The Fate and Composition of In-Stream Organic Carbon

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Out on the streams and ribbons of wind,
The spine of the north stands, dusk begins,
And chasing the light I race the world turning,
To the rocks on the edge at the end of the day.

They've watched as the rain moves in from the west
And the Ring Ouzel shyly returns to his nest,
They've stood as the winter ice melts into spring,
Colours ignited to purple and grey.

And in the visible silence of scattering light
As the forms fade and soften on the crest of the night
I've slept by these gritstones amid the moor sleeping,
Them fast in the darkness as wind shadows sway.

And what of these old songs we hear in the morning,
And what of these new lines at the end of the day?
Through the tide race of living, our rising and falling,
The edge is my island, and I'm cast away.

As we weather our storms there's a still place within me,
As the change of the seasons marks the land's constancy,
So I'll drink in this breath like so many before me,
Who've stood here forever at the brink of the day.

So what of these old songs we sing in the morning,
And what of these new lines we hear on the way?
So chasing the light I'll race the world turning,
To my place on the north edge at the end of the day.

The North Edge, by Robin Beatty, The Old Dance School

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Abstract

The northern peatlands cover only 3% of the Earth, and store between 20 and 30% of the terrestrial carbon pool. In the UK, 15% of the land is covered in peatland, which is estimated to store 2.3 Pg of carbon. Recently, a trend of increasing DOC concentrations in surface waters has been observed in the northern hemisphere, and the in-stream processing and degradation of DOC to CO_2 could represent a major and increasing source of greenhouse gas to the atmosphere.

This thesis measured net DOC loss in unfiltered river water samples across different catchment scales, ranging from 0.005 to 1086 km^2, with the peat content varying from 0 to 100%, and over time scales from 30 hours to 10 days. Experiments were carried out monthly for three years, and considered total loss, photo and aphotic degradation and the rate of each process. The composition of DOC and various source materials was analysed.

There was a clear diurnal cycle in the degradation of DOC, with the rates of decline being much higher during the day and lower over night. The initial rates of DOC degradation were higher in source waters than from large downstream sites. Adding nutrients to the water decreased the initial rate, whereas exposing the water to light increased the rate, compared with water kept in the dark. The apparent quantum yield and activation energy of the degradation were calculated.

The initial rate of DOC degradation was found to be related to the oxidation state of the material, with samples that were more reduced being degraded faster.

The total DOC loss was estimated to be 76%, which equates to a loss of up to 14678 Gg CO_{2eq}/yr from UK peat-covered catchments, which is 2.5% of the UK total GHG emissions, or 0.7% of the global CO_2 emissions from inland waters.

Declaration and copyright

I confirm that no part of the material presented in this thesis has previously been submitted by me or any other person for a degree in this or any university. Where relevant, material from the work of others has been acknowledged.

Signed:

Date:

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1.1. Overview and Project Rationale

The IPCC third assessment (2001) and fourth assessment (2007) carbon (C) cycle diagrams show that the global carbon flux from soils into rivers (0.8 Pg C/yr), transported to the sea, does not change its concentration on route – no carbon is lost between the land and the sea. This has been shown to be inaccurate, with studies showing that up to 50% of the carbon lost from the soil does not reach the catchment outlet (Billett et al., 2010; Worrall et al., 2009). Rivers are not just passive transport mechanisms for carbon; there is a multitude of ways that the carbon interacts with the river biota and environment. The IPCC fifth assessment (2013) has added more detail and a pathway in the carbon cycle for freshwater degassing from lakes/reservoirs, that has a flux of 1 Pg C/yr, and the export from soils to rivers has increased from 0.8 to 1.7 Pg C/yr. There is also removal of carbon by burial in the lakes/reservoirs 0.2 Pg C/yr, and the final flux to the ocean is 0.9 Pg C/yr, more than the estimates in the previous IPCC reports. Still there is no flux from the rivers directly to the atmosphere, but adding a sink of carbon in reservoirs and lakes does reflect the current research thinking that not all the carbon that leaves the soil ends up in the sea.

The aim of this thesis is to start to address this gap in the carbon budget, and quantify the net losses of carbon along the course of the river; the mechanisms by which the carbon is lost; and look at what factors influence the rates and magnitudes of that loss. Peatland ecosystems will be the main focus of this thesis, as they contain large quantities of carbon (approximately 4.5 Pg) and are important components of the carbon cycle, susceptible to large losses (Worrall et al., 2009).

1.2. Research Review

1.2.1. Peatlands and carbon storage

The northern peatlands cover only 3% of the Earth, but they store between 20 and 30% of the terrestrial carbon (C) pool (Gorham, 1991). Due to typically high water tables and low air temperatures, the northern peatlands accumulated carbon during the Holocene (the last 12000 years) at approximately 0.96 Tg C/yr. The C budget of peatlands has been extensively studied in recent years due to increased global interest in the carbon cycle and climate change (e.g. Stutter et al., 2013; Worrall et al., 2003).

In the UK, 15% of the land is covered in peatland, covering between 14000 and 29000 km^2 (Tallis et al., 1997), and is estimated to store 2.3 Pg C (Billett et al., 2010). The majority of this peat is located in upland areas, where the higher altitude has allowed for the formation of deep blanket peats (Evans and Warburton, 2005). There is evidence that the UK peatlands are becoming a source rather than sink of C, losing 27 g $C/m^2/yr$ (Janssens et al., 2005).

The C budget of peatlands includes inputs of carbon from photosynthesis, rainfall and weathering of underlying geology, and outputs as carbon dioxide (CO_2), methane (CH_4) and losses as the result of weathering and erosion (Worrall et al., 2003).

Peatland erosion is a method by which C transfers from the terrestrial pool to the aquatic pool. There are various causes of erosion, including wind, rain, grazing, wildfire and recreational activities (Foulds and Warburton, 2007). Degraded peat, which accounts for 99% of English peatlands, is more easily eroded than intact peat (Evans and Lindsay, 2010).

Soil erosion from England and Wales has been found to mobilise 0.2-0.76 Tg C/yr, of which 0.08-0.29 Tg C/yr was re-deposited on flood plains and 0.12-0.46 Tg C/yr was transported to surface waters (Dawson and Smith, 2007). 10% of blanket peat in the UK has some degree of erosion, and the losses due to erosion of peat have been extensively studied. Most of this work has focussed on the Peak District National Park, due to the widespread areas of bare and degraded peat (Rothwell et al., 2007b), with erosion rates of 267 Mg $C/km^2/yr$ (Evans et al., 2006). Warburton (2003) looked at the erosion rates at Moor House National Nature Reserve (NNR), in the North Pennines, UK, and found losses of 47 Mg $C/km^2/yr$.

The eroded peat that is transported to the surface waters draining the peatland forms part of the fluvial carbon cycle of the peatland, as the exported peat becomes particulate and dissolved matter in the water (Aufdenkampe et al., 2011; Kling et al., 1991). The dissolved fraction is operationally defined as anything smaller than 0.45 μm, and the particulate fraction is anything larger (Evans et al., 2005). Both the particulate and dissolved matter is comprised of organic and inorganic molecules, with the organic components of both being referred to as the Particulate and Dissolved Organic Matter (POM and DOM), respectively. The proportions of these matters that are C are called Particulate and Dissolved Organic Carbon (POC and DOC) respectively. 'OC' refers to organic carbon that can be either particulate or dissolved, or both. POC fluxes are particularly influenced by the state of the peatland, with higher fluxes reported from eroded and bare sites (Evans et al., 2006; Pawson et al., 2008).

1.2.2. Particulate Organic Carbon (POC)

Particulate Organic Carbon (POC) represents approximately 10 to 25% of the fluvial C export from peatlands, and it tends to be more episodic and event driven than DOC (Hope et al., 1997). In their two-week study on Upper North Grain in the Peak District, Pawson et al., (2008) reported that the POC fluxes were much more variable than DOC fluxes. They found that 95% of the POC flux was exported in just 8% of the study time, a period of just over 1 day. The POC flux from a re-vegetated site at Moor House NNR was found to have decreased from 45 $g/m^2/yr$ in 1960 to 18 $g/m^2/yr$ in 1999, showing that the restoration over 40 years has helped to reduce the POC losses (Evans et al., 2006). Holden et al., (2012) found that the average POC flux from another catchment at Moor House NNR was 473 kg/yr, whereas the DOC flux from the same stream was over 20 times higher.

The amount of terrestrial POC exported by rivers is approximately the same as the amount of OC buried on continental shelves. However, very little of the terrestrial OC accumulates in the ocean (Cole and Caraco, 2001).

1.2.3. Dissolved Organic Carbon (DOC)

Dissolved Organic Carbon (DOC) is a universal component of natural waters, comprising of organic compounds, including humic and fulvic acids, in various

stages of degradation (Thurman, 1985). The concentrations of DOC in river waters draining from peatlands in the UK and across Europe and North America has been observed to be increasing, with various reasons presented as the possible cause, including increasing air temperature (Freeman et al., 2001), climate change (Bellamy et al., 2005), increasing atmospheric CO_2 concentrations (Freeman et al., 2004), local disturbances (Robroek et al., 2010), storm events (Austnes et al., 2010), changes in in-stream processing (Dawson et al., 2001b), changes in land management (Clutterbuck and Yallop, 2010), drought (Worrall et al., 2006) and recovery from acidification (Evans et al., 2005). However, none of these individually can account for the recent rise in DOC concentrations observed; this rise, along with the cause of soil carbon content losses are the subject of debate (Evans et al., 2007; Smith et al., 2007).

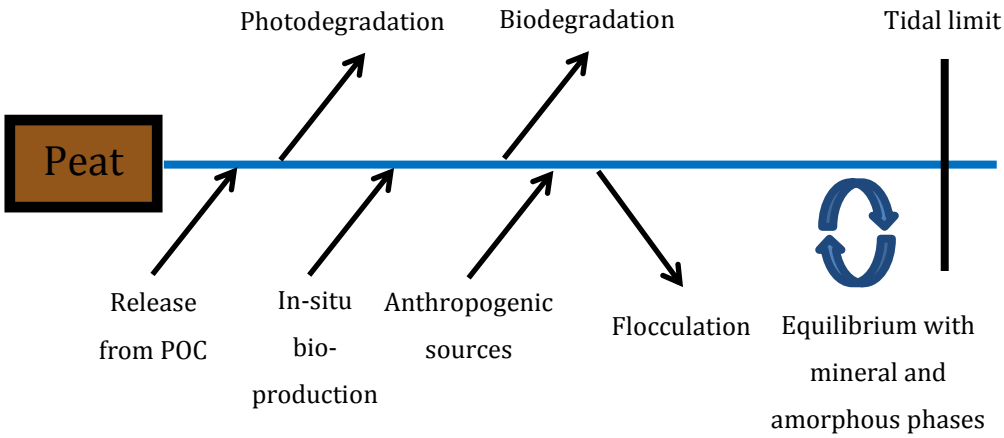
Percentage peat cover and soil carbon content are good predictors of DOC concentrations (Dawson et al., 2004), and DOC fluxes are higher in rivers draining peatlands than draining other land types, suggesting that peatland catchments are contributing quite significantly to the fluvial flux of carbon (Austnes et al., 2010). Hope et al., (1997) found that 65% of the variation in DOC concentrations could be explained by area of surface peat in the catchment. Rivers draining lowland catchments containing no peat tend to have much lower DOC concentrations (Neal et al., 1998).

A number of chemical processes in the water are controlled or influenced by DOC; it plays a part in the complexation and mobilisation of heavy metals (Aitkenhead et al., 1999), the regulation of pH (Dawson et al., 2004) and the availability of nutrients (Qualls and Haines, 1992). It is also a significant energy source for stream biota (Bertilsson and Tranvik, 1998). There are two fractions of DOC, labile and refractory DOC, explained further below (section 1.2.9).

