04	96.22	1.41	250.28	1.17	207.68	3.42	5.33
13/12/2017	4.49	1048.43	0.36	84.06	0.02	4.50	18.71	3.03	707.51	0.04	8.32	85.20	0.50	116.75	0.52	121.42	1.47	6.23
River Tay																		
25/04/2017	3.48	690.85	0.20	39.70	0.02	3.38	11.95	2.09	414.91	0.03	6.32	65.55	0.41	81.39	0.77	152.86	0.43	4.42
23/05/2017	3.20	418.94	0.28	36.66	0.02	2.51	14.39	2.02	264.46	0.04	5.07	52.15	0.29	37.97	0.38	49.75	0.62	5.43
20/06/2017	3.95	1160.83	0.23	67.59	0.03	8.10	8.27	2.38	699.44	0.05	15.68	44.71	0.54	158.70	0.43	126.37	0.65	11.06
19/07/2017	3.92	838.69	0.29	62.05	0.03	6.07	10.27	2.39	511.34	0.05	10.96	46.68	0.50	106.98	0.53	113.39	0.64	4.62
21/08/2017	5.99	2071.23	0.27	93.36	0.02	7.81	11.95	3.46	1196.40	0.06	21.10	56.68	0.61	210.93	0.47	162.52	1.24	5.23
17/09/2017	5.56	2168.21	0.34	132.59	0.02	9.11	14.42	4.10	1598.86	0.09	33.35	47.92	0.47	183.28	0.54	210.58	2.19	6.13
23/10/2017	5.77	3354.37	0.49	284.86	0.03	17.63	16.06	3.89	2261.44	0.05	30.78	73.51	0.64	372.06	0.68	395.32	2.12	5.81
21/11/2017	7.03	3236.73	0.46	211.79	0.02	10.96	19.25	3.20	1473.33	0.04	17.19	85.68	0.78	359.13	2.60	1197.08	1.45	3.60
12/12/2017	3.83	1837.05	0.25	119.91	0.01	7.04	17.08	2.58	1237.49	0.03	13.65	90.62	0.46	220.64	0.53	254.21	0.85	6.35
River Thurso																		
26/04/2017	16.02	230.46	0.57	8.20	0.03	0.48	17.06	9.00	129.47	0.14	1.95	66.48	1.33	19.13	5.11	73.51	1.59	3.72
24/05/2017	9.57	67.41	0.57	4.02	0.04	0.25	16.28	6.90	48.60	0.15	1.06	45.74	0.90	6.34	0.95	6.69	1.87	5.50
19/06/2017	16.03	530.59	0.54	17.87	0.05	1.54	11.61	11.85	392.23	0.24	8.02	48.94	1.14	37.73	1.40	46.34	2.56	4.73
19/07/2017	11.35	104.58	0.75	6.91	0.11	0.98	7.08	8.13	74.91	0.16	1.44	52.04	1.33	12.25	1.00	9.21	3.24	4.97
21/08/2017	20.93	302.72	1.36	19.67	0.11	1.61	12.20	14.35	207.55	0.18	2.64	78.51	2.01	29.07	1.62	23.43	7.41	6.42
24/09/2017	22.53	736.98	1.61	52.66	0.13	4.28	12.27	15.78	516.18	0.27	8.89	58.06	1.54	50.38	1.02	33.37	8.21	5.47
24/10/2017	17.44	545.59	1.74	54.43	0.10	3.18	17.08	12.69	396.99	0.25	7.75	51.23	1.37	42.86	1.64	51.31	11.40	7.35
20/11/2017	11.12	845.38	1.24	94.27	0.06	4.58	20.58	8.79	668.25	0.14	10.59	63.10	0.72	54.74	0.37	28.13	5.74	6.07
11/12/2017	8.41	421.45	0.88	44.10	0.03	1.75	25.27	5.05	253.07	0.07	3.43	73.86	0.95	47.61	1.53	76.67	2.66	4.96



Appendix 5.1: Box and Whisker plots of DOM fractions export for summer (April-September) and winter (October- March) samples from March 2017 to March 2019



Appendix 5.2: Soil C_{org}/N_{total} ratio plotted against organic ^{13}C from Menstrie (red squares) and Auchencorth Moss (black circles) soil samples. Values for organic matter sources were taken from Lamb et al. [2].

Appendix 7.3: Auchencorth Moss and Menstrie summer and winter sampling dates from 2017 and 2018 with their measured of DOC, biopolymers (BP), Dissolved organic nitrogen from biopolymers (DON BP), humics (HS), dissolved organic nitrogen from humics (DON HS) concentration in mg/L, humics aromaticity (SUVA HS), molecular weight from humics and LMW neutrals and LMW acids concentration in mg/L.

Auchencorth Moss										
Date	**DOC**	**BP**	**DON BP**	**HS**	**DON HS**	**SUVA HS**	**Mol-Weight**	**BB**	**LMW neutr.**	**LMW acids**
Summer										
23/05/2017	19.19	1.59	365.1	14.1	274	5.20	926	1.53	1.58	
26/06/2017	10.49	1.19	369.7	7.22	204	5.83	725	0.87	0.77	0.01
04/07/2017	46.57	2.86	164.7	36.1	745	6.04	869	3.41	3.51	0.10
05/07/2017	50.03	2.27	141.0	40.1	794	5.94	894	3.53	3.33	0.05
16/08/2017	59.67	2.70	135.3	40.9	617	6.57	934	4.91	3.82	0.09
28/08/2017	61.50	2.15	56.74	43.9	1021	6.92	952	4.89	4.27	0.09
15/09/2017	58.94	1.34	57.46	45.5	940	5.95	927	4.18	5.31	
26/09/2017	56.68	1.50	68.17	43.7	805	5.82	899	4.23	5.52	
03/04/2018	22.02	0.98	103.6	17.2	379	6.36	954	2.43	1.37	
24/05/2018	12.11	0.68	53.76	4.54	50	5.44	646	1.24		0.24
29/05/2018	7.33	0.25	31.39	3.77	119	5.61	685	1.25	1.94	0.11
31/05/2018	7.12	0.24	37.78	4.51	134	5.65	690	1.24	1.10	0.03
08/06/2018	22.93	2.01	221.0	14.1	357	6.16	817	3.08	3.75	
14/06/2018	13.74	0.99	157.0	9.10	220	5.15	564	1.40	2.19	0.06
19/06/2018	7.97	0.45	64.76	4.83	149	4.93	605	1.22	1.43	0.04
22/06/2018	28.46	0.76	82.88	20.5	468	5.81	807	3.95	3.30	
26/06/2018	1.55	0.15	12.64	0.88	23	6.09	763	0.23	0.14	
02/07/2018	6.25	0.30	63.69	4.14	140	5.65	701	1.11	0.70	
09/07/2018	5.03	0.24	28.47	3.42	79	5.37	654	0.82	0.55	
12/07/2018	4.69	0.13	12.32	3.22	91	4.87	607	0.77	0.57	
16/07/2018	5.87	0.42	56.11	3.74	115	5.26	633	0.90	0.80	0.01
28/08/2018	29.89	3.03	142.3	20.7	1000	6.04	1258	3.77	2.07	0.13
28/09/2018	38.79	1.21	205.1	30.2	857	5.99	1291	4.35	2.61	0.06
Winter										
11/10/2017	50.99	3.05	126.7	35.6	578	5.43	938	4.33	5.15	
12/10/2017	58.68	2.91	125.7	39.5	688	5.50	968	4.60	5.51	
13/10/2017	74.64	2.82	116.2	38.1	630	5.48	965	4.45	5.62	
20/10/2017	42.43	4.33	103.1	43.8	490	5.72	895	4.39	6.20	
30/10/2017	47.02	4.09	152.3	59.6	1102	6.52	886	5.32	5.53	0.07
02/11/2017	45.44	2.68	106.5	34.4	501	7.00	963	4.56	3.82	
29/01/2018	20.17	1.09	152.7	14.7	304	7.06	1075	2.57	1.62	0.15
13/02/2018	20.64	0.81	176.1	15.3	381	7.61	1096	2.65	1.64	0.20
23/03/2018	15.38	0.71	54.95	11.4	273	6.56	941	1.97	1.23	
06/11/2018	41.58	1.09	86.89	31.2	940	6.08	1114	4.13	4.49	0.69
09/11/2018	20.24	1.49	76.41	11.9	315	5.43	926	4.19	2.61	
13/11/2018	49.47	2.59	91.93	38.3	841	5.26	1148	5.63	2.95	0.02

Menstrie, Inch 1										
Date	**DOC**	**BP**	**DON BP**	**HS**	**DON HS**	**SUVA HS**	**Mol-Weight**	**BB**	**LMW neutr.**	**LMW acids**
Summer										
20/07/2017	3.16	0.12	0.01	2.12	75.54	5.79	777	0.43	0.37	0.03
17/08/2017	7.41	0.29	0.03	4.47	95.25	5.92	785	0.67	0.59	0.04
14/09/2017	6.05	0.23	0.02	3.80	99.14	5.05	730	0.55	1.05	
21/09/2017	9.18	0.10	0.05	6.35	159.1	4.79	729	0.86	1.04	
01/06/2018	1.21	0.01		0.45	20.56	5.12	660	0.17	0.53	0.03
02/06/2018	1.33	0.01		0.45	23.18	5.08	935	0.16	0.61	
26/06/2018	0.85	0.01		0.18	7.24	5.61	728	0.08		
29/06/2018	1.96	1.00		0.42	17.54	3.56	581	0.18	0.35	0.01
06/07/2018	1.84	0.05	0.01	0.49	19.69	4.25	545	0.18	1.06	0.06
12/07/2018	1.50	0.03		0.28	17.51	4.26	583	0.18	0.99	0.02
16/07/2018	1.68	0.04		0.99	37.34	5.39	700	0.27	0.24	
Winter										
19/10/2017	3.225	0.14	9.93	2.09	66.27	5.34	758	0.42	0.57	
09/11/2017	1.954	0.04		1.36	36.87	4.80	628	0.30	0.25	
07/12/2017	3.229	0.18	0.01	2.27	41.59	5.44	706	0.43	0.35	
25/01/2018	3.790	0.11	0.16	1.72	76.09	6.10	796	0.77	1.00	0.19
22/02/2018	1.422	0.04	0.02	0.88	35.26	5.22	678	0.30	0.20	
22/03/2018	2.313	0.10	0.01	1.58	52.63	5.56	724	0.37	0.25	
06/11/2018	4.337	0.08	20.71	2.96	123.8	5.81	1050	0.70	0.60	
09/11/2018	4.332	0.10	13.23	2.89	138.8	5.47	1000	0.81	0.52	0.01
13/11/2018	4.526	0.16	17.99	3.01	145.0	5.12	1095	0.76	0.42	