Fluxes of DOC have been measured, estimated and modelled by several authors, (e.g. Kothawala et al., 2014; Wetzel et al., 1977) and estimates of the global DOC river flux vary from 170 Tg C/yr (Harrison et al., 2005) to 400 Tg C/yr (Ludwig et al., 1996). The global pool of DOC is the same order of magnitude as the pool of atmospheric CO_2 (Søndergaard and Middleboe, 1995). For peat-covered catchments in the UK, the estimates of DOC export range from 10.3 to 95.6 Mg C/km^2/yr (Worrall et al., 2012). However, by calculating the flux at the river output, the calculations ignore any processing that may have occurred prior to that point, including loss of dissolved inorganic carbon (DIC), which is known to be

exceptionally high, as lakes and reservoirs tend to be super-saturated with CO_2 compared to the atmosphere (Algesten et al., 2004). The flux calculations also ignore any in-stream processing of DOC and POC (Richey et al., 2002).

Figure 1.1. Schematic diagram of the DOC processing within a peat-sourced stream, adapted from Moody et al. (2013).



Some previous studies have assumed that rivers were passive conduits of DOC, rather than sites of active production and consumption of DOC (e.g. Tipping et al., 2010), but the majority of the literature agrees that some in-stream processing of DOC takes place, even if in only very small proportions (del Giorgio and Pace, 2008). Figure 1.1 shows the processes acting on, and contributing to, DOC in peat sourced natural waters, including flocculation, photodegradation, biodegradation, release from POC, in-situ production and anthropogenic sources. Three of these processes can add DOC to the water: release from POC, in-situ production and anthropogenic sources.

Release of DOC from POC causes decreases in POC concentration, and can be caused by photo- and bio-degradation, as a step towards becoming CO_2 or to remain as DOC (e.g. Billett et al., 2010; Pawson et al., 2006). Autochthonous (in-situ) production of DOC in headwater streams is generally found to be lower than the amount of allochthonous (from outside the stream) DOC (Cole and Caraco, 2001; Eatherall et al., 2000). Autochthonous DOC has been reported as less prone to biodegradation and photosensitisation, meaning that it is resistant to biodegradation and the action of light does not make it biodegradable (Obernosterer

and Benner, 2004). Anthropogenic sources of DOC include waste water treatment and industrial effluent (Kempe, 1984).

Three of the processes in Figure 1.1 would cause the DOC concentrations in the water to decrease: photodegradation, biodegradation and flocculation. Studies have found that of the DOC that enters a river, a large proportion of it is lost in transit, with up to 42% lost (Cole et al., 2007). Worrall et al., (2006) found an average net loss of 40%, whereas Battin et al., (2009a) suggest a lower value of 21% removal. Dawson et al., (2001a) considered a short river reach of 2 km and found between 12 and 18% loss of DOC, and Jonsson et al., (2007) estimated that a higher value of approximately 50% of terrestrially derived OC was mineralised in a lake catchment. These are net losses, as there is likely to be biological production of DOC in the water alongside the processes that decrease the DOC.

Eroding peat catchments are a large source of fluvial DOC and POC, and therefore have a large capacity for in-stream processing of DOC and POC by photo- and bio-degradation, and flocculation. These three processes are described in more detail in sections 1.2.4, 1.2.5 and 1.2.6.

1.2.4. Flocculation and adsorption of DOC

Flocculation is the process by which DOC becomes POC (Figure 1.1), and it is mentioned as a possible reason for the decrease in DOC concentrations in various studies, (e.g. Kopacek et al., 2003; Stubbins et al., 2011) and causes increases in POC concentrations. Flocculation with iron and aluminium can also remove the DOC from solution (Sharp et al., 2006) or at least facilitate aggregation (Maurice et al., 2002). Peat-hosted streams, which are significant sources of DOC (Aitkenhead et al., 2007), tend to be acidic, and although generally low in ionic strength, this can permit Fe and Al to be mobilised, with a consequential potential for flocculation. As stream pH rises through a catchment, this causes Fe and Al-oxyhydroxides to precipitate out of solution; McKnight et al. (1992) showed that such mixing of streams resulted in an average 40% removal of DOC.

1.2.5. Biodegradation of DOC and POC

As mentioned previously in section 1.2.3, DOC serves as an energy source for microbes; this process is biological degradation of DOC (biodegradation; Figure 1.1).

Studies of biodegradation of DOC and DOM have found various losses of DOC in different waters, ranging from 5 to 74%. In upland headwaters in North East Scotland, 5 to 19% of DOM was biodegraded, representing a flux of 854 kg C/km^2/yr (Stutter et al., 2013). Grøn et al., (1992) found that 11% of DOC from an unconfined aquifer was degraded microbially, along with increased bacterial numbers, and Kalbitz et al., (2003) stated that up to 44% of DOM in soil solutions was microbially degraded. Obernosterer and Benner (2004) conducted two-week biodegradation experiments and found an exponential decrease in the DOC concentrations in swamp water from three different sites, with up to 56% of the DOC being mineralised. Servais et al., (1995) carried out several river water incubations in which the DOC and POC concentrations were measured over 45 days. They observed a rapid decrease in DOC in the first day of the incubations, with 6.3 mg C/l consumed in 24 hours, then a slower rate of decrease for the first week, with the DOC concentration stabilising at approximately 20 days. The POC concentrations followed a similar pattern, though the initial decrease was not as fast as the DOC rate. In total, 74% of the initial DOC and 50% of the initial POC was biodegraded in 45 days. Globally, heterotrophic biota in inland waters respire terrestrially sourced OC releasing 1.2 Pg of carbon into the atmosphere each year (Battin et al., 2009b).

1.2.6. Photodegradation of DOC and POC

Photodegradation of DOC is the process by which molecules of DOC are broken down into smaller organic molecules, CO_2 and CO, by light (Figure 1.1; Engelhaupt et al., 2003). The smaller organic molecules can then be broken down further by light, used as an energy source for microbes, or become 'in-edible' (Moran and Zepp, 1997). Miller and Moran (1997) listed the bioavailable products of photodegradation of DOM as formic acid, formaldehyde, acetic acid, acetaldehyde, acetone, propanal, pyruvic acid, citric acid, levulinic acid, glyoxal, methylglyoxal and glyoxylate, all of which are low-molecular-weight compounds and can be readily assimilated by bacteria.

Photodegradation of DOM and photo-bleaching of water (light causing the removal of 'colour' in water) has been extensively studied in various environments: marine (e.g. Estapa and Mayer, 2010; Mopper et al., 1991), estuaries (e.g. Gao and Zepp, 1998; Stubbins et al., 2011), large rivers (e.g. Amon and Benner, 1996b), freshwater wetlands (e.g. Bano et al., 1998), lakes and reservoirs (e.g. Bertilsson and

Tranvik, 1998; Tranvik et al., 2009), and swamps (e.g. Obernosterer and Benner, 2004), however, some studies do not distinguish between photo- and bio-degradation (e.g. Gennings et al., 2001).

Mopper et al. (1991) found that between 12 and 48% of DOC was degraded by sunlight in the 'oceanic mixing cycle'. Miller and Moran (1997) looked at both photo- and bio-degradation in the sea and found significant bacterial growth in the dark, in samples that had previously been exposed to light, compared with samples that had been keep in the dark for the whole experiment. In total, they found 8 to 13% loses of DOC, of which bacterial activity accounted for 4%, and the remaining loss was attributed to photodegradation.

Gao and Zepp (1998) found that estuarine DOC concentrations decreased and the CO and DIC concentrations increased after exposure to artificial light. Southwell et al. (2011) found a 10% loss of organic matter after exposure to light, whereas Stubbins et al. (2011) found total photochemical losses of DOC within the Tyne estuary were small, only 0.13-0.46% of the DOC input. In the Amazon River, DOC losses due to photodegradation were approximately 20% (Amon and Benner, 1996b).

Large bodies of water, such as reservoirs, lakes and the sea, tend to have long residence times, ranging from months to years, meaning that they have been exposed to light for long times. Wu et al. (2005) studied photodegradation in forested headwater streams in Ontario, Canada, stating that they were chosen as they had little previous exposure to light, and therefore the DOM would be more "UV labile" compared to lake water DOM which has already had extensive exposure to light, and would therefore be "insensitive to irradiation", as the lake residence times were between 1.6 and 5.7 years in their catchments. Gennings et al., (2001) used headwater streams for the same reasons. Grzybowski (2000) points out the prior exposure to light for coastal DOM was likely to "exhaust its potential", affecting the photodegradation rates observed for coastal DOM compared with riverine DOM. Larson et al., (2007) note that forested streams are shaded, and so have lower light exposure than lakes, and therefore there is less photo processing of the DOM in the water.

Granéli et al., (1996) reported rates of photodegradation in lakes between 0.009 and 0.4 mg C/l/day, and Amon and Benner (1996b) found Amazonian river DOC consumption rates of 54 μg C/hour in the light, compared to 4.8 μg C/hour in the dark. Anesio and Granéli (2003) found DOC loses between 45 and 50% due to

photochemical mineralisation. These were all studies conducted on filtered water samples and so represent actual photodegradation rates. Some studies do not distinguish between photo- and bio-degradation as the samples are not filtered, such as Gennings et al., (2001) who found that headwater streams lost 72% of DOC to an inorganic product (CO_2 or CO) in 23 days in the light (dark controls lost 9% DOC); the rate was considered to be a first-order reaction. The loss was due to total degradation, rather than solely due to the light exposure as there were microbes present in the water. The production of carbon dioxide is discussed further in section 1.2.7.

It is not only DOC that is susceptible to photodegradation: Estapa and Mayer (2010) found POC loses of 17 to 28% in estuarine sediment water samples irradiated for 18 to 24 hours.

Wu et al., (2005) found higher photodegradation rates of loss of DOC in water of lower pH, as did Anesio and Granéli (2003), suggesting that, as peat-sourced streams tend to be acidic, there should be high rates of loss due to light exposure in these environments. Bano et al., (1998) added nutrient solutions to acidic freshwater wetland samples and found that bacterial production rates were enhanced in irradiated samples by 34% (no nutrients), 63% (nitrates) and 74% (nitrates and phosphates) relative to the samples kept in the dark. They also found that when DOM was exposed to sunlight it was phosphate that limited the rate of bacterial productivity, with the other nutrients resulting in smaller increases than the phosphate treatment. The bacterial productivity was used as a proxy measurement for the photo- and bio-degradation of DOM, as DOM is a substrate for bacterial growth, and shows that the degradation of organic matter is limited by nutrient availability and enhanced by exposure to light and nutrient additions.

Due to the proven effect of light on DOC, it would be expected that the natural day/night cycle would affect the rates of DOC change in natural waters, especially in peatland waters where the concentrations of DOC and POC are high. The rates of loss during daylight would be higher than during darkness, as both photo- and bio-degradation can occur in the light, but only biodegradation will happen at night. The diurnal cycle of in-stream processes is discussed further in section 1.2.8.

1.2.7. Carbon dioxide (CO_2)

There is a growing body of evidence suggesting that the losses of OC from water via photo- and bio-degradation are lost as CO_2, either dissolved in the water as DIC or evolved directly to the atmosphere (e.g. Kling et al., 1991; Stutter et al., 2013). Therefore these two processes were investigated as they have the potential to contribute CO_2 to the atmosphere.

Aufdenkampe et al., (2011) linked the CO_2 evolved from water to terrestrial OC, and comments that the rates of erosion will affect the global-scale C fluxes. Pawson et al., (2006) states that POC can be readily transformed directly into CO_2, or indirectly via DOC. Köhler et al., (2002) recovered 70% of the loss of Total OC (TOC = POC + DOC) as CO_2 from closed-system incubations exposed to artificial light. Similar incubations in the dark found little or no change in TOC concentrations.

1.2.8. Diurnal cycles

If photodegradation is an important process that could control the fate of DOC and POC, then the turnover of DOC and POC should show a diurnal cycle, due to the exposure to light during the day. Diurnal cycles of phosphate and iron concentrations were shown by Moran and Zepp (1997) and Gao and Zepp (1998), respectively, and Kaplan and Bott (1982), Engelhaupt et al., (2003), and Worrall et al. (2013a) found diurnal cycles of DOC and DOM concentrations. Kaplan and Bott (1982) found that the DOC concentrations increased during the day from a pre-dawn minimum to a late afternoon maximum, then fell again after sunset.

Studies of the fluxes of in-stream processes that do not consider diurnal cycles risk under/over estimating of fluxes as a result (e.g. Köhler et al., 2002; Moran et al., 2000), as discussed by Walling and Webb (1985) and Worrall et al. (2013a). The problems arise when fluxes are extrapolated from samples taken daily when the variable being measured could be subject to a diurnal cycle, especially if it is the result of a light or temperature dependent reaction, as most in-stream biological processes are. Worrall et al. (2013a) found that 87% of nitrate measurements from rivers across the UK were taken between 9 am and 5 pm, during the working day. These samples cannot be representative of the average flux or concentration for that day unless samples were also taken during the night, when the rates of change for the production and consumption of nitrates will be different to the rates during daylight. Seasonal variations in daylight length and temperature will also affect the

rates of change (Kaplan and Bott, 1982). DOC and POC release from soils can be event-driven, and so rainfall can cause short-term increases in the concentrations of both that could cause over-estimates of fluxes if samples were taken during storm events (Austnes et al., 2010).

Calculating fluxes of DOC based on samples taken more frequently than daily will allow for the diurnal cycle to be taken into account, and could reveal detail about the in-stream carbon dynamics and relative rates of photo- and bio-degradation.

1.2.9. Labile and refractory DOC

DOC is broadly composed of two fractions, a labile fraction that is easily degraded, and a refractory/recalcitrant fraction that has longer turnover times and is less bio-available (Young et al., 2005); the composition of the DOC is thought to affect the rates of photo- and bio-degradation (Amon and Benner, 1996a). Microbes and light break down the labile DOC, whereas the refractory pool accumulates in the water, leading to up to 80% of DOC in inland waters being refractory (Wetzel and Tuchman, 2005). The labile pool is comprised of low molecular weight carbohydrates, amino acids, peptides, small carboxylic acids and alcohols (Kulovaara et al., 1996; Sachse et al., 2005), whereas the refractory component has higher molecular weights, is more aromatic, and composed of lignin and humic compounds (Tranvik et al., 2009). The two components can be broadly separated by molecular weight (e.g. Abdulla et al., 2010a; Amon and Benner, 1996a), allowing for characterisation of the fractions.

Peatland headwater catchments have 'fresh' and labile DOC that has not been exposed to light for long periods of time, and so they are good environments in which to study the turnover and composition of DOC.

1.2.10. Composition of DOC

The name 'DOC' is a widely used, rarely defined term, often described as comprising of organic compounds such as fulvic and humic acids. It is operationally defined as anything smaller than 0.45 μm, but what is actually is composed of is less well known.

Studies have attempted to classify the exact composition and character of DOC, using various different methods of analysis, including ^{13}C Nuclear Magnetic Resonance (NMR; e.g. Abdulla et al., 2010b; Wetzel et al., 1995), Fourier Transform Infrared Spectroscopy (FTIR; e.g. Davis et al., 1999; Mursito et al., 2010), thermal analysis (e.g. Peuravuori et al., 1999; Plante et al., 2009), Pyrolysis Gas Chromatography/Mass Spectrometry (Pyrolysis GC/MS; e.g. Christy et al., 1999; Leinweber et al., 2001) and elemental analysis (e.g. Provenzano and Senesi, 1999; Worrall et al., 2013b). DOC from different sources were analysed by Aitkenhead and McDowell (2000) who found that the C:N ratio of DOC was a good predictor of the DOC flux at the biome scale, with predicted fluxes being within 4.5% of the measured DOC flux. However, less work has been done linking structural aspects of DOC with measured degradation rates. One study that links physical and chemical characteristics of DOC to degradation is Baldock et al. (2004), where ^{13}C NMR was used to model the composition of DOC and explain biochemical recalcitrance and decomposition rates.

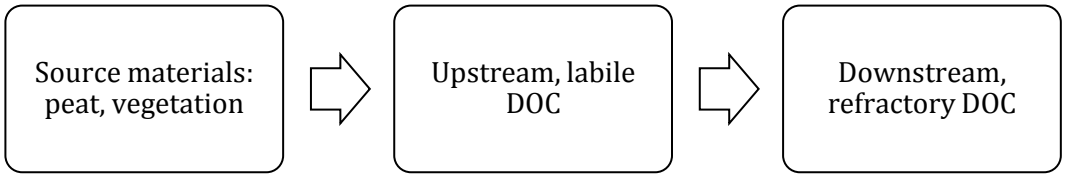
Various studies have used specific absorbance at different wavelengths (e.g. Austnes et al., 2010; Glatzel et al., 2003) or a ratio of absorbance at two different wavelengths (e.g. E2:E3 (Klavins et al., 2008), E4:E6 (Davis et al., 1999; Wilson et al., 2011) as proxy measurements of DOC composition, as these can offer some information on the humic and fulvic content. Lower E2:E3 ratios reflect higher molecular weights and aromaticity (Yang and Xing, 2009). Chen (1977) studied the E4:E6 ratio and concluded that the ratio "is governed primarily by the particle sizes and weights" of humic and fulvic acids, and is related to the pH, oxygen and carbon content, and the abundance of -COOH groups in the molecule. The study agreed with the results of Kononova (1966), that a high E4:E6 ratio reflects a low degree of aromatic condensation, with large proportions of aliphatic structures. E4:E6 and E2:E3 ratios can only be used to infer information about the composition but not about the rate of degradation of the DOC.

The number of studies conducted investigating the composition of DOC shows the complexity of the substance, that there is not one set method to be used that can show what it is, where it came from or how best to quantify or qualify it.

From the literature it can be concluded that there are several different types of DOC, partially explained in section 1.2.9, that differ in many aspects, but can be broadly categorised as 'labile' and 'refractory'; however the chemical differences between these categories are not widely known. It is known that upland streams

contain higher proportions of allochthonous DOC, which is more labile, and that lower down stream the proportion of autochthonous DOC increases, which is more refractory (Cole and Caraco, 2001; Eatherall et al., 2000). It is hypothesised that the DOC composition changes as it travels downstream, as in Figure 1.2. The reason for these changes may simply be the changing influence of the auto/allochthonous contributions to the DOC concentrations in the river; however there is no method to distinguish between these two processes.

Figure 1.2. The hypothesized pathway of DOC composition from headwater to sea.



1.2.11. Current state of knowledge

As outlined above, there are numerous studies of the photo- and bio-degradation of DOC and POC; however there are some gaps in these studies:

- The photo- and bio-degradation studies mentioned above were often specifically looking at one or the other, with water samples being kept in the dark (e.g. del Giorgio and Pace, 2008; Raymond and Bauer, 2001a), or filtered/sterilised to remove microorganisms, (e.g. Lindell et al., 2000; Soumis et al., 2007).
- Some experiments have not distinguished between photo- and bio-degradation, looking at total losses of DOC without differentiating between or quantifying either process (e.g. Cole and Caraco, 2001; Gennings et al., 2001).
- Studies have been conducted in a variety of ecosystems, with several looking at ecosystems where the residence time of the water has been much longer than the average residence time of rivers in the UK (e.g. lakes, reservoirs, the sea; Anesio and Granéli, 2003; Tranvik et al., 2009). Where the residence time is long, the DOC will be old, and therefore less reactive. Studies from

shorter residence time systems, such as UK rivers, should be on very fresh fluvial organic matter.

- Studies have used artificial light, or 'solar simulators' to ensure the water is exposed to a known quantity and quality of light (e.g. Benner and Kaiser, 2011; Köhler et al., 2002), however these are often replicating the natural summer radiation, and so would not be representative of the whole year, and may not include all wavelengths present in natural light.

Stutter et al. (2013) conducted a study on the degradation of DOC from peat-sourced, small headwater streams in Scotland and found biodegradability of DOM ranged from 5 to 19% in batch incubations. However, after analysing the initial DOC concentration in the water, subsequent samples were not taken for two days, giving the labile DOC plenty of time to breakdown and be lost as CO_2 before the next samples were analysed. Also, the initial DOC concentration was obtained by adding fulvic acid or glucose to sterile river water at a standard pH, rather than using 'natural' DOC, making the data gained useful, but perhaps not representative of the source DOC.

The C budget of peatlands cannot be complete until the full impact of in-stream processing of DOC and POC is included, as the existing budgets have no allowance for the ultimate fate of this carbon, other than burial in the sea (e.g. 2013), which has been shown to be the case for only the minority of terrestrial carbon (Battin et al., 2009b). There are also gaps in knowledge regarding the effect that changes in DOC composition can have on the degradation rates of DOC (Leinweber et al., 2001; Reiche et al., 2010). Therefore, the aims of this thesis were to fill these research gaps, by investigating and quantifying the net losses of fluvial C from a peat-hosted river, and analysing the effect of DOC composition on the rates of loss. This was achieved through a series of monthly degradation experiments spanning time scales between 30 hours and 10 days, alongside monthly analysis of DOC composition. All measurements of DOC loss are the net changes, as it was not possible to distinguish between autochthonous and allochthonous DOC.

1.3. Aims and Objectives

Given the current state of knowledge, this thesis aimed to quantify the rates of biodegradation and photodegradation of carbon from a peatland headwater. The aims of the first three experimental chapters (Chapters 2, 3, and 4) were to investigate the rates of OC bio- and photo-degradation over three different time scales and in two different river systems. The aim of the Chapter 5 was to look at how the composition of DOC, and various types of organic matter, influenced the degradation of DOC in the river. The aims of Chapters 6 and 7 were to investigate the effects of nutrients on the rates of DOC degradation. All experiments were designed to be fully factorial, so as to better inform the statistical analysis.

The thesis can be broadly divided into three experimental sections, DOC degradation, DOC composition and nutrient experiments, as outlined in Table 1.1.

Table 1.1. The objective and approach of the six experimental chapters.

Chapter	Objective	Approach
2	Investigate long time scale degradation	Monthly ten-day experiments on the River Tees, with light and dark treatments.
3	Investigate shorter time scale degradation	Quarterly, in-situ, 30-hour experiments on the River Ashop, with light and dark treatments, and sub-daily sampling.
4	Investigate medium time scale degradation, combining techniques from Chapters 2 and 3.	Monthly 70-hour experiments on the River Tees, with light and dark treatments, and sub-daily sampling.
5	Investigate the composition of DOC and relate it to the rates of degradation.	Combined analysis of DOM, POM, vegetation, peat and litter samples with water samples and rates of DOC degradation to produce a model of the factors affecting DOC degradation in the river.
6	Investigate the effects of nutrients on the rates of DOC degradation, to see if the nutrient concentrations were limiting the biodegradation of DOC.	Bi-monthly 70-hour experiments on the River Tees, with three nutrient treatments.
7	Investigate the effects of nutrients in a naturally high nutrient lowland river, and the effect of the confluence of the upland and lowland rivers on the DOC dynamics.	Bi-monthly 70-hour experiments on the River Tees, with three nutrient treatments.

The conclusion drew together all the analysis from the previous chapters to provide a summary of the key findings, and suggests possible areas for further work.

Chapter 2:
The rate of loss of DOC through a catchment[1]

2.1. Introduction and Aims

As outlined in Section 1.2 (Chapter 1), peatlands represent a large store of carbon, and in the UK alone store 2.3 Pg C (Billett et al. 2010). Peatlands therefore contribute a large amount of carbon to the fluvial system, with percentage peat cover in a catchment being a good predictor of DOC concentrations in peat-sourced rivers. Upland rivers with short residence times have high concentrations of labile DOC and therefore the potential for in-stream processing, such as bio- and photo-degradation of DOC. A review of the existing literature on DOC, in-stream processing, and labile and refractory DOC are outlined in sections 1.2.3, 1.2.4, 1.2.5, 1.2.6 and 1.2.9 in Chapter 1.