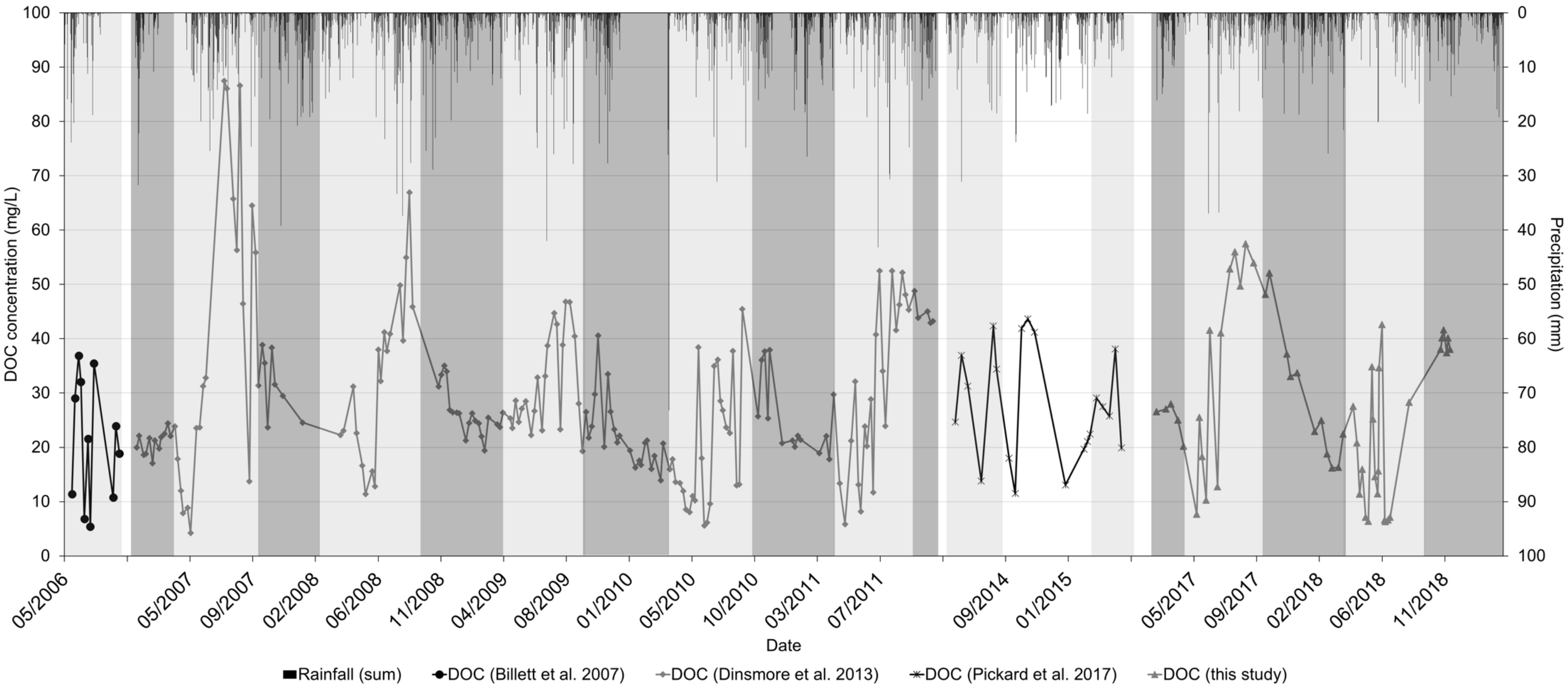
Location		Auchencorth Moss																	Menstrie, unplanted area							
Date		23/03/2018									11/03/2019								11/03/2019							
Depth	cm	0	6	10	30	35	45	50	65	80	0	10	15	25	30	35	40	60	0	10	20	25	30	45	50	66
weight	g	2.80	1.99	2.06	2.00	2.17	2.04	2.02	2.01	2.00	2.02	2.08	2.00	2.00	2.03	2.03	2.03	2.03	1.98	1.99	1.99	1.99	2.01	2.01	2.04	2.04
MilliQ	mL	22	22	22	20	22	22	20	22	20	22	22	22	22	22	22	20	20	22	22	22	22	22	22	22	22
DOC	ppm	773.0	311.6	32.8	15.5	54.2	48.1	48.5	34.8	112.5	665.5	599.4	143.7	152.6	25.8	26.6	64.1	52.0	535.2	521.6	253.7	232.0	57.0	63.7	23.4	21.8
	mg/g	12548.9	7118.5	723.5	386.7	1136.3	1072.4	1201.0	786.3	2811.7	14974.3	13099.7	3266.8	3468.3	578.1	596.1	1578.0	1281.3	12285.7	11915.0	5795.4	5299.2	1289.4	1441.4	520.5	485.4
biopolymers	ppm	92.4	44.5	1.6	0.7	6.5	6.1	2.3	2.7	6.9	44.1	28.7	20.7	17.3	1.8	2.0	3.7	3.7	35.0	32.8	25.3	17.6	5.9	5.8	2.5	2.3
	ppm/g*L	1500.5	1016.1	36.0	16.7	135.9	136.8	57.2	60.3	172.1	991.3	626.9	471.2	394.1	39.8	45.7	90.4	91.1	803.3	748.2	577.4	401.2	134.4	130.7	56.4	51.8
DON	ppb	3138.6	2531.7	155.3	652.8	798.2	1599.5	2495.8	273.5	578.9	2120.5	1172.7	1190.9	1262.9	128.2	156.5	n.q.	3257.4	3456.7	1755.0	1408.0	943.3	482.3	590.3	244.8	276.7
C/N		30.1	18.3	10.3	1.1	8.1	3.8	1.0	11.7	11.8	20.8	24.5	17.4	13.7	13.9	13.0	--	1.1	10.1	18.7	18.0	18.6	12.3	9.8	10.3	8.4
humics	ppm	284.3	123.3	13.8	3.9	24.6	25.8	17.9	12.1	33.4	92.7	91.0	32.9	39.2	8.7	8.4	21.4	21.0	111.2	105.6	64.3	63.5	21.5	21.5	9.6	9.3
	mg/g	4615.7	2817.0	303.9	97.4	515.3	575.7	443.5	274.4	834.0	2086.6	1987.9	748.2	889.8	195.8	188.2	527.6	517.5	2552.3	2412.2	1469.1	1449.6	486.4	487.1	214.0	206.2
DON	ppb	5898.5	2849.5	304.9	891.0	829.4	553.4	648.3	585.7	899.7	2510.8	2628.9	960.7	1042.5	216.9	218.6	7464.1	719.5	2694.3	3363.4	1771.7	1919.6	705.8	702.4	386.7	379.4
C/N		48.5	43.3	45.3	10.8	30.5	46.7	27.7	20.8	37.1	36.9	34.6	34.3	37.6	40.3	38.5	2.9	29.2	41.3	31.4	36.3	33.1	30.5	30.7	24.8	24.4
Aromaticity	L/(mg*m)	4.2	4.5	4.4	4.9	3.3	6.0	6.3	2.9	4.6	2.0	1.9	4.0	3.3	5.1	5.3	7.4	7.2	2.4	2.4	2.3	2.4	2.0	2.0	3.5	3.6
Mol-Weight	g/mol	856	743	663	649	612	758	762	607	782	656	616	740	756	759	729	868	872	713	719	748	843	655	699	618	681
building blocks	ppm	68.1	27.0	3.5	1.1	5.7	5.2	3.9	3.6	10.4	69.1	67.3	14.8	14.3	3.6	3.3	5.0	4.5	55.8	53.1	27.0	29.0	5.9	6.8	2.4	2.9
	mg/g	1105.0	617.8	76.5	27.9	120.1	116.1	96.6	82.4	258.8	1554.9	1470.0	335.7	324.3	79.5	73.7	122.1	110.6	1280.2	1213.4	615.6	661.3	133.2	154.7	53.2	64.1
LMW neutrals	ppm	52.8	18.3	2.8	1.5	6.4	3.7	3.6	4.1	16.1	222.4	218.2	34.9	35.9	5.2	5.2	3.8	3.1	157.3	150.1	41.1	39.6	11.0	11.1	2.8	2.8
	mg/g	857.9	417.4	62.1	36.3	134.6	81.7	89.2	93.2	401.9	5003.6	4769.3	792.6	815.7	116.1	116.9	94.7	77.3	3610.4	3429.1	937.7	905.6	249.6	250.6	62.8	61.3
LMW acids	ppm				0.2					1.0	24.9	23.5	2.7	2.3					24.2	24.3	6.4	6.2	0.1			
	mg/g				3.9					24.7	560.2	513.9	62.1	52.1					555.9	556.0	146.4	141.3	3.3			
TC	%w/w	33.9	33.3	2.0	7.1	7.2	19.5	1.0	2.6	1.0	43.9	43.9	29.4	29.4	1.7	1.7	1.2	1.2	39.4	39.4	9.6	9.6	4.9	4.9	2.1	2.1
TOC	%w/w	29.1	28.3	1.4	4.3	3.6	18.8	0.7	2.1	0.7	34.9	34.9	21.5	21.5	1.0	1.0	0.6	0.6	31.1	31.1	6.6	6.6	2.6	2.6	0.9	0.9
TN	%w/w	1.9	1.8	0.1	0.4	0.5	0.8	0.1	0.2	0.0	2.4	2.4	1.7	1.7	0.1	0.1	0.1	0.1	1.8	1.8	0.8	0.8	0.4	0.4	0.2	0.2
C/N		15.7	16.0	11.7	10.4	7.6	22.6	13.1	13.4	19.3	14.2	14.2	12.9	12.9	10.8	10.8	9.8	9.8	17.5	17.5	8.2	8.2	6.0	6.0	5.5	5.5
Bulk ^{13}C	$d^{13}C_{V\text{-}PDB}$ (‰)	-27.6	-28.2	-28.0	-28.4	-27.8	-27.2	-26.5	-27.1	-24.3	-28.1	-28.1	-28.4	-28.4	-28.1	-28.1	-27.2	-27.2	-28.7	-28.7	-27.2	-27.2	-26.8	-26.8	-26.4	-26.4
Organic ^{13}C	$d^{13}C_{V\text{-}PDB}$ (‰)	-27.2	-28.4	-28.2	-28.5	-27.8	-27.1	-26.7	-27.1	-24.4	-28.1	-28.1	-28.4	-28.4	-28.4	-28.4	-27.1	-27.1	-28.6	-28.6	-27.3	-27.3	-26.9	-26.9	-26.3	-26.3
Bulk ^{15}N	$d^{15}N_{air}$ (‰)	1.4	3.2	4.1	5.4	2.5	0.0	1.8	2.6	2.3	-0.1	-0.1	4.4	4.4	4.1	4.1	1.2	1.2	-1.0	-1.0	5.2	5.2	7.0	7.0	5.8	5.8

Appendix 5.4: All results from LC-OCD-OND analyses of the soil extracts and data from soil analysis by Iso Analytical, UK.

Appendix 5.5: Auchencorth Moss and Menstrie results from the 3-component UV absorbance carbon model by Carter et al. [98] and Adams et al. [172] including measured dissolved organic carbon (DOC), hydrophilic DOC and LMW neutrals (iDOM) concentrations from the LC-OCD-OND as well as measured UV absorbance values from 270 nm and 350 nm. The model improvements are: (1) measured LMW neutrals data used as third non-UV absorbing component and (2) LMW neutrals and the low-UV absorbing fraction of biopolymers used as the third component in the model.

	Measured values						Carter et al.				Adams et al.					Model improvements	
Field site	DOC (mg/l)	CDOC (mg/L)	measured iDOM	Abs 270nm	Abs 350nm	R	fA	E270 sample	E350 sample	modelled DOC	fB	fC	E270 sample	E350 sample	modelled DOC	modelled DOC (1)	modelled DOC (2)
Black Burn, Auchencorth Moss	19.19	17.19	1.58	286.09	105.45	2.71	0.56	45.59	16.80	7.08	0.14	0.30	42.46	17.13	7.54	8.32	9.92
	10.49	8.88	0.77	133.22	52.53	2.54	0.69	52.83	20.83	3.33	0.16	0.15	51.28	20.99	3.40	3.37	5.78
	48.56	43.15	4.92	372.23	134.54	2.77	0.53	43.92	15.87	9.28	0.14	0.33	40.42	16.24	10.01	14.13	12.39
	48.29	47.06	3.44	408.12	148.08	2.76	0.54	44.24	16.05	10.03	0.14	0.33	40.81	16.41	10.80	13.44	13.18
	58.68	54.35	6.20	226.88	83.62	2.71	0.56	45.59	16.80	5.78	0.14	0.30	42.46	17.13	6.15	11.54	8.52
	45.44	42.76	3.82	464.16	180.49	2.57	0.66	51.10	19.87	9.89	0.16	0.18	49.19	20.07	10.24	13.25	12.61
	20.17	19.08	1.62	204.13	75.60	2.70	0.57	46.02	17.04	5.24	0.14	0.29	42.99	17.36	5.55	6.37	7.93
	20.64	19.83	1.64	211.18	79.08	2.67	0.59	47.07	17.63	5.29	0.15	0.27	44.27	17.92	5.57	6.41	7.95
	15.38	14.67	1.23	142.51	53.82	2.65	0.60	47.89	18.08	3.78	0.15	0.25	45.28	18.36	3.95	4.38	6.33
	22.02	21.04	1.37	191.07	68.29	2.80	0.51	43.03	15.38	5.24	0.13	0.35	39.33	15.77	5.66	6.22	8.04
	17.64	11.42	10.11	84.61	39.05	2.17	1.39	90.11	41.58	1.74	0.20	-0.58	96.21	40.94	1.68	10.99	4.06
	6.88	7.08	1.81	57.84	24.75	2.34	0.95	66.63	28.52	1.67	0.18	-0.13	67.98	28.37	1.66	2.66	4.03
	6.63	6.89	1.02	66.27	28.43	2.33	0.96	67.21	28.84	1.79	0.18	-0.14	68.68	28.68	1.77	1.99	4.14
	22.35	20.92	3.49	206.77	91.11	2.27	1.09	73.94	32.58	3.60	0.18	-0.27	76.78	32.28	3.50	6.19	5.87
	12.80	12.75	2.13	130.58	57.80	2.26	1.11	75.21	33.29	2.54	0.19	-0.30	78.31	32.96	2.47	3.80	4.85
	7.44	7.52	1.33	102.04	46.75	2.18	1.33	87.06	39.88	1.98	0.19	-0.52	92.55	39.31	1.91	2.44	4.28
	27.76	27.70	3.07	289.04	103.80	2.78	0.52	43.41	15.59	7.46	0.14	0.34	39.79	15.97	8.07	10.33	10.44
	1.55	1.25	0.14	156.69	67.34	2.33	0.97	67.58	29.04	3.12	0.18	-0.15	69.12	28.88	3.07	2.41	5.44
	6.25	5.95	0.70	55.69	23.99	2.32	0.98	68.14	29.35	1.62	0.18	-0.16	69.80	29.18	1.60	1.50	3.98
	5.03	4.79	0.55	51.19	22.69	2.26	1.12	75.59	33.50	1.48	0.19	-0.30	78.77	33.17	1.45	1.20	3.83
	4.36	4.56	0.53	50.53	21.92	2.31	1.01	69.81	30.28	1.53	0.18	-0.19	71.81	30.07	1.51	1.23	3.88
	5.47	5.45	0.75	61.15	27.14	2.25	1.12	75.97	33.71	1.61	0.19	-0.31	79.23	33.37	1.58	1.52	3.95
	29.89	26.65	2.07	286.24	110.17	2.60	0.64	49.92	19.21	6.54	0.15	0.21	47.75	19.44	6.80	8.06	9.17