The objectives of this chapter were to measure the net loss of DOC from the upland River Tees in northern England, looking at a stretch from source to sea along a short residence time river system. The longer residence-times of lakes, which receive DOC from rivers, mean that the DOC may be several days old and so already refractory by the time it is studied in the lake. Investigating at an upland river with a shorter residence time means that the DOC should be younger and more easily degraded. The aim was to also assess the controls on DOC degradation and loss, by measuring various covariates and analysing the DOC changes in the context of all variables measured. Once the variables that had a significant effect on the DOC concentrations were identified, they were used to estimate the extent of loss of DOC across a catchment, using modelling to extrapolate to the larger catchment scale.

[1] This chapter is based on a paper that has been published in the Journal of Hydrology:

Moody, C.S., Worrall, F., Evans, C.D., Jones, T.G., 2013. The rate of loss of dissolved organic carbon (DOC) through a catchment. Journal of Hydrology 492, 139–150.

2.2. Approach and Methodology

For this chapter, in-situ degradation measurements of DOC were conducted, from the headwater to the former tidal limit of a major UK river, the Tees, whose headwaters are peat-covered and where DOC fluxes have been extensively studied (e.g. Worrall et al., 2008). The in-situ experiments were conducted so that it was possible to measure total net loss of DOC; loss of DOC in darkness (and therefore by difference the loss due to photolytic processes); and the rate of each of these processes. Results from degradation experiments were used to construct empirical rate laws that were combined with a time series of headwater DOC concentration and estimates of in-stream residence times so that estimates of total DOC loss from the catchment could be made.

2.2.1. Field sites

This chapter considers four sites along the River Tees, northern England (Figure 2.1, Table 2.1). The River Tees flows 132 km from its source at Moor House National Nature Reserve (NNR), before draining into the North Sea, although the estuary is cut off by a total exclusion tidal barrage. Four sites were chosen from upstream of the barrage that differed by almost orders of magnitude in their upstream catchment area, each of which was co-located with a river flow gauging station. The two lowest order stream sites (Cottage Hill Sike, CHS and Trout Beck, TB – Table 2.1) are within the Moor House NNR, the most extensively studied of all UK peatlands (Billett et al., 2010), with 100% and 91% deep peat cover within their respective catchments. The Moor House NNR is part of the Environmental Change Network (ECN) monitoring programme which means that DOC concentration has been monitored in the streamwater at these sites weekly since 1993 (Worrall et al., 2009). Equally, the most downstream site (Broken Scar, DBS) is co-located with a water treatment works where water colour (not DOC concentration) has been measured daily since 1970 (Worrall et al., 2008).

The Trout Beck (TB) catchment altitude range is between 841.7 m and 533 m AOD, and is underlain by predominantly moderate permeability bedrock with generally low permeability superficial deposits. The land cover is dominated by peat (90.8%) with the rest of the catchment being 0.3% woodland and 7.9%

grassland. The average rainfall data for the period of 1961 to 1990 was 1902 mm/yr for the catchment.

The second largest site, at Middleton-in-Teesdale (MIT), has a catchment area of 242.1 km^2, and is 211.2 m AOD. The underlying geology is moderate permeability bedrock, largely carboniferous limestone, with low and mixed permeability superficial deposits. The land cover is 47.6% peat, 48.5% grassland, 1.1% woodland, 0.1% arable/horticultural and 0.1% urban. The average rainfall data for the period of 1961 to 1990 was 1533 mm/yr for the catchment.

The most downstream site, DBS, has a catchment area of 818.4 km^2, and is 37.2 m AOD. The catchment land use is 4.4% woodland, 13% arable and horticulture, 45.4% grassland, 0.4% urban and 33.9% peat. The maximum altitude in the catchment is 884.8 m AOD, and the average rainfall data for the period of 1961 to 1990 was 1141 mm/yr. The majority of the underlying geology of the catchment is moderate permeability millstone grit and carboniferous limestone, with mixed permeability superficial deposits of peat and boulder clay.

Figure 2.1. Location of the Tees catchment and monitoring sites.

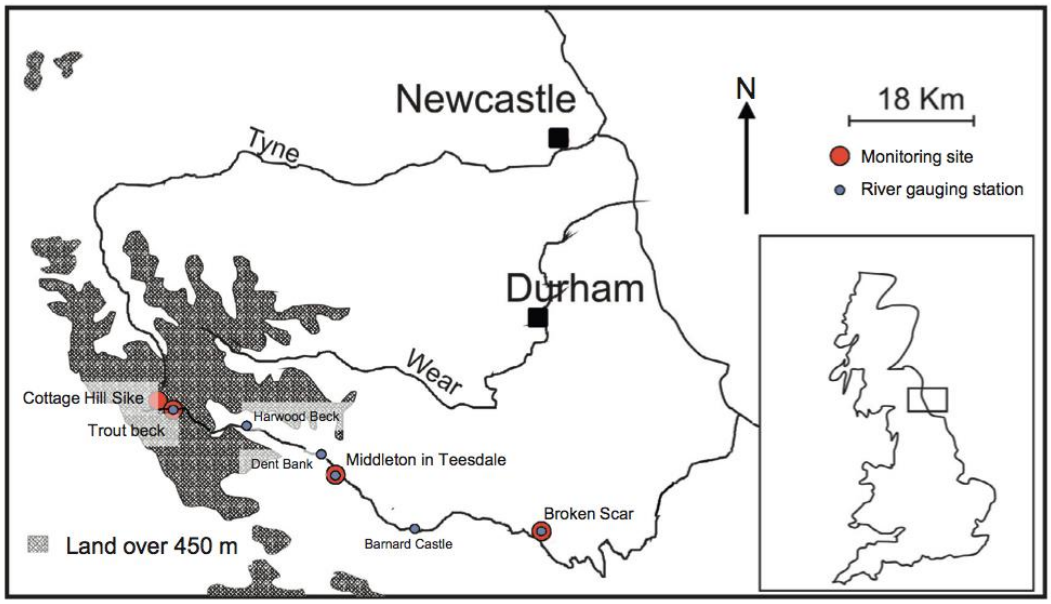


Table 2.1. Areas and locations of all field sites, with catchment areas and percentage peat (mountain/heath/bog) covers are taken from literature.

Site	Site code	National grid reference	Catchment area (km^2)	Peat cover (%)
Cottage Hill Sike	CHS	NY 744 327	0.2	100
Trout Beck	TB	NY 759 336	11.4	90.8
Middleton-in-Teesdale	MIT	NY 950 250	242.1	47.6
Broken Scar	DBS	NZ 259 137	818.4	33.9

2.2.2. Degradation measurement

The degradation measurements were made outside of the laboratory, in ambient light and temperature conditions (rather than indoors under artificially controlled conditions).

The experimental design considered degradation in light and dark so as to distinguish between possible components of degradation (e.g. photo-induced degradation), and measured degradation over timescales relevant to river residence times. Experiments were conducted each month over the course of a year in order to experience a range of both meteorological conditions and DOC concentrations and compositions. The samples were not pre-filtered to exclude particulates, because this meant that the experiment considered the net fate of DOC and could include production from POC or adsorption by it.

Water samples were taken on a monthly basis from the four sites on the River Tees (Table 2.1). December and January samples were only obtained from two sites; poor weather conditions prevented the two sites within the Moor House NNR from being visited. Each degradation experiment spanned 10 days with sacrificial sampling taking place on day 0, 1, 2, 5 and 10, and light and dark treatments for each site. Duplicate samples, where two quartz tubes were sacrificially sampled instead of one, were included within each degradation experiment and over the course of the year all combination of factors were replicated. No day_0 samples were replicated, but 44% of all other measurements were replicates (285 of 646 samples). Replication was limited by practical constraints of the number of quartz tubes available and the time taken to process DOC analysis.

A data logger with a PAR (photosynthetically active radiation) meter and thermocouple recorded the radiation levels and air temperature at 15 minute intervals throughout the 10-day period of each month's experiment. Radiation and temperature conditions were summarised as the average conditions over the period for each sample and PAR measurements were summed to give the total radiation experienced by a sample. These were treated in this way because a sample after 10 days may have experienced the same average radiation as a sample after 1 day but will have received a larger total radiation dose. By including radiation and temperature variables it was possible to estimate the apparent quantum yield and the activation energy for DOC photodegradation.

The water was then poured into acid-washed, quartz glass tubes, stoppered with a rubber bung at the bottom, and loosely stoppered at the top. Quartz glass allows all light wavelengths to pass through it. Dark samples were wrapped in foil to prevent exposure to light. All samples were outside in trays, with all tubes lying at an angle to prevent rainfall entering and the sample evaporating or pouring out. The angling of the tubes also stopped the light samples being shaded by the top bung and exposed a larger surface area of water to light. The samples were moved to different positions daily to avoid any bias in shading from nearby trees.

2.2.3. Sample analysis

Upon each day of sampling the respective quartz tube for each site, each treatment and replicates, where appropriate, were sacrificially sampled and sub-samples frozen for subsequent analysis for DOC concentration: it is assumed that the freezing and thawing of samples did not alter DOC concentrations.

Samples for DOC analysis were defrosted and filtered to 0.45 μm and the DOC concentration measured using the wet oxidation method described in Bartlett and Ross (1988). DOC concentrations were calibrated using standards of oxalic acid with known concentrations, and only calibration curves with an r^2 of 0.95 or above were used. The Bartlett and Ross method is accurate between 2 and 60 mg/l DOC and samples were diluted so as to be within this range. Samples with a higher DOC concentration were diluted using deionised water, which was also used as a blank. Absorbance at 400, 465 and 665 nm was measured and the 'E4:E6' ratio (abs at 465 nm/abs at 665 nm) recorded. Absorbance at 400 nm is a basic (visible) colour reading and the specific visible light absorbance was taken as the absorbance at 400

nm divided by the DOC concentration of the sample. The E4:E6 ratio is a measure of DOC composition, with higher ratios indicating a greater degree of humification (Thurman, 1985). All optical measurements were performed using a UV–Vis spectrophotometer, with a 1 cm cuvette. Blanks of deionised water were used.

Suspended sediment concentration was measured in samples at the beginning and end of the experiments. Samples were filtered through pre-weighed, 0.45 μm glass fibre filters; dried to 105 °C and the filter paper re-weighed to give the concentration of particulate matter. The composition of the particulate matter was not analysed and particulate concentrations were only measured in a sample of 50 ml volume.

A number of additional water analyses were performed in order to provide covariate information. Alkalinity or acidity was measured by titration at the beginning and end of each experiment. An acidity or alkalinity titration was carried out (in the field on day 0), titrating 20 ml of river water against either 0.1 M sodium hydroxide (NaOH) or 0.005 M hydrochloric acid (HCl), using five drops of phenolphthalein or bromophenol blue, respectively, as chemical indicators of pH change. Three titrations were carried out for each site and treatment, and the average volume of acid/alkali used was recorded. Conductivity, pH, and water temperature of samples as it left each quartz glass vial were measured by electrode methods. Ion Chromatography was used to measure the concentrations of certain anions: fluoride, bromide, chloride, nitrate, phosphate and sulphate. The system used for the ion chromatography (Metrohm 761 Compact IC and 813 Compact Autosampler) was accurate down to 50 ppb. Cations such as Fe and Al were not included in the analysis. However, the stream water at CHS on the Tees is regularly sampled as part of the monitoring programme of the Environmental Change Network (www.ecn.ac.uk – Sykes and Lane, , 1996).

The concentrations of DOC and the specific absorbance were analysed in both absolute and relative terms where the relative value for each sample in an experiment was expressed as the ratio of the measured value to measurement on day_0 for the same experimental run.

2.2.4. Statistical methodology

The design of the experiment incorporates four factors: Month, Day, Site and Treatment. Each factor has a number of levels: Month has 12 levels (one for each

calendar month): Day has five levels (days 0, 1, 2, 5 and 10); Site has four levels (CHS, TB, MIT and DBS); and Treatment has two levels (light and dark).

An analysis of variance (ANOVA) was used to assess the significance of all four factors and where possible the interactions between the factors were also determined. Furthermore, the analysis was repeated including covariates (ANCOVA). The covariates were: pH, conductivity, absorbance at 400 nm, E4:E6 ratio; anion concentrations; and light and temperature variables. The instantaneous river flow at the time of sampling was not available for this chapter. The ANOVA and ANCOVA were performed separately so as to explore what effects existed and whether they could be explained by the available covariates. The magnitude of the effects, in this case generalized ω^2 (Olejnik and Algina, 2003), of each significant factor and interaction were calculated. Post-hoc testing of the results was made for pairwise comparisons between factor levels using the Tukey test in order to assess where significant differences lay. There are several assumptions associated with using the ANOVA approach. Firstly, the Levene test was used to assess homogeneity of variance with respect to the factors in ANOVA; if this test failed then data were log-transformed. It should be noted that ANOVA is robust against the assumptions of homogeneity of variance and normality of the data. Secondly, the Anderson–Darling test was used to ensure that the data were normally distributed; if not the data were log-transformed. Thirdly, to avoid type I errors all probability values are given even if significance was assessed at the 95% level.

2.2.5. Empirical modelling

The statistical analysis of the Tees data was used to direct the development of empirical models of DOC loss. Multiple linear regression was used to develop the empirical model based upon terms identified from the ANOVA and including interaction terms. Only variables whose effect was significant at least at 95% probability of not being zero were included in the developed model with the further caveat that final models were also chosen so as to be physically interpretable. The month factor was transformed into the sinusoidal function:

$$\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) \qquad \textit{(Eq. 2.1)}$$

where *m* is the month number (January = 1 to December = 12). Some of the variables were transformed for the sake of physical-interpretability, e.g. reciprocal of the absolute temperature. When statistically significant multiple regression equations were derived a partial regression analysis was performed to assess the importance of each significant term.

The modelling of net catchment losses required an estimate of the in-stream residence, and therefore this chapter used the approach of Worrall et al., (2013a) in order to calculate the in-stream residence time from source to a monitoring point lower in the catchment (Table 2.2). Further modelling methods can be found in Moody et al., (2013).

Monitoring of stream water DOC concentration in the catchment headwaters was weekly, and so observed concentrations were paired with the flow measurement for the same time at the Trout beck, and then in-stream residence time calculated for that flow. Given the in-stream residence time for a given initial concentration of DOC it was possible to calculate the export from the DOC source and the expected loss to tidal limit of the river, i.e. the point at which the river enters the estuary.

2.3. Results and Discussion

It was possible to generate a sample size of 690 DOC concentrations with complete covariate information and within the context of the factorial design. Summary of the water chemistry at the two sites at the extremes of the sites over the 10 days of the experiment period in daylight conditions is given in Table 2.3. The iron (Fe) and aluminium (Al) concentrations for the headwater stream (CHS) are below those reported for photo-aggregation by Maurice et al., (2002).

Some of the covariates showed the same pattern between Day 0 and Day 10, at both of the two sites shown in Table 2.3. The suspended sediment concentration decreased, and the conductivity, pH, absorbance at 400 nm, bromide and fluoride concentrations increased. All the anion concentrations increased at CHS, whereas the bromide and fluoride were the only two to increase at DBS. The E4:E6 ratio decreased at CHS and increased at DBS. Of these changes, only seven were significantly different between Day 0 and Day 10 across both sites: the suspended sediment decrease at CHS ($p=0.0023$) and DBS ($p=0.0053$), the conductivity

($p=0.0041$), pH ($p<0.0001$), nitrate ($p=0.0437$), and sulphate ($p=0.0410$), increases at CHS, and the pH increase at DBS ($p<0.0001$).

The concentrations of the conservative ions (bromide and chloride) were investigated further to show how much natural variability there was in the dataset. An ANOVA on the whole dataset showed there were no significant differences between the Site, Time and Treatment for bromide, however there were significant differences for the chloride, which was all due to the differences between the concentrations at the four sites. Post hoc tests showed that this was all down to the differences between DBS and the three other sites.

The changes in anion concentrations, pH and conductivity suggest that some of the water was likely to have evaporated, increasing the concentrations, or that some of the anions were utilised in the production of biomass. However, there were no significant changes in the conservative ions (bromide and chloride) between the beginning and end of the experiments, suggesting that there was little evaporation of the water, and the other changes were likely due to natural variability. The changes in the average absorbance at 400 nm and the E4:E6 ratio suggest that the DOC underwent some compositional changes during the experiment.

The alkalinity data was not used in further analysis as to produce accurate DIC measurements requires cation data, which was not available from the ECN for the dates sampled, and could not be analysed from the water sampled.

Table 2.2. Details of River Tees gauging stations as required for calibration of in-stream residence time.

Gauging station	Catchment area (km^2)	Mainstream river length (km)	Altitude (m asl)	1085 Slope (m/km)
Broken Scar	818	79	37	6.9
Barnard Castle	509	51	133	9.8
Middleton	242	32	211	12.9
Dent Bank	217	29	227	17.8
Harwood Beck	25.1	9.7	374	26.5
Trout Beck (Moor House)	11.4	5.1	533	35.8

Gauging station	Bankfull discharge (Q_{bf} – m^3/s)	Bankfull width (W_{bf} – m)	Bankfull depth (d_{bf} – m)
Broken Scar	384	12	2.44
Barnard Castle	257	10.4	2.04
Middleton	115	9.4	2.19
Dent Bank	93	9.3	2.36
Harwood Beck	19	8.8	1
Trout Beck (Moor House)	4	6.1	0.53

Table 2.3. The average and coefficient of variation (CV - %) of the 12 variables measured from both sites (CHS and DBS), averaged across all sampling months. The table shows the initial (Day 0) and end (Day 10) concentrations for each variable from the light treatment.

	Cottage Hill Sike (CHS)				Broken Scar (DBS)			
	Day 0		Day 10		Day 0		Day 10	
Determinant	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Suspended sediment (mg/l)	95	103	21	45	28	45	10	52
Conductivity (µS/cm)	35	38	57	36	317	90	528	151
pH	4.5	13	6.3	5	7	6	7.3	7
DOC (mg C/l)	112	67	24	77	30	55	9	83
Abs_{400}	0.14	37	0.16	35	0.06	47	0.08	26
E4:E6	6.1	31	5.7	34	4.8	69	5.6	88
Bromide (mg/l)	0	62	0.02	210	0.22	92	0.37	89
Chloride (mg/l)	5.9	71	9.3	136	51.8	130	24.4	163
Fluoride (mg/l)	0.2	42	0.5	119	0.3	39	0.6	56
Nitrate (mg/l)	0.3	70	0.6	58	13.2	156	7.6	188
Phosphate (mg/l)	0.9	45	1.3	127	2.6	167	1.8	173
Sulphate (mg/l)	4.4	123	10.4	80	39.7	93	25.7	85
Iron (mg/l)*	0.62	70						
Aluminium (mg/l)*	0.09	71						

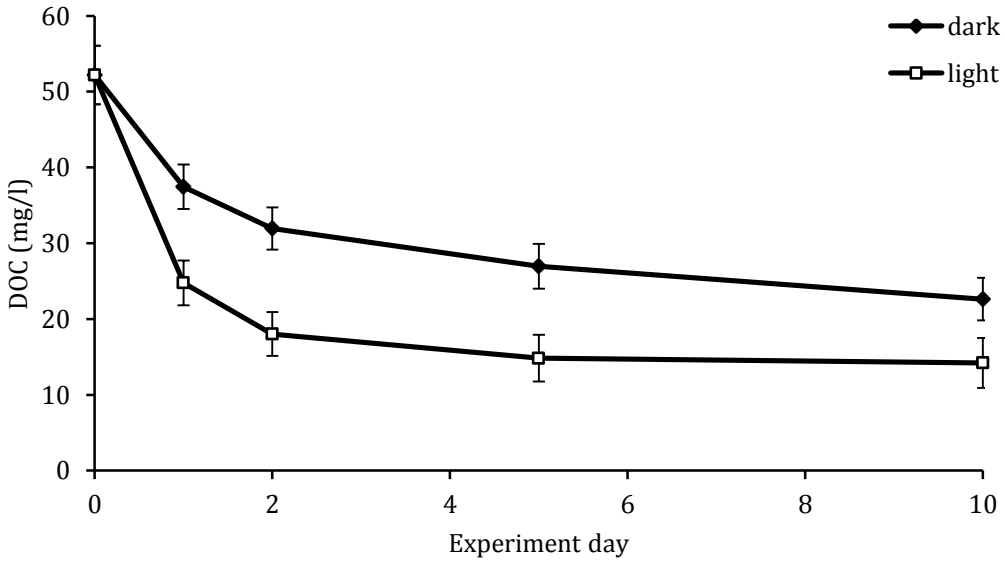
* Average values taken from all sampling reported for CHS from Environmental Change Network monitoring

2.3.1. DOC concentrations

For nearly every month of measurement the DOC concentration in both treatments decreased. The average DOC concentration over time over all sites showed a steep initial decline, although the rate of decline was still not zero after 10 days (Figure 2.2) suggesting that further decreases would have occurred if the experiments had continued for longer. The average decline in DOC concentration across all months for all sites for samples in daylight was from 51 to 14 mg C/l after 10 days: when concentrations were judged relative to the day_0 DOC concentration (DOC_0) at each site then the average decline over 10 days was 76%. For experiments only in the dark the average decline over a 10-day period was 47%. The average difference across all sites and all times between samples in light and dark was 11.8 mg C/l with day_{10} DOC concentrations of samples kept in the light being on average 29% lower than those kept in the dark when judged relative to the DOC_0 concentration. Larson et al., (2007) compared DOC concentrations in samples of stream water kept in light and dark conditions for 24 hours of normal sunlight and found an average decrease between 5% and 10%.

Of all the experiments run, there were 66 samples (out of a total of 690 samples) where an increase in DOC concentration was observed. In 14 of the cases there was a higher day_{10} DOC concentration (DOC_{10}) than DOC_0. Given that no raw water samples were filtered prior to inclusion in the experiment it was possible that particles or the microbial population within the sample generated DOC over the course of the experiments. Samples where there was an increase in DOC over the course of the experiment were not removed from the analysis.

Figure 2.2. The main effects plot of DOC concentration change across all sites for light and dark over the ten days. Error bars are the standard errors.



2.3.1.1. ANOVA on DOC concentrations

The Anderson–Darling test showed that neither the distribution of DOC concentration nor relative DOC concentration for the experiments conducted in the light and those in the dark met the condition of normality, therefore all subsequent ANOVA were performed on log-transformed data, which did exhibit a normal distribution. Conversely, the Anderson–Darling test of the photo-induced degradation data (i.e. the difference in concentration between experiments performed in the light and dark) was normally distributed and so this was not transformed further.

When the relative concentration data for all treatments (daylight and dark) were considered without covariates, all single factors were found to be significant (Table 2.4). The least important single factor was Site (explaining only 0.4% of the variance in the original dataset). One of the reasons for using relative DOC concentration was to minimise the difference between sites, and so this result indicates that this was largely effective. Post-hoc testing showed that the difference between sites was largely associated with the difference between the CHS and MIT, but not between CHS and DBS. There were no significant interactions between the Site factor and any other factor. The most important factor was Day, i.e. the time over the experiment with all days being significantly different from each other. The

second most important factor was the difference between Treatments, with the relative DOC concentration in the light being 48% lower than those kept in the dark. Indeed the most important interaction was that between Day and Treatment factors which reflects the difference in the curves illustrated in Figure 2.2. There was a significant effect due to Month but this may reflect the importance of the DOC_0 concentration for the degradation rate (with faster degradation rates associated with higher initial concentrations) rather than a seasonal cycle in degradation behaviour per se, which also explains the significant interactions between the Month factor and the Day and the Treatment factors. Overall the ANOVA of the relative DOC concentration explains 62.7% of the variance in the original data, i.e. the error term represents 37.3% of the variance. This error term represents the unexplained variance which was not only due to sampling or measurement error but also variables, factors or their interactions that were not or could not be included: inclusion of covariates should decrease this term.