Field site	DOC (mg/l)	CDOC (mg/L)	measured iDOM	Abs 270nm	Abs 350nm	R	fA	E270 sample	E350 sample	modelled DOC	fB	fC	E270 sample	E350 sample	Modelled DOC	modelled DOC (1)	modelled DOC (2)
Inch 1, Menstrie	1.95	1.91	0.25	19.26	6.98	2.76	0.53	44.12	15.99	1.24	0.14	0.33	40.66	16.35	1.28	0.73	3.65
	3.23	3.05	0.35	30.14	10.99	2.74	0.54	44.65	16.28	1.48	0.14	0.32	41.31	16.63	1.53	1.08	3.91
	1.42	1.39	0.20	26.38	7.52	3.51	0.30	31.57	9.00	1.64	0.07	0.63	24.99	9.69	1.86	1.26	4.23
	2.31	2.21	0.25	22.39	6.46	3.47	0.31	31.97	9.23	1.50	0.08	0.62	25.50	9.90	1.68	1.13	4.06
	2.39	2.29	0.35	19.30	5.18	3.72	0.27	29.76	7.99	1.45	0.06	0.68	22.63	8.74	1.66	1.21	4.03
	1.65	1.18	0.78	5.94	2.11	2.82	0.50	42.47	15.07	0.94	0.13	0.37	38.64	15.47	0.96	0.93	3.33
	0.49	0.37	0.10	7.66	2.74	2.79	0.52	43.23	15.49	0.98	0.13	0.35	39.56	15.87	1.00	0.29	3.37
	1.83	0.96	0.34	6.33	2.15	2.94	0.45	39.60	13.47	0.96	0.12	0.43	35.09	13.94	0.98	0.52	3.36
	1.72	1.79	0.99	5.60	1.97	2.84	0.49	41.86	14.73	0.94	0.13	0.38	37.89	15.15	0.95	1.14	3.33
	1.54	1.46	0.92	6.13	2.38	2.58	0.66	50.79	19.70	0.93	0.15	0.19	48.81	19.90	0.93	1.05	3.30
	1.68	1.50	0.22	10.74	3.92	2.74	0.54	44.72	16.32	1.04	0.14	0.32	41.40	16.67	1.06	0.48	3.44

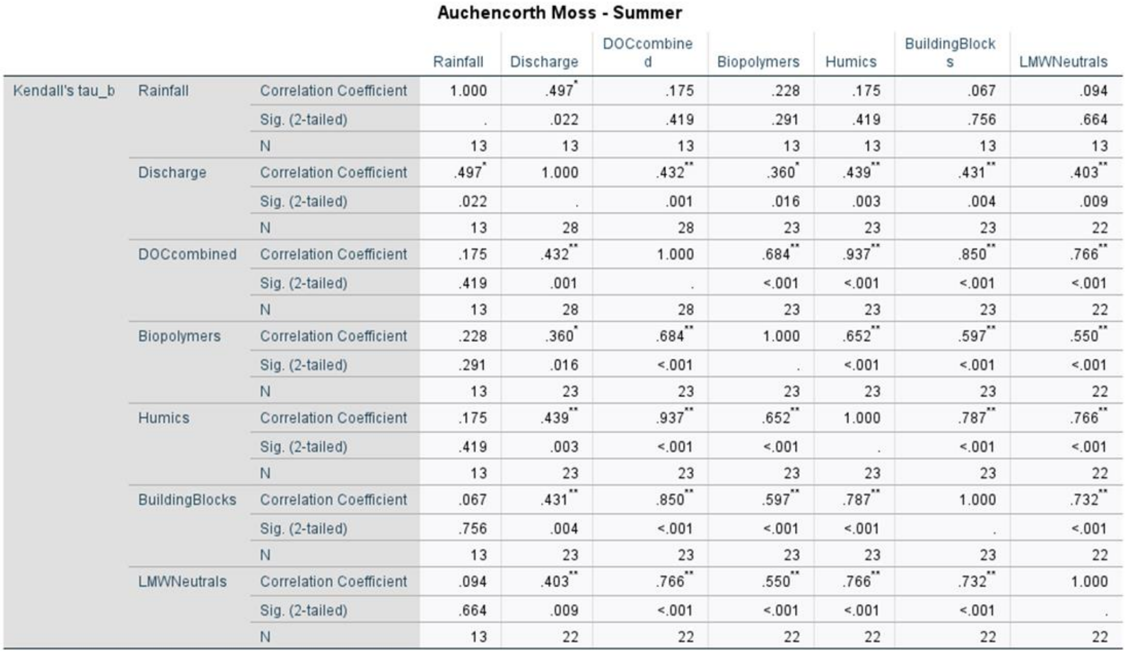


Appendix 5.6: Monthly-biweekly DOC concentration in Black Burn (Auchencorth Moss). The data from May 2006 to August 2006 is from Billett et al. [333], January 2007 to November 2011 from Dinsmore et al. [142], May 2015 to May 2016 from Pickard et al. [326] and February 2017 to March 2019 from this study. Precipitation in mm is from the Auchencorth Moss Atmospheric Observatory (AU) – CEDA Archive (The Natural Environment Research Council's Data Repository for Atmospheric Science and Earth Observation)

Appendix 5.7: Mann Kendall correlation results of pCO_2 Efflux ratio (partial pressure carbon dioxide in river water minus atmosphere) versus the four main DOC fractions for the peatland and peat-podzol headwater. Correlation coefficient representing R^2 and significance the p-value.

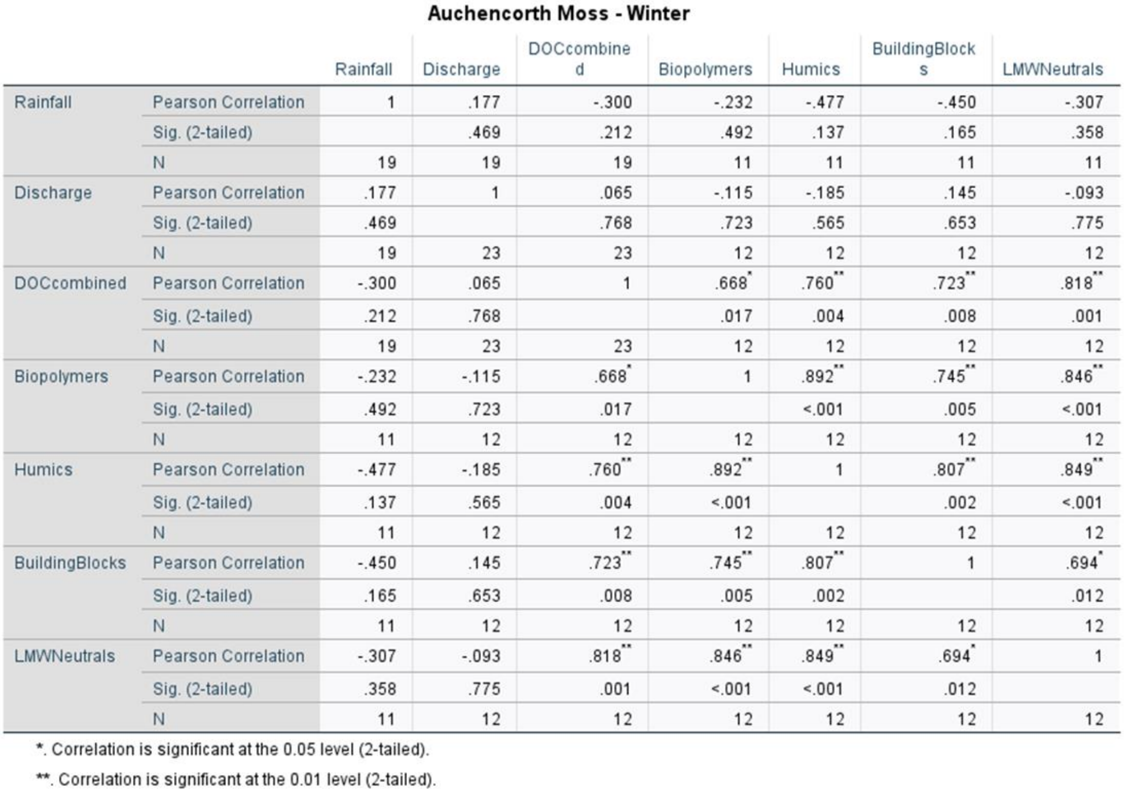
Peatland headwater	**pCO2 Efflux (river - atmosphere)**	
	Kendall's tau_b correlation coefficient	Significance
Biopolymers	0.133	0.471
Humics	0.25	0.177
Building Blocks	0.15	0.418
LMW neutrals	0.383	0.038
Peat-podzol headwater	**pCO2 Efflux (river - atmosphere)**	
	Kendall's tau_b correlation coefficient	Significance
Biopolymers	0.028	0.917
Humics	-0.028	0.917
Building Blocks	-0.479	0.075
LMW neutrals	-0.141	0.6

Appendix 5.8: Results from the Mann Kendall (Kendall's tau_b) test for rainfall, discharge, as well as DOC, biopolymers, humics, building blocks and LMW neutrals concentration for summer and winter data from Auchencorth Moss.



Auchencorth Moss - Summer

			Rainfall	Discharge	DOCcombined	Biopolymers	Humics	BuildingBlocks	LMWNeutrals
Kendall's tau_b	Rainfall	Correlation Coefficient	1.000	.497*	.175	.228	.175	.067	.094
		Sig. (2-tailed)	.	.022	.419	.291	.419	.756	.664
		N	13	13	13	13	13	13	13
	Discharge	Correlation Coefficient	.497*	1.000	.432**	.360*	.439**	.431**	.403**
		Sig. (2-tailed)	.022	.	.001	.016	.003	.004	.009
		N	13	28	28	23	23	23	22
	DOCcombined	Correlation Coefficient	.175	.432**	1.000	.684**	.937**	.850**	.766**
		Sig. (2-tailed)	.419	.001	.	<.001	<.001	<.001	<.001
		N	13	28	28	23	23	23	22
	Biopolymers	Correlation Coefficient	.228	.360*	.684**	1.000	.652**	.597**	.550**
		Sig. (2-tailed)	.291	.016	<.001	.	<.001	<.001	<.001
		N	13	23	23	23	23	23	22
	Humics	Correlation Coefficient	.175	.439**	.937**	.652**	1.000	.787**	.766**
		Sig. (2-tailed)	.419	.003	<.001	<.001	.	<.001	<.001
		N	13	23	23	23	23	23	22
	BuildingBlocks	Correlation Coefficient	.067	.431**	.850**	.597**	.787**	1.000	.732**
		Sig. (2-tailed)	.756	.004	<.001	<.001	<.001	.	<.001
		N	13	23	23	23	23	23	22
	LMWNeutrals	Correlation Coefficient	.094	.403**	.766**	.550**	.766**	.732**	1.000
		Sig. (2-tailed)	.664	.009	<.001	<.001	<.001	<.001	.
		N	13	22	22	22	22	22	22