Inclusion of covariates into the ANOVA did increase the proportion of the variance explained, by 4% (Table 2.4). However inclusion of covariates did not make any of the factors or interactions insignificant; on the contrary, inclusions of significant covariates increased the importance of the differences between sites even when relative DOC concentration was being tested. The most important covariate was the specific absorbance, which significantly declined with increasing DOC concentration. The second most important covariate was the DOC_0 concentration, where relative concentration declined faster with increasing DOC_0 concentration. This suggests that degradation rate was composition and concentration dependent. No other covariates were found to be significant in this analysis.

Table 2.4. Results of ANOVA for relative DOC concentrations across both daylight and dark treatments (na = not applicable; and ns = not significant).

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	0.00	9.6
Specific Absorbance	na	-	0.00	24.0
Site	0.04	0.4	0.04	2.4
Treatment	0.00	8.9	0.00	6.1
Day	0.00	12.6	0.00	11.4
Month	0.00	6.4	0.00	1.9
Treatment*Day	0.00	3.7	0.00	2.0
Treatment*Month	0.00	3.5	0.00	9.5
Day*Month	0.00	2.3	ns	-
Error		37.3		33.0

Guided by the results of the ANOVA it was possible to give the best-fit equation for the change in the DOC concentration (ΔDOC):

$$ln\Delta DOC = \underset{(0.04)}{1.05} lnDOC_0 + \underset{(0.04)}{0.28} lnt - \underset{(0.05)}{0.29}\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) - \underset{(0.18)}{1.15}$$

n=264, r^2=0.76 *(Eq. 2.2)*

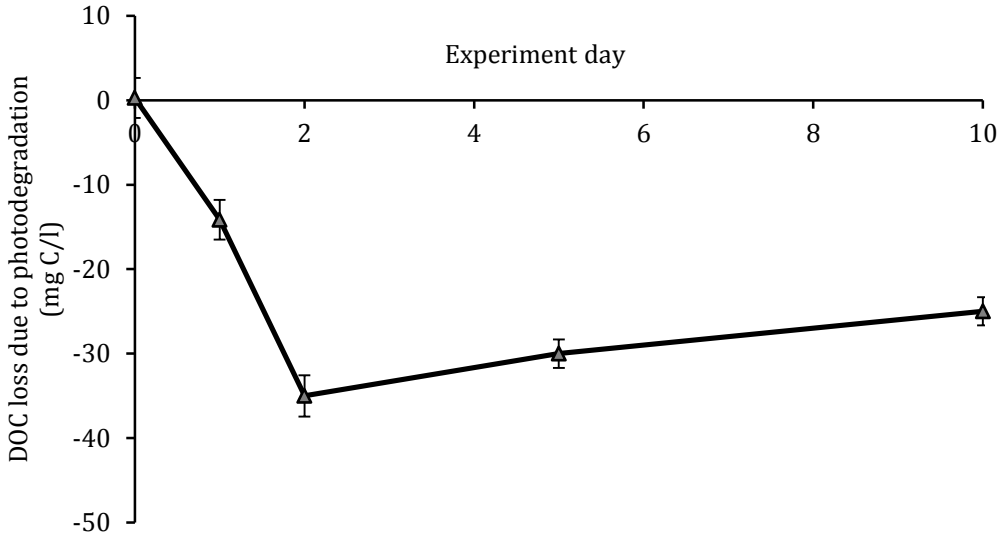
where DOC_0 is the DOC concentration on day zero of each experiment (mg C/l); t the time since the start of the experiment (days); m the month number (January = 1 to December = 12). Only variables that were found to be significantly different from zero at least at a probability of 95% were included. The values in brackets give the standard errors on the coefficients and the constant term. The partial regression analysis shows that the most important variable is $lnDOC_0$ (partial regression coefficient of 0.66) with the other terms of approximately equal importance.

2.3.2. Photo-induced degradation

The difference between the dark and light concentrations in each experiment was taken as the estimate of the impact of photic processes. The extent of photo-induced

degradation could be estimated in 313 cases and the loss due to photo-induced degradation varied from 48 mg C/l to -11 mg C/l (i.e. as above in some experiments the DOC concentration was observed to increase, implying photo-induced production).

Figure 2.3. The main effects plot of the change in loss due to photodegradation over the course of the experiment. Error bar is given as the standard error.



The ANOVA shows that all single factors were significant but that there were no significant interactions between those factors (Table 2.5). Only one variable, and no others, was found to be a significant covariate – the DOC_0 concentration. The Month factor, although significant, shows no clear seasonal cycle which may imply that hydroclimatic conditions on the day of sampling (e.g. riverflow) are more important than the season of the year. The Day factor showed a significant maximum in the difference due to photo-induced degradation after 2 days (Figure 2.3, also apparent in Figure 2.2), which then declines to the 10-day period.

Table 2.5. Results of ANOVA for the difference in the DOC concentration between daylight and dark treatments. (na = not applicable; and ns = not significant).

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	0.00	1.8
Site	0.00	21.8	0.00	8.0
Day	0.00	7.1	0.00	8.7
Month	0.00	28.1	0.00	30.0
Error		42.9		51.2

Given the results of the ANOVA it was possible to identify the best-fit equation for the loss due to photo-induced degradation (ΔDOC_{photo}):

$$\Delta DOC_{photo} = \underset{(9.0)}{35.2} + \underset{(0.06)}{0.47}DOC_0 - \underset{(3.0)}{13.4}lnDOC_0 - \underset{(255)}{933}\frac{Abs_{400}}{DOC_0}$$

n=313, r^2=0.52 *(Eq. 2.3)*

where Abs_{400} is the absorbance at 400 nm. The most important term in Eq. 2.3 is $lnDOC_0$ with a partial regression coefficient of 0.69, followed by Abs_{400}/DOC_0 with the least important term being DOC_0 having a partial regression coefficient of 0.035. The significance of the absorbance at 400 nm suggests that there is an effect of the composition of the DOC on the photo-induced degradation.

It should be noted that neither temperature nor PAR variables were found to be significant covariates in any of the above approaches. However, it was possible to estimate the apparent quantum yield (AQY) in 158 of the experiments and this was found to vary between 9.6 and -1.7 mmol C/mol photons (again there were periods of photo-induced production as opposed to photo-induced degradation) – on an energy basis this equates to a maximum AQY of 1.9 mg CO_2/kJ. Most values of AQY in the literature are defined for single wavelengths (e.g. Boyle et al., 2009) or for inappropriate end-products making them less transferrable to this analysis (e.g. Stubbins et al., 2011). Osburn et al. (2009) measured AQY for DOC values between 1 and 3 mmol C/mol photons, for samples at the mouth of the Mackenzie River (>1,800,000 km^2). Soumis et al. (2007) give photoreactivity of DOC in sterile lake

water as between 15.5 and 35.8 mg CO_2/kJ. This larger photoreactivity may be due to the experiment being performed in sterile containers that remove any biotic process and so photic processes are the only process operating.

The ANOVA of the AQY showed significant effects due to Day, Month and with DOC_0 as a covariate. Month was the most important factor with a peak in December and a minimum between February and June. This suggests that some months were associated with proportionately more photo-induced production than other months. This seasonal cycle could appear to be the inverse of the day length or solar declination, both of which would have peaked in June rather than December when the days are shortest and the sun's declination to the horizon at its lowest. It should be remembered that AQY is a measure of the photo-induced degradability and not the amount of photo-induced degradation, i.e. the DOC in December was more photodegradable.

2.3.3. Rate of DOC degradation

The rate of degradation of DOC was considered relative to the individual treatments, i.e. (i) the rate of degradation in the light (i.e. total degradation); (ii) the rate of degradation in the dark; and (iii) the difference between the two treatments which was taken as the rate of photic processes. For samples in the light, the degradation rate varied from 30.1 mg C/l/day to -3.5 mg C/l/day, i.e. increases or no change in DOC concentrations were observed in 60 cases.

2.3.3.1. Rate of degradation in the light

The ANOVA of the rate of degradation for samples in the light showed that all factors were significant (Table 2.6). When no covariates were included then all three factors were found to be significant (no treatment factor was included because only experiments in the light were being considered). Once covariates were included then neither Site nor Month factors were found to be significant. The lack of significance for the Site factor means that the different parts of the river did not have inherently different degradation rates. Equally, the lack of a significant difference between months of sampling suggests that there is no seasonal cycle in degradability. When covariates were included then both $\ln DOC_0$ and 1/Temp were found to be significant and no others, although collectively they explained only 8% of the original variance.

Figure 2.4. Main effects plot of rate of DOC loss in light conditions over time in the experiment. Error bar is given as the standard error.

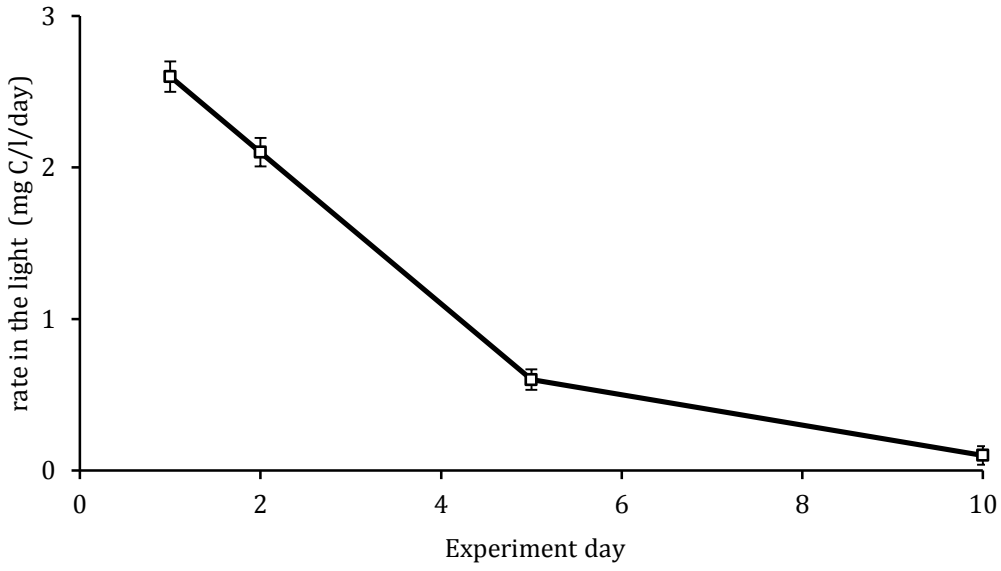


Table 2.6. The results of ANOVA of the degradation rate of DOC in the light.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
1/Temp	na	-	0.00	1
$lnDOC_0$	na	-	0.00	6.9
Site	0.00	3.3	ns	-
Day	0.00	47.3	0.00	48.3
Month	0.00	9.5	ns	-
Error		39.9		43.9

Given the results of the ANOVA the best fit equation for degradation rate in daylight was:

$$ln\,rate_{light} = \underset{(5.7)}{13.5} + \underset{(0.12)}{0.74}lnDOC_0 - \underset{(0.1)}{1.28}lnt - \frac{\underset{(1567)}{3824}}{T}$$

n=167, r^2=0.61 *(Eq. 2.4)*

where T is the absolute temperature of the experiment (Kelvin). The residuals of Eq. 2.4 were normally distributed. The most important term in Eq. 2.4 is $lnDOC_0$ with a partial regression coefficient of 0.51 and the least important term being $1/T$ with a partial regression coefficient of 0.035. Although the visual inspection of the residuals of Eq. 2.4 show no obvious changes, the main effects plot of $lnrate_{light}$ vs. t (Figure 2.4) would suggest that, although a straight line fit was significant, a combination of two straight lines would be better, with one fast rate equation covering the period up to approximately 4–5 days and one after 5 days. The significance of the reciprocal of absolute temperature in Eq. 2.4 means that it was possible to estimate the activation energy of the degradation given a value of the universal gas constant as 0.692 J/K/g C and in which case this would be 2.6 ± 1.2 kJ/g C.

2.3.3.2. Rate of degradation in the dark

It was possible to calculate the rate of degradation in the dark in 258 experiments, which ranged from a decrease of 19.4 mg C/l/day to -6 mg C/l/day, i.e. increase or no change in DOC concentrations were observed in 77 cases.