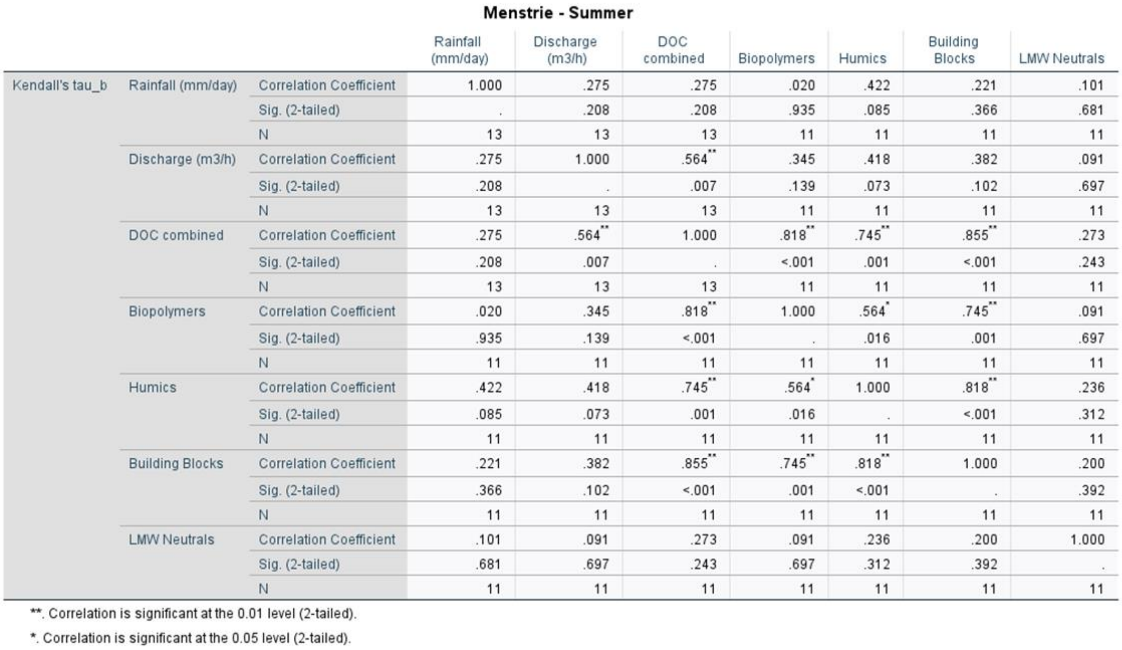
Auchencorth Moss - Winter

		Rainfall	Discharge	DOCcombined	Biopolymers	Humics	BuildingBlocks	LMWNeutrals
Rainfall	Pearson Correlation	1	.177	-.300	-.232	-.477	-.450	-.307
	Sig. (2-tailed)		.469	.212	.492	.137	.165	.358
	N	19	19	19	11	11	11	11
Discharge	Pearson Correlation	.177	1	.065	-.115	-.185	.145	-.093
	Sig. (2-tailed)	.469		.768	.723	.565	.653	.775
	N	19	23	23	12	12	12	12
DOCcombined	Pearson Correlation	-.300	.065	1	.668*	.760**	.723**	.818**
	Sig. (2-tailed)	.212	.768		.017	.004	.008	.001
	N	19	23	23	12	12	12	12
Biopolymers	Pearson Correlation	-.232	-.115	.668*	1	.892**	.745**	.846**
	Sig. (2-tailed)	.492	.723	.017		<.001	.005	<.001
	N	11	12	12	12	12	12	12
Humics	Pearson Correlation	-.477	-.185	.760**	.892**	1	.807**	.849**
	Sig. (2-tailed)	.137	.565	.004	<.001		.002	<.001
	N	11	12	12	12	12	12	12
BuildingBlocks	Pearson Correlation	-.450	.145	.723**	.745**	.807**	1	.694*
	Sig. (2-tailed)	.165	.653	.008	.005	.002		.012
	N	11	12	12	12	12	12	12
LMWNeutrals	Pearson Correlation	-.307	-.093	.818**	.846**	.849**	.694*	1
	Sig. (2-tailed)	.358	.775	.001	<.001	<.001	.012	
	N	11	12	12	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 5.9: Results from the Mann Kendall (Kendall's tau_b) test for rainfall, discharge, as well as DOC, biopolymers, humics, building blocks and LMW neutrals concentration for summer and winter data from Menstrie.

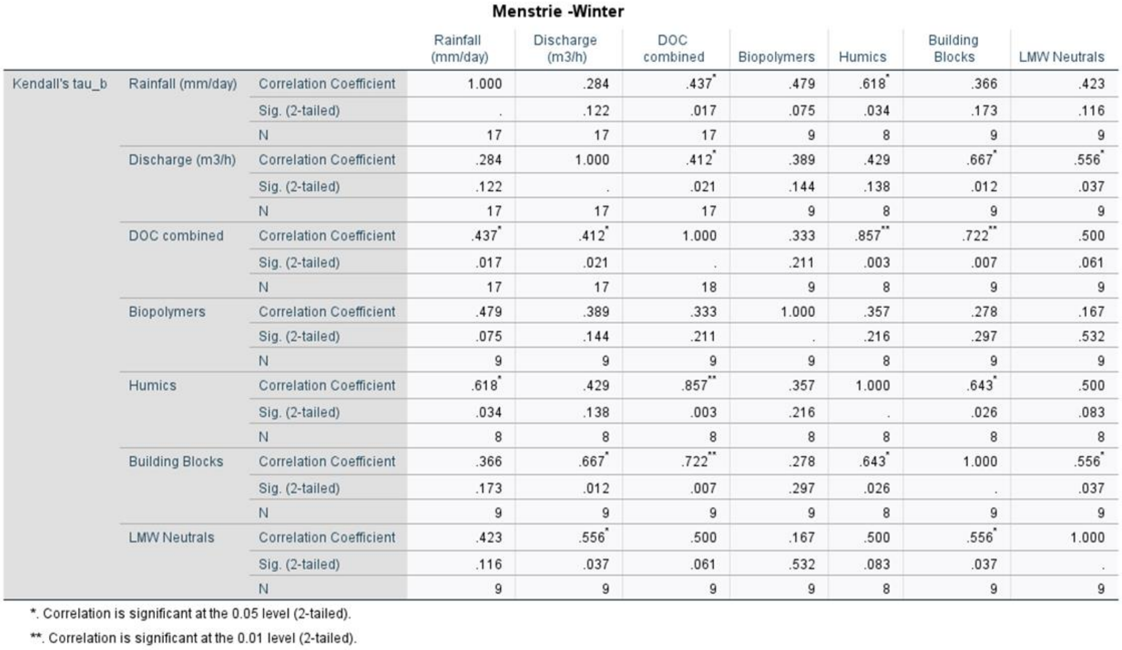


Menstrie - Summer

			Rainfall (mm/day)	Discharge (m3/h)	DOC combined	Biopolymers	Humics	Building Blocks	LMW Neutrals
Kendall's tau_b	Rainfall (mm/day)	Correlation Coefficient	1.000	.275	.275	.020	.422	.221	.101
		Sig. (2-tailed)	.	.208	.208	.935	.085	.366	.681
		N	13	13	13	11	11	11	11
	Discharge (m3/h)	Correlation Coefficient	.275	1.000	.564**	.345	.418	.382	.091
		Sig. (2-tailed)	.208	.	.007	.139	.073	.102	.697
		N	13	13	13	11	11	11	11
	DOC combined	Correlation Coefficient	.275	.564**	1.000	.818**	.745**	.855**	.273
		Sig. (2-tailed)	.208	.007	.	<.001	.001	<.001	.243
		N	13	13	13	11	11	11	11
	Biopolymers	Correlation Coefficient	.020	.345	.818**	1.000	.564*	.745**	.091
		Sig. (2-tailed)	.935	.139	<.001	.	.016	.001	.697
		N	11	11	11	11	11	11	11
	Humics	Correlation Coefficient	.422	.418	.745**	.564*	1.000	.818**	.236
		Sig. (2-tailed)	.085	.073	.001	.016	.	<.001	.312
		N	11	11	11	11	11	11	11
	Building Blocks	Correlation Coefficient	.221	.382	.855**	.745**	.818**	1.000	.200
		Sig. (2-tailed)	.366	.102	<.001	.001	<.001	.	.392
		N	11	11	11	11	11	11	11
	LMW Neutrals	Correlation Coefficient	.101	.091	.273	.091	.236	.200	1.000
		Sig. (2-tailed)	.681	.697	.243	.697	.312	.392	.
		N	11	11	11	11	11	11	11

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

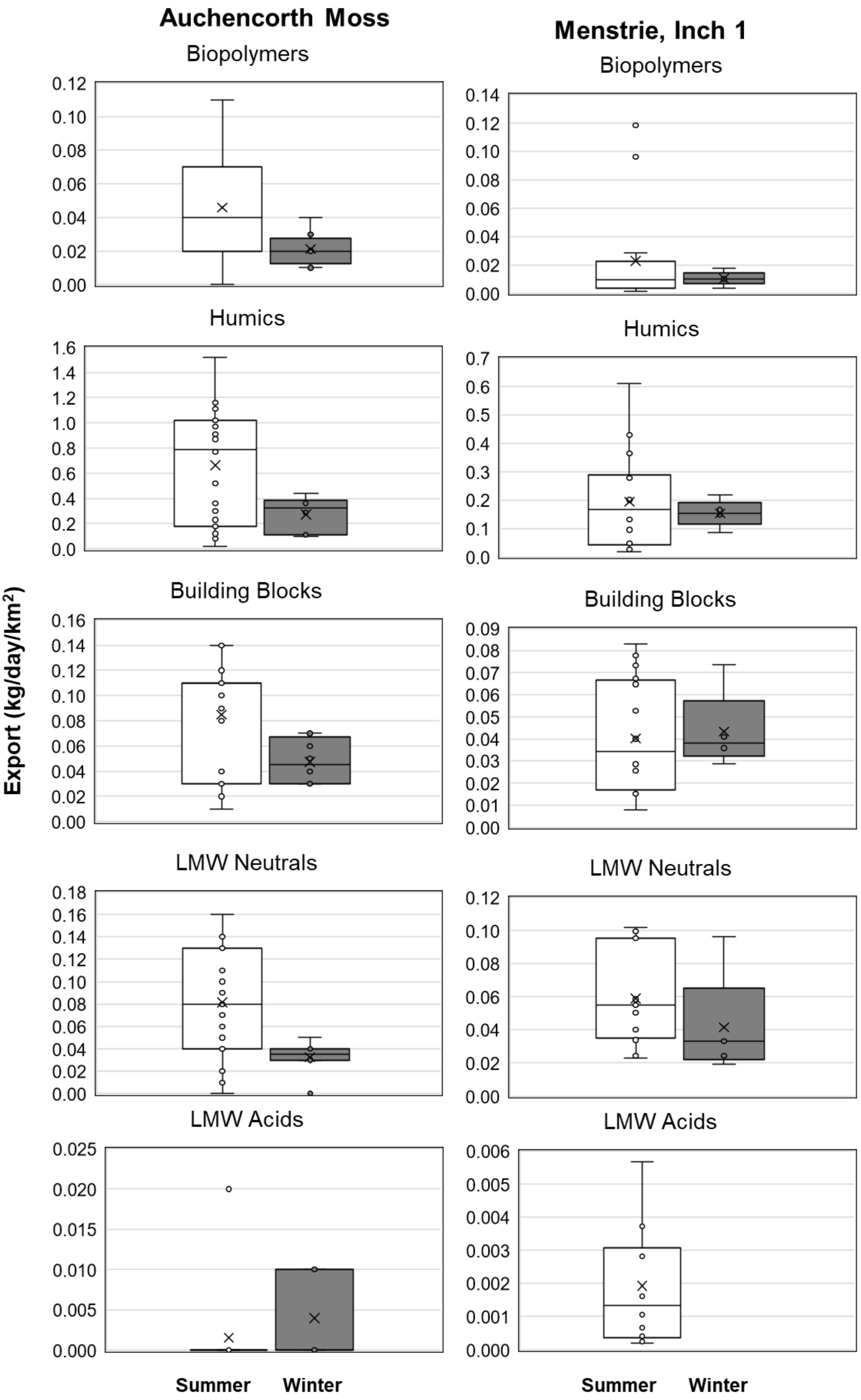


Menstrie -Winter

			Rainfall (mm/day)	Discharge (m3/h)	DOC combined	Biopolymers	Humics	Building Blocks	LMW Neutrals
Kendall's tau_b	Rainfall (mm/day)	Correlation Coefficient	1.000	.284	.437*	.479	.618*	.366	.423
		Sig. (2-tailed)	.	.122	.017	.075	.034	.173	.116
		N	17	17	17	9	8	9	9
	Discharge (m3/h)	Correlation Coefficient	.284	1.000	.412*	.389	.429	.667*	.556*
		Sig. (2-tailed)	.122	.	.021	.144	.138	.012	.037
		N	17	17	17	9	8	9	9
	DOC combined	Correlation Coefficient	.437*	.412*	1.000	.333	.857**	.722**	.500
		Sig. (2-tailed)	.017	.021	.	.211	.003	.007	.061
		N	17	17	18	9	8	9	9
	Biopolymers	Correlation Coefficient	.479	.389	.333	1.000	.357	.278	.167
		Sig. (2-tailed)	.075	.144	.211	.	.216	.297	.532
		N	9	9	9	9	8	9	9
	Humics	Correlation Coefficient	.618*	.429	.857**	.357	1.000	.643*	.500
		Sig. (2-tailed)	.034	.138	.003	.216	.	.026	.083
		N	8	8	8	8	8	8	8
	Building Blocks	Correlation Coefficient	.366	.667*	.722**	.278	.643*	1.000	.556*
		Sig. (2-tailed)	.173	.012	.007	.297	.026	.	.037
		N	9	9	9	9	8	9	9
	LMW Neutrals	Correlation Coefficient	.423	.556*	.500	.167	.500	.556*	1.000
		Sig. (2-tailed)	.116	.037	.061	.532	.083	.037	.
		N	9	9	9	9	8	9	9