For the rate of degradation in the dark the ANOVA shows that all factors were significant (Table 2.7). Once covariates were included then Site was found not to be significant, however, unlike when considering the rate of the reaction in the light there was still a significant role for the Month factor, i.e. there was a seasonal cycle in aphotic degradability. The main effects plot of the Month factor shows that degradability peaked in July and October at 6.11 mg C/l/day, and was at a minimum in November at 0.28 mg C/l/day (Figure 2.5). There is a superficial similarity between the rate of degradation and the annual average temperature during each month's experiment but the temperature did not show the local maxima in July and October. When covariates were included then both $\ln DOC_0$ and 1/Temp were again found to be significant: no others were found to be significant.

Figure 2.5. Main effects plot of the seasonal cycle in the rate of DOC loss in dark conditions over time in the experiment (1 = January, 12= December). Error bars are the standard errors.

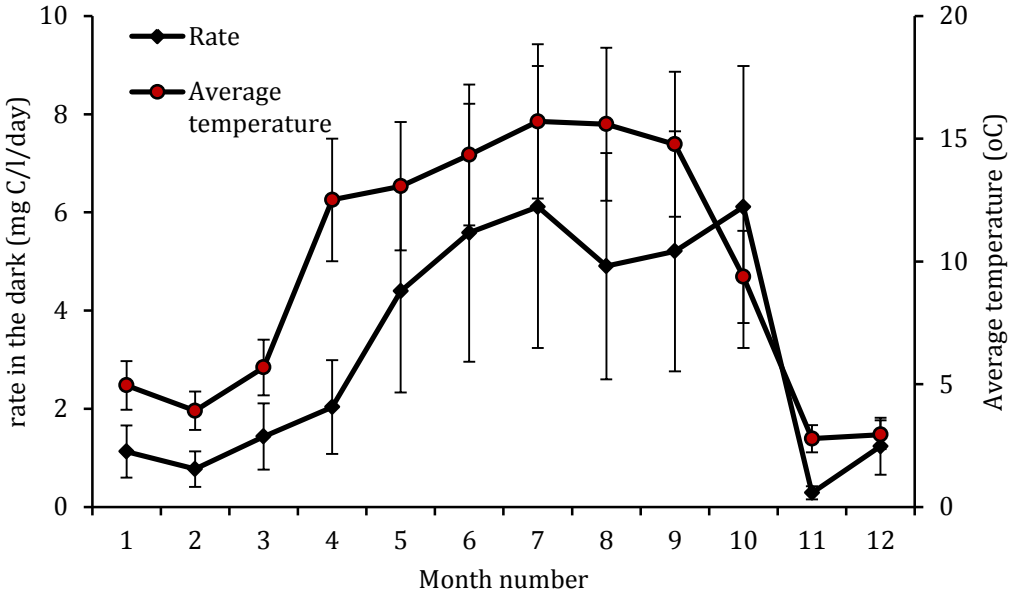


Table 2.7. ANOVA of the degradation rate of DOC in the dark.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
1/Temp	na	-	0.04	1.0
$lnDOC_0$	na	-	0.00	6.5
Site	0.00	4.8	ns	-
Day	0.00	23.8	0.00	29.3
Month	0.00	28.3	0.00	14.3
Error		43.2		48.9

Given the results of the ANOVA the best fit equation for degradation rate in darkness was:

$$lnrate_{dark} = \underset{(0.11)}{0.71 lnDOC_0} - \underset{(0.10)}{0.7 lnt} - \underset{(0.17)}{0.42 sin\left(\frac{\pi m}{6}\right)} - \underset{(0.20)}{0.59 cos\left(\frac{\pi m}{6}\right)} + \underset{(2783)}{\frac{3267}{T}}$$

n=178, r^2=0.45 *(Eq. 2.5)*

where all terms are defined as above. The residuals of Eq. 2.5 were normally distributed. The most important term in Eq. 2.5 is $lnDOC_0$ with a partial regression coefficient of 0.29 and the least important term being $1/T$ with a partial regression coefficient of 0.008. As above the main effects plot of $lnrate_{dark}$ vs. t suggests that a more complex rate law than a single rate law. Again it was possible to estimate the activation energy of the degradation and in which case this would be 2.3 ± 1.8 kJ/g C, i.e. not significantly different from the estimate based on the degradation rate in the light. It is difficult to find studies that measure activation energy in comparative systems, but Alperin et al., (1994) give a value of 6.7 kJ/g C for DOC in marine sediments; a higher value may be expected for DOC that is likely to have been older and more recalcitrant than that found in rivers.

2.3.3.3. Rate of photo-induced degradation

The rate of the photo-induced degradation could be calculated from 168 experiments and varied from 27.3 mg C/l/day to -4.3 mg C/l/day, i.e. in 39 cases an increase was observed. All three factors were found to be significant but again the Site factor was not found to be significant when covariates were included (Table 2.8). As before the Day factor was found to be the most important, though there was a significant seasonal cycle where the rate peaked in September at 7.7 mg C/l/day with a minimum in June at 1.1 mg C/l/day. The covariates found to be significant were not only DOC_0 but also cumulative PAR.

Table 2.8. ANOVA of the photodegradation rate of DOC.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
ΣPAR	na	-	0.00	1.8
$lnDOC_0$	na	-	0.00	1.0
Site	0.05	1.8	ns	-
Day	0.00	40.5	0.00	30.1
Month	0.00	13.5	0.00	3.3
Error		44.2		63.0

Given the results of the ANOVA the best fit equation for the photo-induced degradation rate was:

$$lnrate_{photo} = \underset{(14.8)}{31.4} - \underset{(4.5)}{10.51 lnDOC_0} - \underset{(2.0)}{2.02 lnt} + \underset{(3.9)}{9.1 sin\left(\frac{\pi m}{6}\right)} - \underset{(0.003)}{0.005 \frac{\sum PAR}{t}}$$

n=94, r^2=0.29 *(Eq. 2.6)*

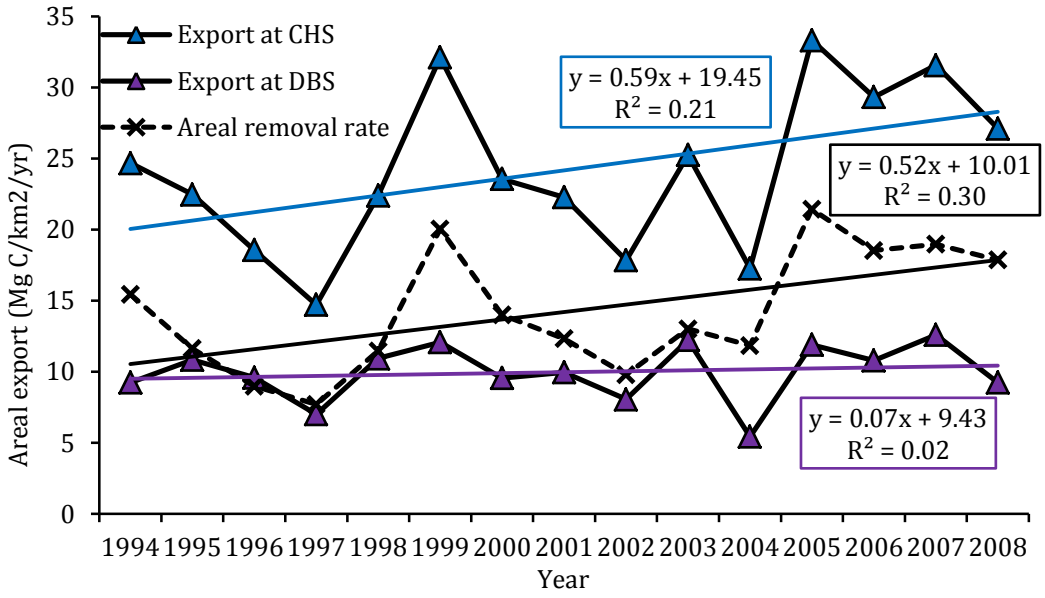
where all terms are defined as above. The significant effect of the term in $\frac{\sum PAR}{t}$ does suggest that we could measure significant AQY. The most important term in Eq. 2.6 was *lnt* (partial regression coefficient of 0.39) with no other term having a partial regression coefficient greater than 0.07. By using partial regression it was possible to examine the relationship between $lnrate_{photo}$ and $\frac{\sum PAR}{t}$, which does suggest that the rate of photo-induced degradation declined with increasing ΣPAR. This implies that there was a progressive decrease in the sensitivity of remaining DOC to photo-induced degradation, i.e. that photobleaching had occurred, and that this was associated not only with time but also with increased light intensity.

2.3.4. Empirical modelling on the Tees

The results of the modelling of the flow rates estimated in-stream residence time for water between TB and the tidal limit varied from 12.9 to 127.2 hours. Between 1994 and 2009 the annual flux of DOC at CHS varied from 14.7 to 33.3 Mg C/km^2/yr. For each measurement of DOC concentration at CHS the flow measurement at the TB gauging station was used to calculate the in-stream residence time. Given an initial concentration and an estimate of the in-stream residence time it was possible to calculate the loss of DOC and the export that would represent. Based on the in-stream residence time and Eq. 2.2, then the equivalent flux at the tidal limit would be between 5.4 and 12.6 Mg C/km^2/yr, which gives an equivalent removal rate of 7.7 and 21.4 Mg C/km^2/yr, a removal rate of between 48% and 69% (Figure 2.6). There was a significant trend in the DOC flux from CHS, which increased at average rate across the whole period of 0.59 Mg C/km^2/yr^2 (3.0%/yr^2) but no significant trend was observed for the flux at DBS over the same period. Therefore, it is perhaps not

surprising that there was a significant increase in the predicted removal rate of 0.52 Mg C/km²/yr² (5.0%/yr²). The increase in the predicted removal rate is in line with the increase observed for the flux of DOC at source, and so therefore the observations of DOC degradation for this catchment imply that the river is capable of removing most or all of the increase in DOC export from the source, before it reaches the sea. This in turn implies that observed increases in DOC flux from peat soils across the northern hemisphere could translate into large increases in loss of CO_2 to the atmosphere. However, in this case this would assume no other changes in sources in the rest of the catchment such as no changes in urban or agricultural sources.

Figure 2.6. Estimated annual loss of DOC across the Tees catchment, with regression lines for export from CHS and DBS, and the areal removal rate.



2.3.5. Limitations and implications

One particular process that this experiment has not quantified is the processing of the particulates. The samples were deliberately not filtered prior to experiment, to allow for the possibility of interaction between particulates and DOC, but because of the small volume of samples it was not possible to test the composition of the particulates over the course of the experiments. However, suspended sediment concentrations were measured in at the beginning and end of each experiment,

meaning that it was possible to assess the change in particulate concentration. It was possible to show, over a 10-day period in 35 cases, no increases in suspended sediment concentration were observed, with removal rates ranging from 0.2 to 15.6 mg C/l/day. Without compositional information it is difficult to infer the extent to which the particulate carbon content has changed. However, for the CHS there is no mineral soil in the catchment and so any suspended sediment can be assumed to be organic. There were nine cases where it was possible to compare the day_0 and day_{10} samples at CHS, and this gave a loss of POC between 7.5 and 29.4 mg C/l/day (assuming a carbon content of 45%), which is a removal rate of between 38% and 87% over 10 days. Of course this assumes that our experimental set up mimics the settling out of POC into a streambed, and the analysis does not indicate whether the POC was converted to directly to CO_2 or to DOC. Nevertheless, the absence of any evidence of increasing particulate concentrations in any of the experiments argues strongly that the widespread reductions in DOC observed were not due to flocculation or precipitation.