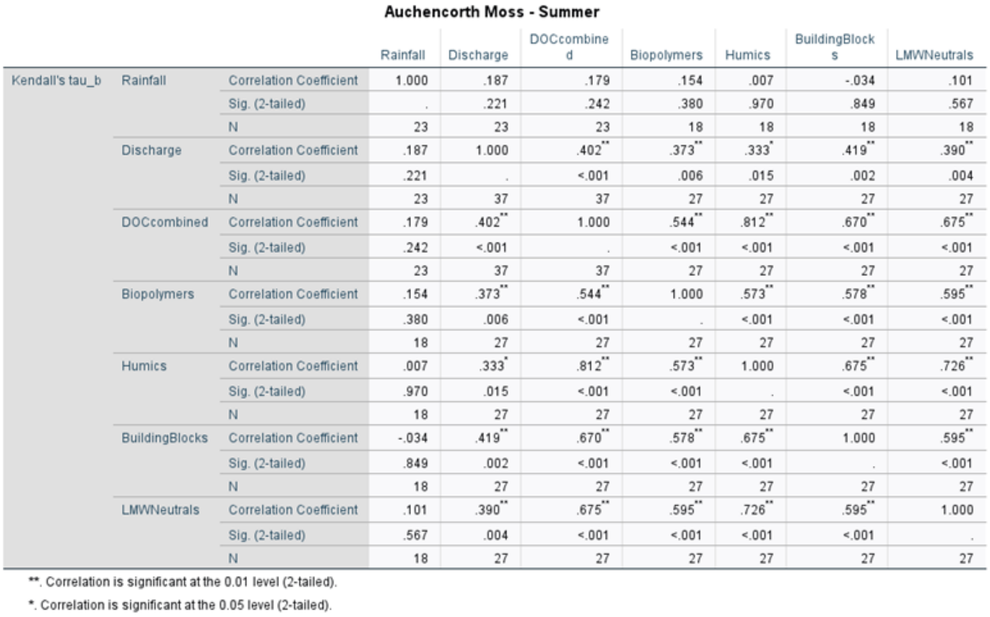
*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).



Appendix 5.10: Box and Whisker plots of DOM fractions export for a new periodicity with summer being from June to November and winter from December to May.

Appendix 5.11: Results from the Mann Kendall (Kendall’s tau_b) test for rainfall, discharge, as well as DOC, biopolymers, humics, building blocks and LMW neutrals concentration for the newly defined summer (June-November) and winter (December-May) data from Auchencorth Moss.

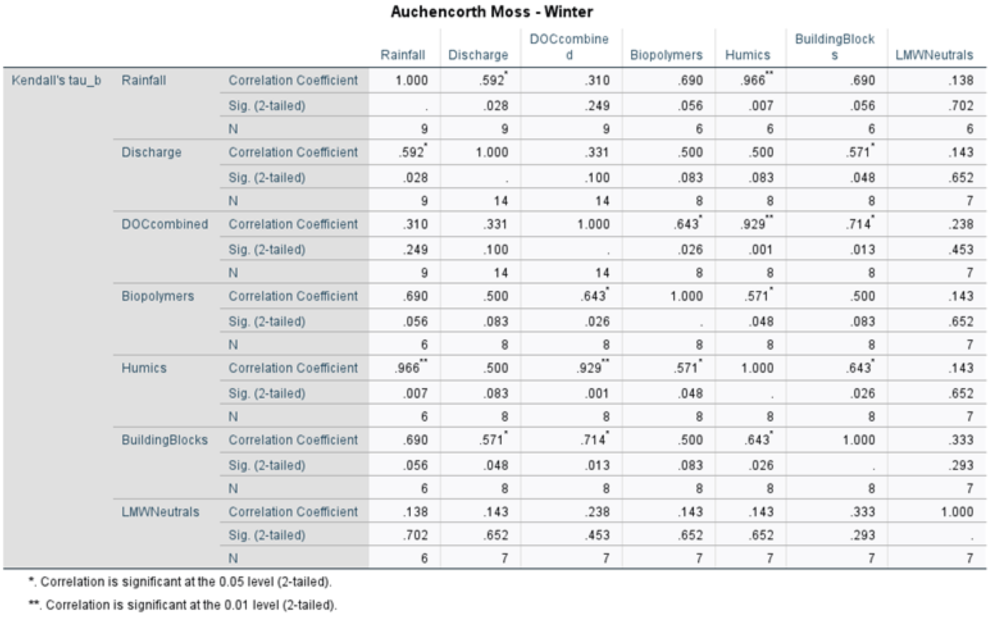


Auchencorth Moss - Summer

			Rainfall	Discharge	DOCcombined	Biopolymers	Humics	BuildingBlocks	LMWNeutrals
Kendall's tau_b	Rainfall	Correlation Coefficient	1.000	.187	.179	.154	.007	-.034	.101
		Sig. (2-tailed)	.	.221	.242	.380	.970	.849	.567
		N	23	23	23	18	18	18	18
	Discharge	Correlation Coefficient	.187	1.000	.402**	.373**	.333*	.419**	.390**
		Sig. (2-tailed)	.221	.	<.001	.006	.015	.002	.004
		N	23	37	37	27	27	27	27
	DOCcombined	Correlation Coefficient	.179	.402**	1.000	.544**	.812**	.670**	.675**
		Sig. (2-tailed)	.242	<.001	.	<.001	<.001	<.001	<.001
		N	23	37	37	27	27	27	27
	Biopolymers	Correlation Coefficient	.154	.373**	.544**	1.000	.573**	.578**	.595**
		Sig. (2-tailed)	.380	.006	<.001	.	<.001	<.001	<.001
		N	18	27	27	27	27	27	27
	Humics	Correlation Coefficient	.007	.333*	.812**	.573**	1.000	.675**	.726**
		Sig. (2-tailed)	.970	.015	<.001	<.001	.	<.001	<.001
		N	18	27	27	27	27	27	27
	BuildingBlocks	Correlation Coefficient	-.034	.419**	.670**	.578**	.675**	1.000	.595**
		Sig. (2-tailed)	.849	.002	<.001	<.001	<.001	.	<.001
		N	18	27	27	27	27	27	27
	LMWNeutrals	Correlation Coefficient	.101	.390**	.675**	.595**	.726**	.595**	1.000
		Sig. (2-tailed)	.567	.004	<.001	<.001	<.001	<.001	.
		N	18	27	27	27	27	27	27

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).



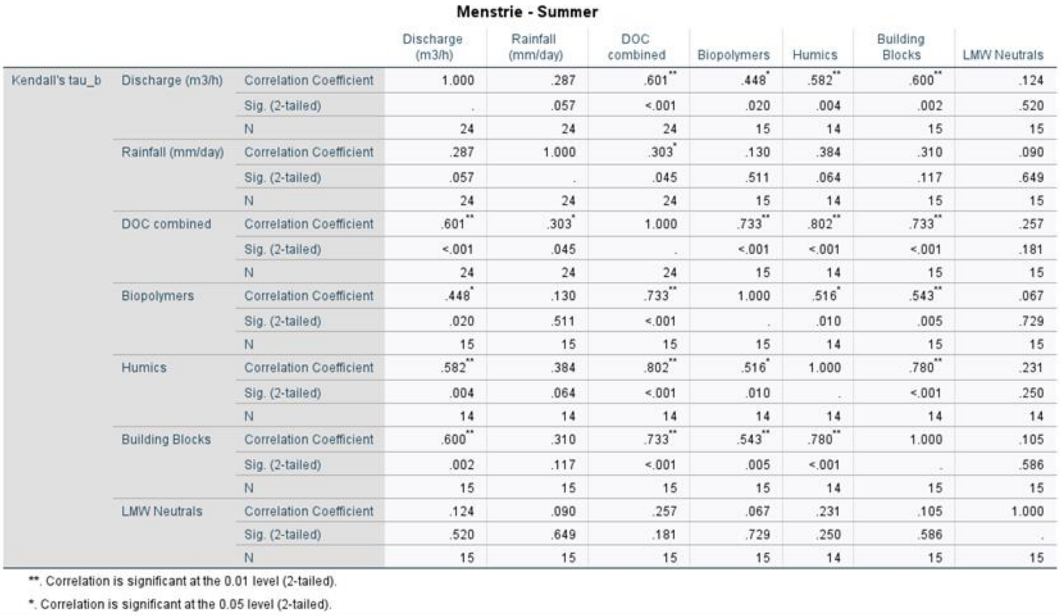
Auchencorth Moss - Winter

			Rainfall	Discharge	DOCcombined	Biopolymers	Humics	BuildingBlocks	LMWNeutrals
Kendall's tau_b	Rainfall	Correlation Coefficient	1.000	.592*	.310	.690	.966**	.690	.138
		Sig. (2-tailed)	.	.028	.249	.056	.007	.056	.702
		N	9	9	9	6	6	6	6
	Discharge	Correlation Coefficient	.592*	1.000	.331	.500	.500	.571*	.143
		Sig. (2-tailed)	.028	.	.100	.083	.083	.048	.652
		N	9	14	14	8	8	8	7
	DOCcombined	Correlation Coefficient	.310	.331	1.000	.643*	.929**	.714*	.238
		Sig. (2-tailed)	.249	.100	.	.026	.001	.013	.453
		N	9	14	14	8	8	8	7
	Biopolymers	Correlation Coefficient	.690	.500	.643*	1.000	.571*	.500	.143
		Sig. (2-tailed)	.056	.083	.026	.	.048	.083	.652
		N	6	8	8	8	8	8	7
	Humics	Correlation Coefficient	.966**	.500	.929**	.571*	1.000	.643*	.143
		Sig. (2-tailed)	.007	.083	.001	.048	.	.026	.652
		N	6	8	8	8	8	8	7
	BuildingBlocks	Correlation Coefficient	.690	.571*	.714*	.500	.643*	1.000	.333
		Sig. (2-tailed)	.056	.048	.013	.083	.026	.	.293
		N	6	8	8	8	8	8	7
	LMWNeutrals	Correlation Coefficient	.138	.143	.238	.143	.143	.333	1.000
		Sig. (2-tailed)	.702	.652	.453	.652	.652	.293	.
		N	6	7	7	7	7	7	7

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 5.12: Results from the Mann Kendall (Kendall's tau_b) test for rainfall, discharge, as well as DOC, biopolymers, humics, building blocks and LMW neutrals concentration for the newly defined summer (June-November) and winter (December-May) data from Menstrie.



Menstrie - Summer

			Discharge (m3/h)	Rainfall (mm/day)	DOC combined	Biopolymers	Humics	Building Blocks	LMW Neutrals
Kendall's tau_b	Discharge (m3/h)	Correlation Coefficient	1.000	.287	.601**	.448*	.582**	.600**	.124
		Sig. (2-tailed)	.	.057	<.001	.020	.004	.002	.520
		N	24	24	24	15	14	15	15
	Rainfall (mm/day)	Correlation Coefficient	.287	1.000	.303*	.130	.384	.310	.090
		Sig. (2-tailed)	.057	.	.045	.511	.064	.117	.649
		N	24	24	24	15	14	15	15
	DOC combined	Correlation Coefficient	.601**	.303*	1.000	.733**	.802**	.733**	.257
		Sig. (2-tailed)	<.001	.045	.	<.001	<.001	<.001	.181
		N	24	24	24	15	14	15	15
	Biopolymers	Correlation Coefficient	.448*	.130	.733**	1.000	.516*	.543**	.067
		Sig. (2-tailed)	.020	.511	<.001	.	.010	.005	.729
		N	15	15	15	15	14	15	15
	Humics	Correlation Coefficient	.582**	.384	.802**	.516*	1.000	.780**	.231
		Sig. (2-tailed)	.004	.064	<.001	.010	.	<.001	.250
		N	14	14	14	14	14	14	14
	Building Blocks	Correlation Coefficient	.600**	.310	.733**	.543**	.780**	1.000	.105
		Sig. (2-tailed)	.002	.117	<.001	.005	<.001	.	.586
		N	15	15	15	15	14	15	15
	LMW Neutrals	Correlation Coefficient	.124	.090	.257	.067	.231	.105	1.000
		Sig. (2-tailed)	.520	.649	.181	.729	.250	.586	.
		N	15	15	15	15	14	15	15

**. Correlation is significant at the 0.01 level (2-tailed).

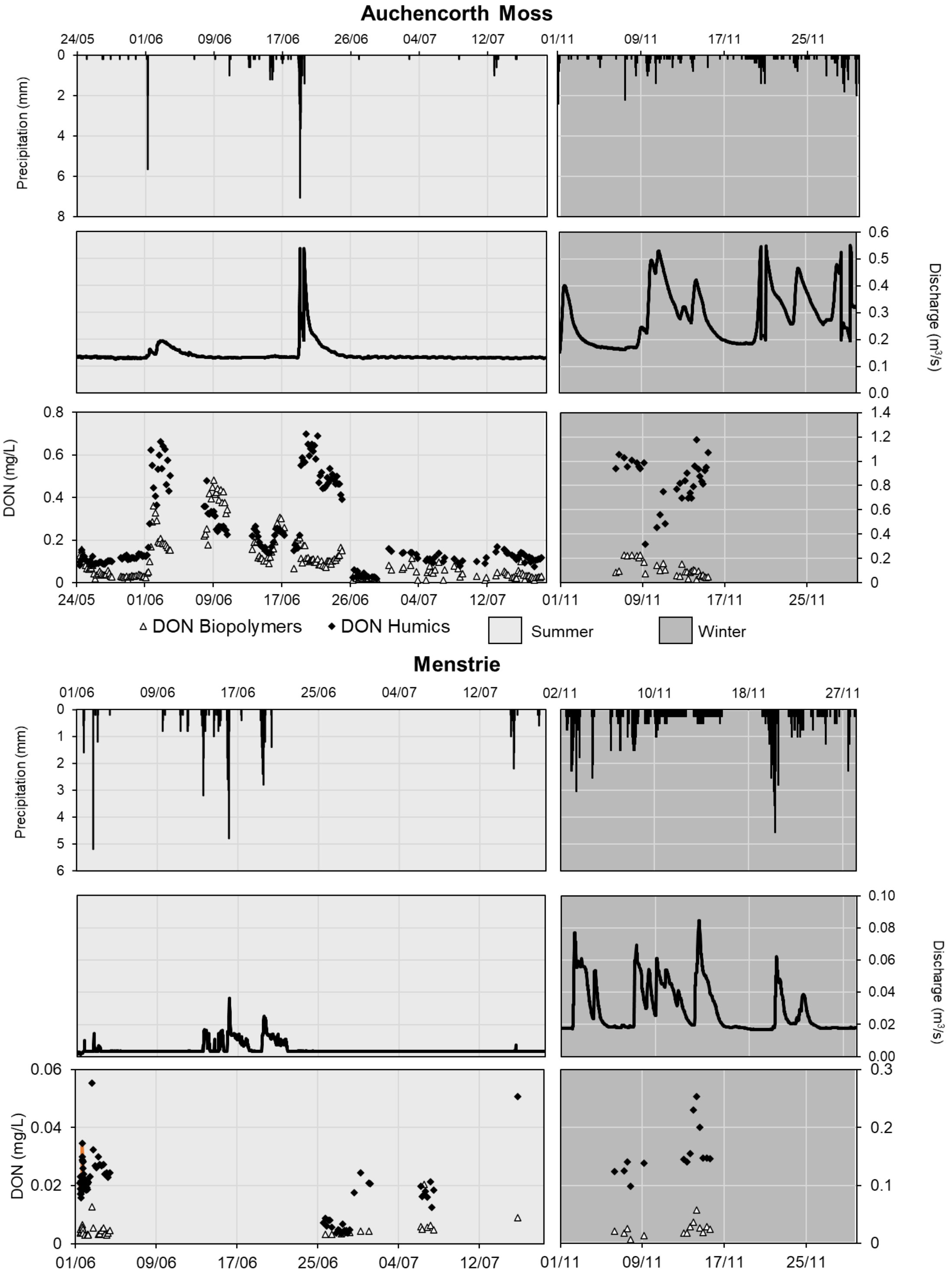
*. Correlation is significant at the 0.05 level (2-tailed).

Menstrie - Winter

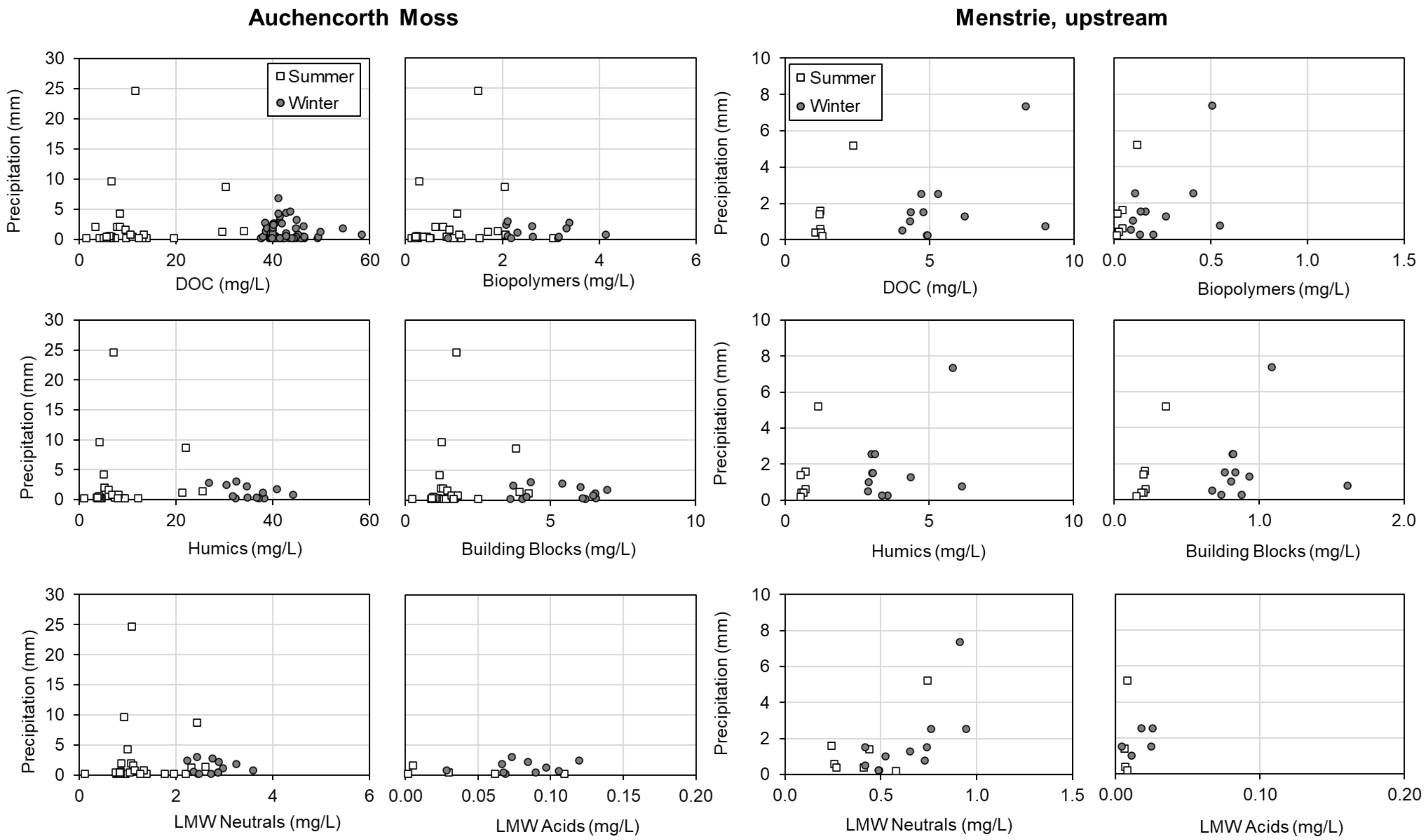
			Discharge (m3/h)	Rainfall (mm/day)	DOC combined	Biopolymers	Humics	Building Blocks	LMW Neutrals
Kendall's tau_b	Discharge (m3/h)	Correlation Coefficient	1.000	.414	.600	.600	.400	.800	.600
		Sig. (2-tailed)	.	.251	.091	.142	.327	.050	.142
		N	6	6	6	5	5	5	5
	Rainfall (mm/day)	Correlation Coefficient	.414	1.000	.552	.738	.949*	.527	.316
		Sig. (2-tailed)	.251	.	.126	.077	.023	.207	.448
		N	6	6	6	5	5	5	5
	DOC combined	Correlation Coefficient	.600	.552	1.000	.800	.600	1.000*	.800
		Sig. (2-tailed)	.091	.126	.	.050	.142	.	.050
		N	6	6	7	5	5	5	5
	Biopolymers	Correlation Coefficient	.600	.738	.800	1.000	.800	.800	.600
		Sig. (2-tailed)	.142	.077	.050	.	.050	.050	.142
		N	5	5	5	5	5	5	5
	Humics	Correlation Coefficient	.400	.949*	.600	.800	1.000	.600	.400
		Sig. (2-tailed)	.327	.023	.142	.050	.	.142	.327
		N	5	5	5	5	5	5	5
	Building Blocks	Correlation Coefficient	.800	.527	1.000**	.800	.600	1.000	.800
		Sig. (2-tailed)	.050	.207	.	.050	.142	.	.050
		N	5	5	5	5	5	5	5
	LMW Neutrals	Correlation Coefficient	.600	.316	.800	.600	.400	.800	1.000
		Sig. (2-tailed)	.142	.448	.050	.142	.327	.050	.
		N	5	5	5	5	5	5	5

*. Correlation is significant at the 0.05 level (2-tailed).

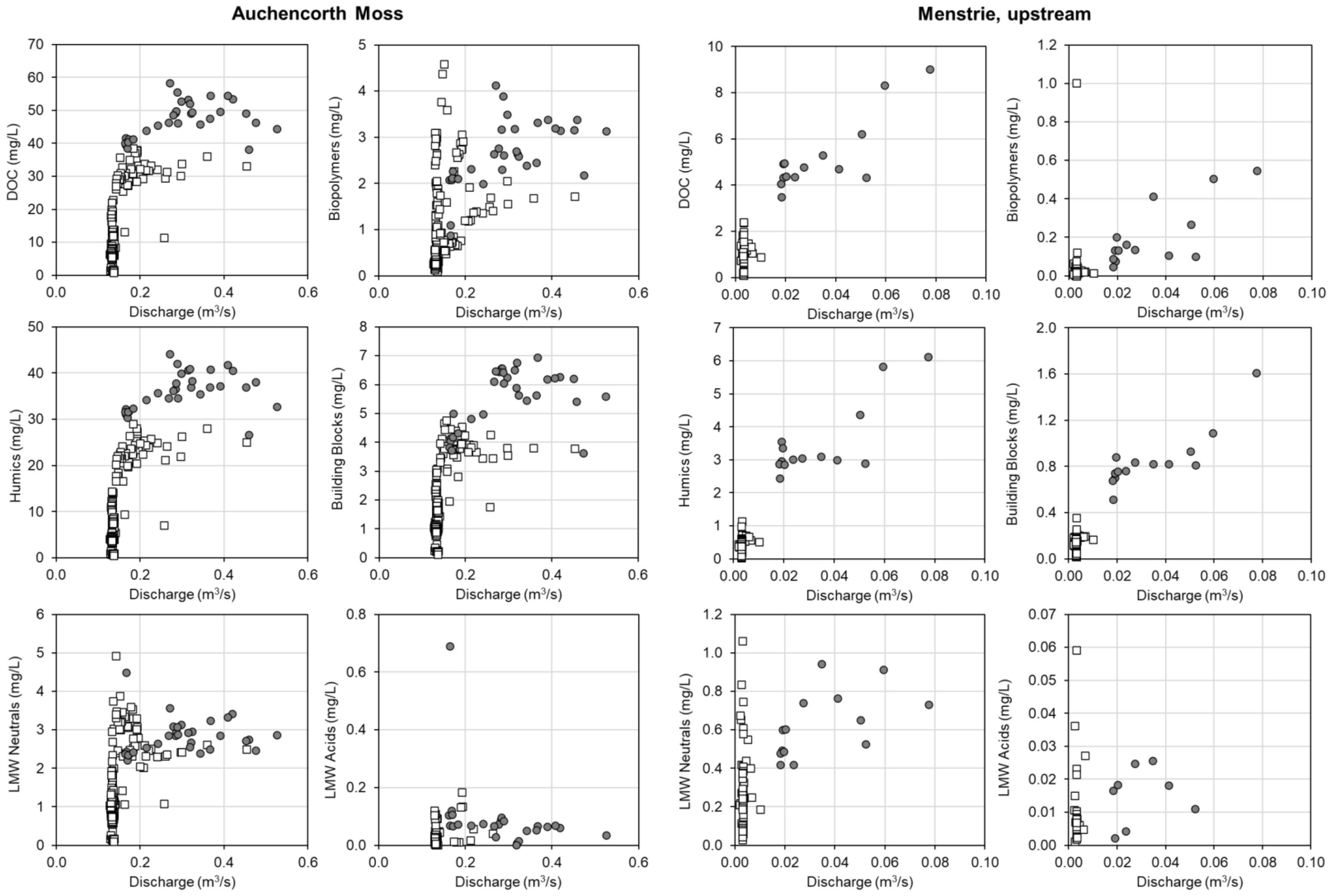
**. Correlation is significant at the 0.01 level (2-tailed).



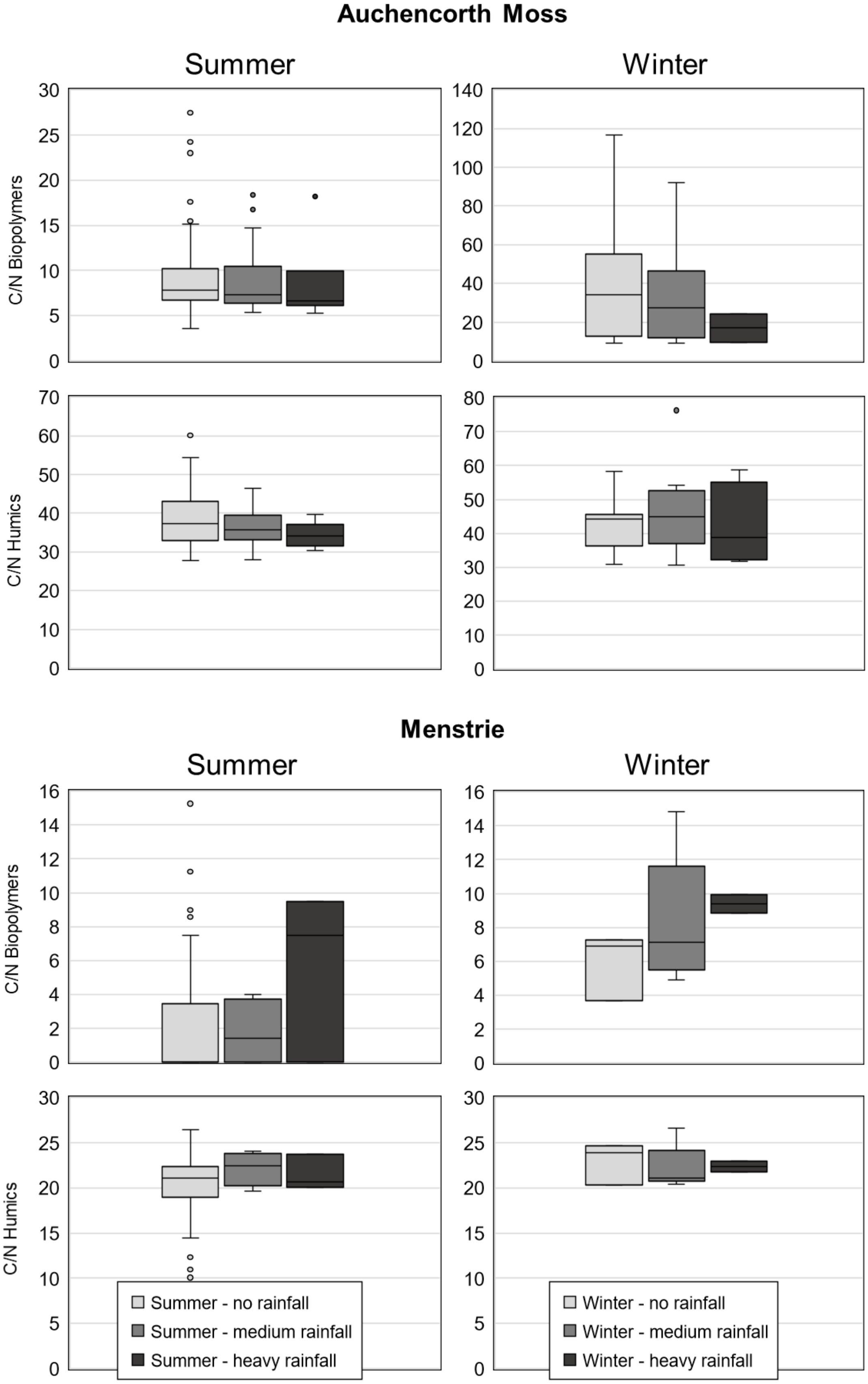
Appendix 6.1: Summer (light grey background) and winter (dark grey background) high-resolution timeseries of precipitation, river discharge and dissolved organic nitrogen concentration in mg/L from biopolymers (white triangle) and humics (black diamonds) for Black Burn in Auchencorth Moss and Inch 1 in the Menstrie catchment.



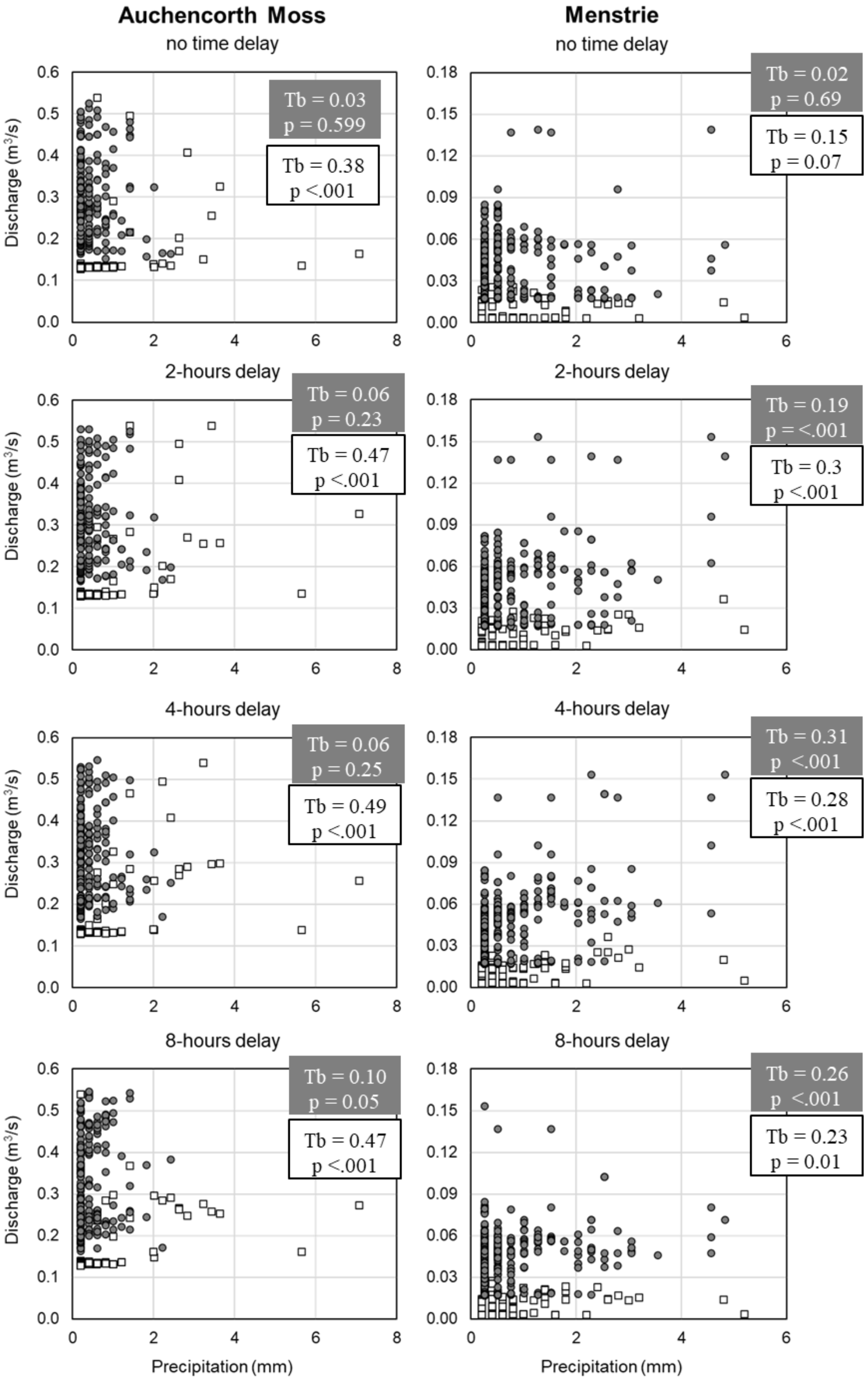
Appendix 6.2: DOC and its fractions against precipitation from Auchencorth Moss and Menstrie during winter (grey dots) and summer (white square) data points).



Appendix 6.3: DOC and its fractions against discharge from Auchencorth Moss and Menstrie during winter (grey dots) and summer (white square) data points).

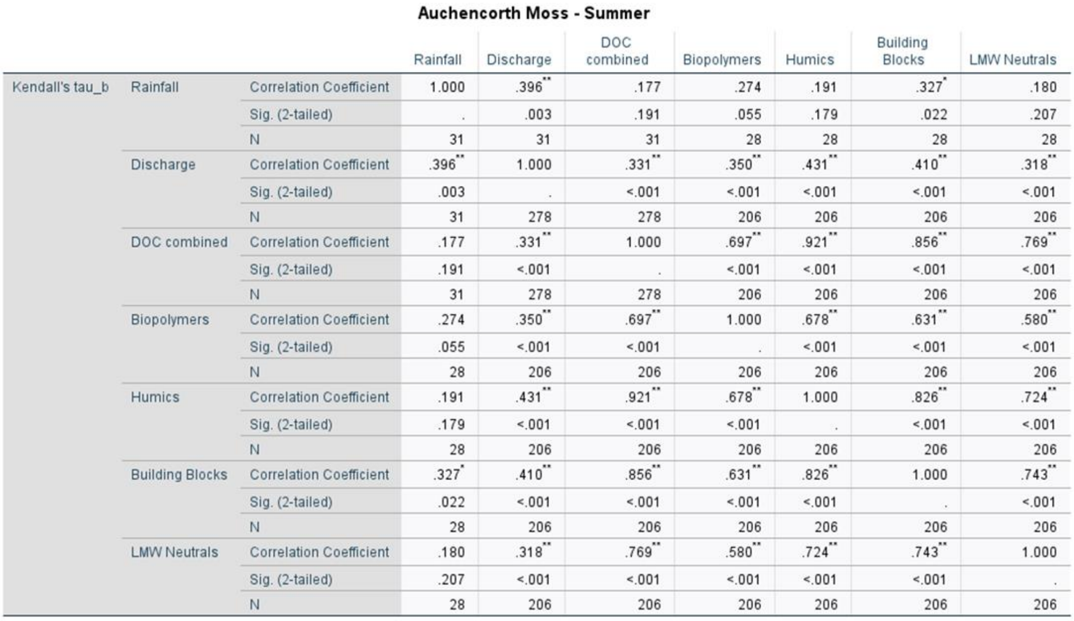


Appendix 6.4: Carbon-Nitrogen ratios from the biopolymers and humics fraction for Black Burn, Auchencorth Moss and Inch 1, Menstrie during summer and winter months. The samples were grouped in regard to the amount of precipitation received within 4 hours before sampling. Medium rainfall is defined as 0.2- 1.9 mm precipitation within 4 hours and heavy rainfall is defined as > 1.9 mm rainfall received within 4 hours before sampling.



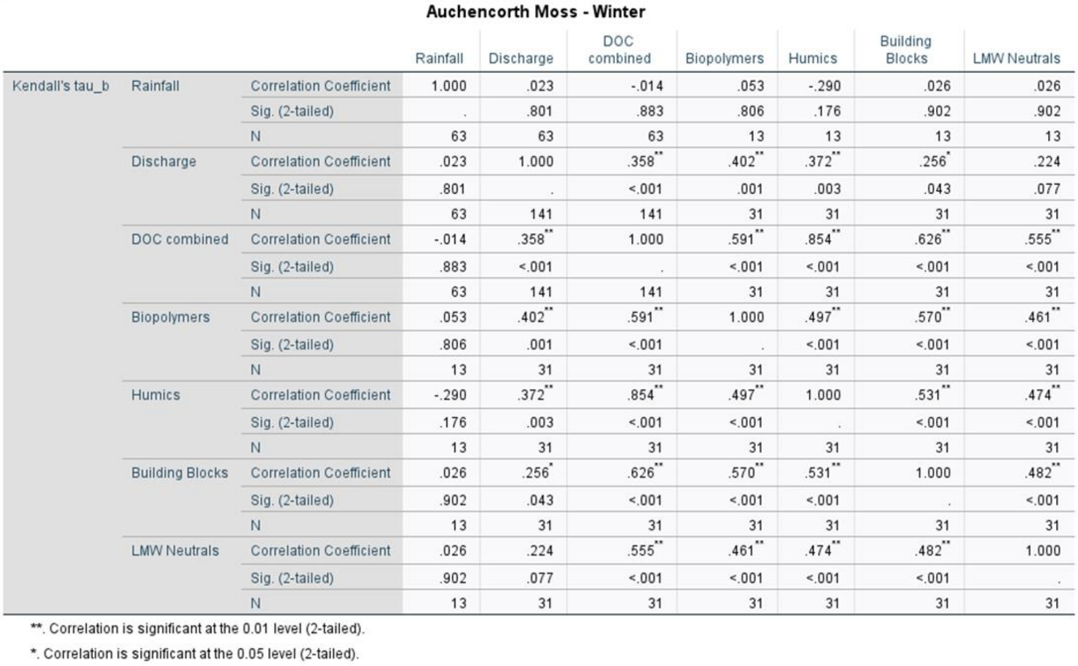
Appendix 6.5: Correlation between precipitation and discharge (30-min resolution) with not time delay, a 2-hour time delay, a 4-hour time delay and 8-hour time delay in the discharge data. Tb is the Kendall Tau_b Correlation coefficient and p is the is the probability number of a statistical hypothesis test, with p-value < 0.05 meaning that there is stronger evidence in favour of the alternative hypothesis. The grey box with white text show Kendall Tau_b results for winter samples and white boxes show summer results.

Appendix 6.6: Results from the Mann Kendall (Kendall's tau_b) test for rainfall, discharge, as well as DOC, biopolymers, humics, building blocks and LMW neutrals concentration for summer and winter data from Auchencorth Moss.



Auchencorth Moss - Summer

			Rainfall	Discharge	DOC combined	Biopolymers	Humics	Building Blocks	LMW Neutrals
Kendall's tau_b	Rainfall	Correlation Coefficient	1.000	.396**	.177	.274	.191	.327*	.180
		Sig. (2-tailed)	.	.003	.191	.055	.179	.022	.207
		N	31	31	31	28	28	28	28
	Discharge	Correlation Coefficient	.396**	1.000	.331**	.350**	.431**	.410**	.318**
		Sig. (2-tailed)	.003	.	<.001	<.001	<.001	<.001	<.001
		N	31	278	278	206	206	206	206
	DOC combined	Correlation Coefficient	.177	.331**	1.000	.697**	.921**	.856**	.769**
		Sig. (2-tailed)	.191	<.001	.	<.001	<.001	<.001	<.001
		N	31	278	278	206	206	206	206
	Biopolymers	Correlation Coefficient	.274	.350**	.697**	1.000	.678**	.631**	.580**
		Sig. (2-tailed)	.055	<.001	<.001	.	<.001	<.001	<.001
		N	28	206	206	206	206	206	206
	Humics	Correlation Coefficient	.191	.431**	.921**	.678**	1.000	.826**	.724**
		Sig. (2-tailed)	.179	<.001	<.001	<.001	.	<.001	<.001
		N	28	206	206	206	206	206	206
	Building Blocks	Correlation Coefficient	.327*	.410**	.856**	.631**	.826**	1.000	.743**
		Sig. (2-tailed)	.022	<.001	<.001	<.001	<.001	.	<.001
		N	28	206	206	206	206	206	206
	LMW Neutrals	Correlation Coefficient	.180	.318**	.769**	.580**	.724**	.743**	1.000
		Sig. (2-tailed)	.207	<.001	<.001	<.001	<.001	<.001	.
		N	28	206	206	206	206	206	206



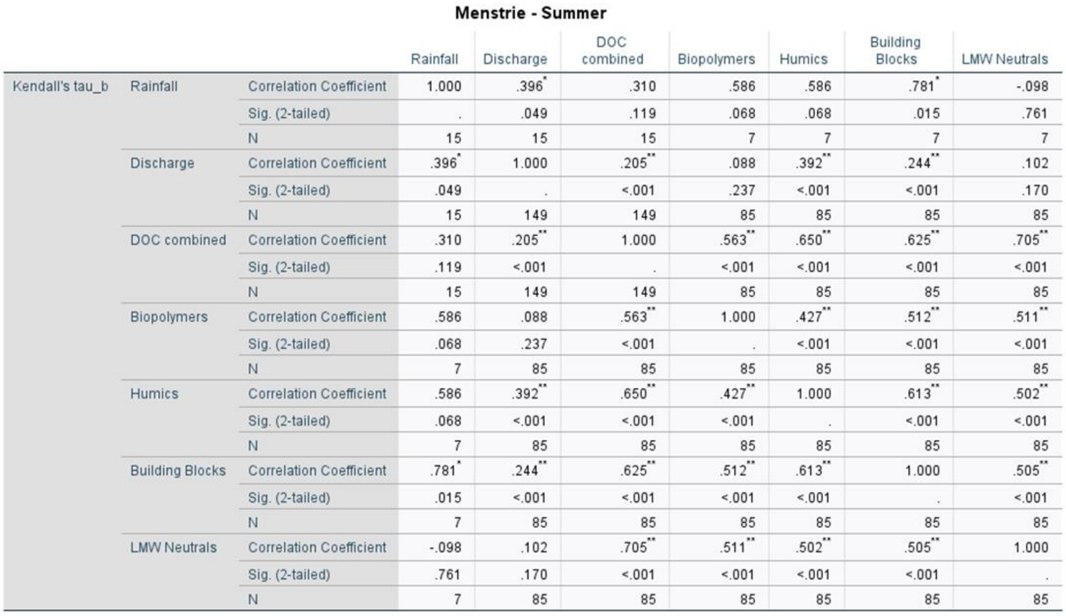
Auchencorth Moss - Winter

			Rainfall	Discharge	DOC combined	Biopolymers	Humics	Building Blocks	LMW Neutrals
Kendall's tau_b	Rainfall	Correlation Coefficient	1.000	.023	-.014	.053	-.290	.026	.026
		Sig. (2-tailed)	.	.801	.883	.806	.176	.902	.902
		N	63	63	63	13	13	13	13
	Discharge	Correlation Coefficient	.023	1.000	.358**	.402**	.372**	.256*	.224
		Sig. (2-tailed)	.801	.	<.001	.001	.003	.043	.077
		N	63	141	141	31	31	31	31
	DOC combined	Correlation Coefficient	-.014	.358**	1.000	.591**	.854**	.626**	.555**
		Sig. (2-tailed)	.883	<.001	.	<.001	<.001	<.001	<.001
		N	63	141	141	31	31	31	31
	Biopolymers	Correlation Coefficient	.053	.402**	.591**	1.000	.497**	.570**	.461**
		Sig. (2-tailed)	.806	.001	<.001	.	<.001	<.001	<.001
		N	13	31	31	31	31	31	31
	Humics	Correlation Coefficient	-.290	.372**	.854**	.497**	1.000	.531**	.474**
		Sig. (2-tailed)	.176	.003	<.001	<.001	.	<.001	<.001
		N	13	31	31	31	31	31	31
	Building Blocks	Correlation Coefficient	.026	.256*	.626**	.570**	.531**	1.000	.482**
		Sig. (2-tailed)	.902	.043	<.001	<.001	<.001	.	<.001
		N	13	31	31	31	31	31	31
	LMW Neutrals	Correlation Coefficient	.026	.224	.555**	.461**	.474**	.482**	1.000
		Sig. (2-tailed)	.902	.077	<.001	<.001	<.001	<.001	.
		N	13	31	31	31	31	31	31

**. Correlation is significant at the 0.01 level (2-tailed).

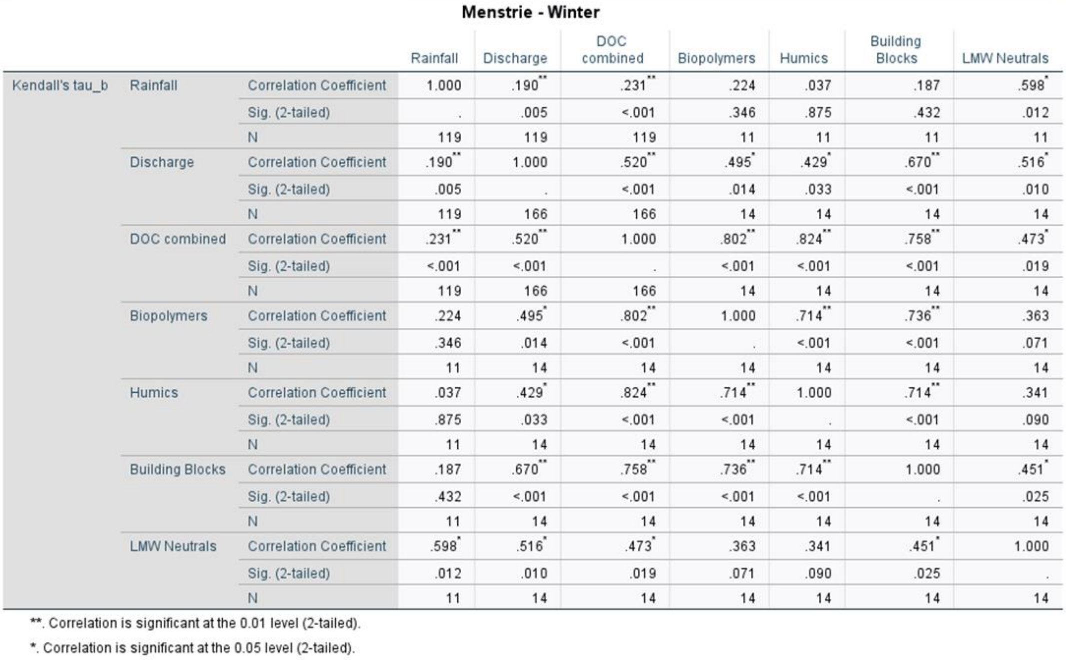
*. Correlation is significant at the 0.05 level (2-tailed).

Appendix 6.7: Results from the Mann Kendall (Kendall's tau_b) test for rainfall, discharge, as well as DOC, biopolymers, humics, building blocks and LMW neutrals concentration for summer and winter data from Menstrie.



Menstrie - Summer

			Rainfall	Discharge	DOC combined	Biopolymers	Humics	Building Blocks	LMW Neutrals
Kendall's tau_b	Rainfall	Correlation Coefficient	1.000	.396*	.310	.586	.586	.781*	-.098
		Sig. (2-tailed)	.	.049	.119	.068	.068	.015	.761
		N	15	15	15	7	7	7	7
	Discharge	Correlation Coefficient	.396*	1.000	.205**	.088	.392**	.244**	.102
		Sig. (2-tailed)	.049	.	<.001	.237	<.001	<.001	.170
		N	15	149	149	85	85	85	85
	DOC combined	Correlation Coefficient	.310	.205**	1.000	.563**	.650**	.625**	.705**
		Sig. (2-tailed)	.119	<.001	.	<.001	<.001	<.001	<.001
		N	15	149	149	85	85	85	85
	Biopolymers	Correlation Coefficient	.586	.088	.563**	1.000	.427**	.512**	.511**
		Sig. (2-tailed)	.068	.237	<.001	.	<.001	<.001	<.001
		N	7	85	85	85	85	85	85
	Humics	Correlation Coefficient	.586	.392**	.650**	.427**	1.000	.613**	.502**
		Sig. (2-tailed)	.068	<.001	<.001	<.001	.	<.001	<.001
		N	7	85	85	85	85	85	85
	Building Blocks	Correlation Coefficient	.781*	.244**	.625**	.512**	.613**	1.000	.505**
		Sig. (2-tailed)	.015	<.001	<.001	<.001	<.001	.	<.001
		N	7	85	85	85	85	85	85
	LMW Neutrals	Correlation Coefficient	-.098	.102	.705**	.511**	.502**	.505**	1.000
		Sig. (2-tailed)	.761	.170	<.001	<.001	<.001	<.001	.
		N	7	85	85	85	85	85	85



Menstrie - Winter

			Rainfall	Discharge	DOC combined	Biopolymers	Humics	Building Blocks	LMW Neutrals
Kendall's tau_b	Rainfall	Correlation Coefficient	1.000	.190**	.231**	.224	.037	.187	.598*
		Sig. (2-tailed)	.	.005	<.001	.346	.875	.432	.012
		N	119	119	119	11	11	11	11
	Discharge	Correlation Coefficient	.190**	1.000	.520**	.495*	.429*	.670**	.516*
		Sig. (2-tailed)	.005	.	<.001	.014	.033	<.001	.010
		N	119	166	166	14	14	14	14
	DOC combined	Correlation Coefficient	.231**	.520**	1.000	.802**	.824**	.758**	.473*
		Sig. (2-tailed)	<.001	<.001	.	<.001	<.001	<.001	.019
		N	119	166	166	14	14	14	14
	Biopolymers	Correlation Coefficient	.224	.495*	.802**	1.000	.714**	.736**	.363
		Sig. (2-tailed)	.346	.014	<.001	.	<.001	<.001	.071
		N	11	14	14	14	14	14	14
	Humics	Correlation Coefficient	.037	.429*	.824**	.714**	1.000	.714**	.341
		Sig. (2-tailed)	.875	.033	<.001	<.001	.	<.001	.090
		N	11	14	14	14	14	14	14
	Building Blocks	Correlation Coefficient	.187	.670**	.758**	.736**	.714**	1.000	.451*
		Sig. (2-tailed)	.432	<.001	<.001	<.001	<.001	.	.025
		N	11	14	14	14	14	14	14
	LMW Neutrals	Correlation Coefficient	.598*	.516*	.473*	.363	.341	.451*	1.000
		Sig. (2-tailed)	.012	.010	.019	.071	.090	.025	.
		N	11	14	14	14	14	14	14

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).