Based on BOD measurements from rivers across England and Wales, Worrall et al., (2007) estimated an average 29% removal of DOC, although this estimate was based upon an assumption of a fixed 5-day residence time. Worrall et al., (2006) working on the River Tees calculated the DOC export at a range of scales to show an average net loss of 40% of DOC from source to outlet. Worrall et al., (2012) developed an empirical model of net watershed loss based upon data from 169 catchments and applying the method to the Tees catchment suggests a removal rate of 58%. Therefore, the estimates of removal rates are not dissimilar to previous less detailed estimations, and indeed not dissimilar to estimates of global in-stream removal (42% – Cole et al., 2007). Worrall et al., (2012) estimated the flux of DOC from the UK was 909 ± 354 Gg C/yr (2.2–5.2 Mg C/km^2/yr), so applying the removal rates measured in this chapter suggests that the flux of DOC at source in the UK would have been between 1067 and 4074 Gg C/yr (4.4–16.7 Mg C/km^2/yr). Rates of DOC loss through the UK's fluvial network would be between 512 and 2811 Gg C/yr (2.1–11.5 Mg C/km^2/yr), which represents a greenhouse gas emission of between 1880 and 10320 Gg CO_2eq/yr. Even the lower of these estimates would represent 1% of the UK's national total GHG budget.

Although this chapter's data have been able to develop empirical rate law for the loss of DOC, it is clear from that if we are to further understand the turnover of DOC in the rivers then it will be necessary to consider changes on hourly timescales

rather than daily, and to better constrain in-stream residence times across regions. The findings suggests that there is a strong influence of radiation on the loss of DOC which would create a strong diurnal cycle in the loss or processing of DOC, which in the short residence times of rivers has two implications: firstly, that without a good knowledge of in-stream residence time it will be difficult to judge how much DOC is lost. Second, a strong diurnal cycle in northern latitudes also implies that there should be a strong annual cycle in loss of DOC, even with a fixed in-stream residence time. The findings also suggest that there are at least two broad types of DOC, with one rapidly turning over into the other, at the same time as the particulate organic matter is itself turning over producing DOC. The interplay of at least these three processes means that we need to consider each of these on sub-daily timescales.

2.4. Conclusions

The main findings of this chapter are outlined in Table 2.9. This chapter has found that for peat-derived DOC in the river network the average loss of DOC in light conditions was 73% over a 10-day period, but with the majority of the loss occurring in the first 2 days. There were significant differences in the loss of DOC between the sites, and the initial DOC was often a significant covariate in the analyses, showing that the site differences in DOC type and concentration play a large role in determining the DOC degradation rates. The rate of DOC degradation declined over time, suggesting a decrease in the 'degradability', or an increase in the recalcitrance, of DOC. When the rate is extrapolated across the catchment the annualised removal rate was between 48% and 69% of the flux of DOC at its soil source. These measured removal rates are for DOC close to its source in rivers with residence times of only several days, and not for longer residence times systems or for the relatively old DOC found downstream in a larger river network. The results suggest that rivers could be sources of CO_2 equivalent to several percent of a national GHG inventory.

Table 2.9. The main findings of Chapter 2.

Sites studied	Teesdale: CHS, TBG, MIT, DBS
Range of catchment areas (km^2)	0.2 – 818.4
Range of percentage peat cover (%)	33.9 – 100
Duration of experiments	Monthly, for 10 days
Sample size	690
Net DOC loss in the light treatment (%)	76
Net DOC loss in the dark treatment (%)	47
Range of rate of DOC loss in the light treatment (mg C/l/day)	30.1 – -3.5
Range of rate of DOC loss in the dark treatment (mg C/l/day)	19.4 – -6.0
Significant covariates:	
Change in DOC concentrations	Initial DOC, specific absorbance
Light rate	Initial DOC
Dark rate	Initial DOC, inverse temperature
What to investigate next?	Diurnal cycles of DOC degradation, due to the significant differences in DOC degradation in the light and dark treatments.

Chapter 3:
Short-term degradation of DOC in the Peak District

3.1. Introduction and Aims

The results of Chapter 2 showed that there were significant differences between the changes in DOC concentration in the light and the dark treatments, and therefore there was significantly more DOC lost from the water samples exposed to light. This would suggest that there would be a diurnal cycle to the DOC concentrations and rates of loss. A review of the existing literature on diurnal cycles in outlined in Section 1.2.8 in Chapter 1.

The Peak District National Park has a long history of human influence on the land. The Industrial Revolution in the 19th century caused the near surface soils between Manchester and Sheffield to be highly contaminated with lead and other metal deposits. This has led to high rates of erosion and therefore high and episodic POC fluxes, and high DOC fluxes (Evans et al., 2006).

The aim of this series of experiments was to measure the DOC concentrations in a small peat-sourced river, to the compare the rates of decline in light and dark treatments as a replication of the work being conducted on the River Tees. The experiments were conducted over shorter timescales than in the previous chapter and with a higher frequency sampling relative to the diurnal cycle.

3.2. Approach and Methodology

3.2.1. Field sites

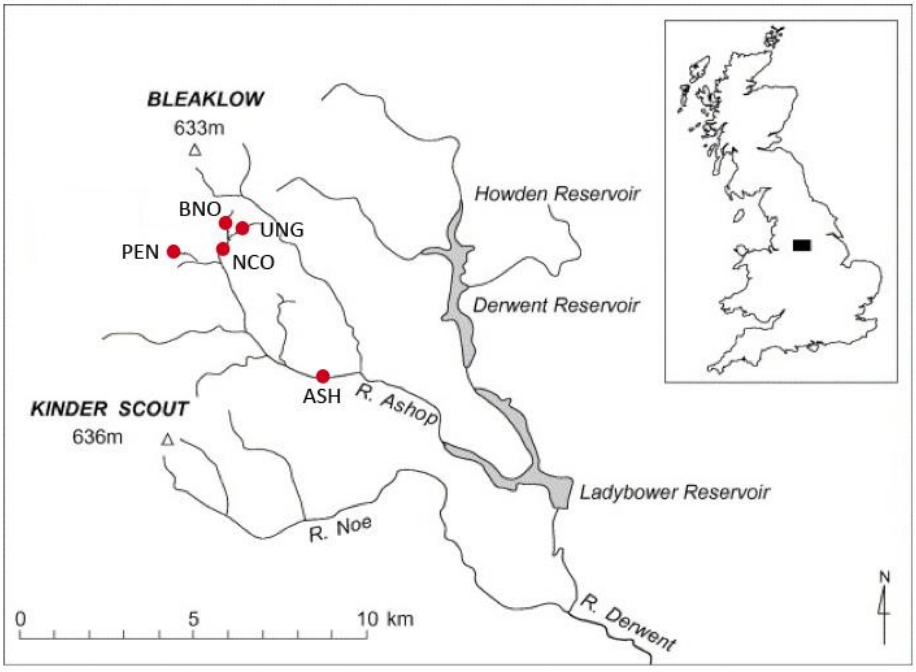
For this chapter five sites were chosen in the River Ashop catchment in the Peak District National Park, UK, upstream of Ladybower reservoir (Figure 3.1). The furthest downstream site (ASH: SK 131 894; Table 3.1.) was located approximately 5 km upstream from the reservoir. Upper North Grain is a tributary of the River Ashop, just to the east of where the Pennine Way crosses Snake Pass, and the location of three sample sites (BNO (SK 103 936), UNG (SK 105 935) and NCO (SK 103 934)): BNO was the main tributary joining from the north-west; UNG from the

north-east; and NCO was just below the confluence of these two. Due to its position on Bleaklow Plateau, Upper North Grain (UNG) is the field site for various other studies, looking at lead contamination and erosion (Rothwell et al., 2007b; Rothwell et al., 2008), as well as carbon balance studies (Billett et al., 2010). The most upstream site with the smallest catchment area was PEN (SK 092 931), an entirely peat-sourced headwater, lying in the triangle created by Snake Pass, the Pennine Way and the Doctor's Gate path.

Table 3.1. Areas and locations of all field sites. Catchment areas and percentage peat covers are taken from literature. The gaps indicate unknown data.

Site	Site code	Grid reference	Catchment area (km^2)	Peat cover (%)
Penguin's Drift	PEN	SK 092 931	0.005	100
Baskerville North	BNO	SK 103 936	-	100
Upper North Grain	UNG	SK 105 935	0.38	100
Newton Confluence	NCO	SK 103 934	-	100
River Ashop	ASH	SK 131 894	11.0	-

Figure 3.1. Map showing the location of the sites on the river, and the River Ashop within the UK, adapted from Rothwell et al. (2007a).



3.2.2. Degradation measurements

The degradation measurements were made in-situ in the Peak District, under ambient light and temperature conditions, rather than in the artificial light of the laboratory. Methods were similar to those used in Chapter 2 for the River Tees. However, due to practical constraints imposed by distance from Durham the experiments only lasted 30 hours.

The experiment considered two treatments, one in which degradation experiments were always exposed to ambient light (thus experiencing both night and day time conditions); and one in which all experiments were exposed to ambient temperature but were covered and therefore always dark. These treatments, henceforward referred to as light (always in ambient conditions) and dark (never in the light), were employed so as to distinguish between components of degradation (i.e. the difference between light and dark degradation rates is the photo-induced degradation). Experiments were conducted quarterly (in February, May, August and November) on each site over the course of a year to experience a range of both meteorological conditions; and DOC concentrations and compositions. So as not to exclude particulates the samples were not pre-filtered, therefore this chapter could consider the net fate of DOC and could include production from POC or adsorption by it.

Each of the quarterly degradation experiment spanned approximately 30 hours with sacrificial sampling taking place at hours 0 (called 't_0'), 1, 2 and 4, and dusk on day 1 (approximately 8 hours), and then at dawn (approximately 24 hours) and dusk on day 2 (hour 30, called 't_{30}'); light and dark treatments were taken for each site in each season. No t_0 samples were replicated, but 9% of all other measurements were replicated (28 of 315 samples). Replication was limited by practical constraints of the number of quartz tubes available and the time taken to process DOC analysis to ensure the short timescales at the beginning of the experiment.

All samples were kept outside for the whole 30 hours of the experiment. The air temperature was measured at 15-minute intervals throughout the 30-hour period of each season's experiment. The temperature conditions were summarised according to the method in Chapter 2.

3.2.3. Sample analysis

To achieve the temporal resolution required for this experiment, samples for DOC analysis from degradation experiments were filtered to 0.45 μm, and then "fixed" with concentrated sulphuric acid. This technique was used because addition of concentrated sulphuric acid is the first step in the analysis of DOC concentration measured using the wet oxidation method described in Bartlett and Ross (1988). The samples were then analysed for DOC as in Chapter 2.

At each sampling time a duplicate sample was filtered to 0.45 μm, and used for further analysis, including absorbance (at 400, 465 and 665 nm), E4:E6, conductivity, pH, water temperature and ion chromatography. Alkalinity and acidity was measured in the field at the start and end of each experiment.

Suspended sediment concentration was measured in each experiment in samples taken at hour 0 (t_0) and hour 30 (t_{30}). Samples were filtered through pre-weighed, 0.45 μm, glass fibre filters; dried to 105 °C and the filter paper re-weighed to give the concentration of suspended sediment. No further analysis was performed on the suspended sediment, but in catchments where there is no mineral soil it can be assumed that all suspended sediment is organic. Four of the sites have 100% peat cover within their catchment; only the ASH site contains mineral soil (Figure 3.1). Therefore the POC can be estimated in PEN, BNO, UNG and NCO, assuming a carbon content of 45% of the suspended organic matter (Worrall et al., 2003).

The measured concentrations of DOC were analysed in both absolute and relative terms where the relative value for each sample in an experiment was expressed as the ratio of the measured value to measurement at t_0 for the same site on that experimental run.

3.2.4. Statistical methodology

The experiment used a fully factorial design, with four factors: Season, Time, Treatment and Site. Each factor had a number of levels: Season had four (February, May, August and November), Time had seven (hour 0, 1, 2, 4, 7.25, 24.53, 30), Treatment had two (light and dark), and Site had five (PEN, BNO, UNG, NCO and ASH). The sample times were the averaged values (each has a standard error) that represent the samples taken on the first day (hours 0, 1, 2, 4, 7.25) and dawn and

dusk on day 2 (hours 24.53 and 30). A similar analysis progression was used as in Chapter 2, including ANOVA and ANCOVA.

The change in DOC concentration and rate of degradation of DOC were considered relative to the individual treatments; e.g. (i) the rate of degradation in the light (total degradation); (ii) the rate of degradation in the dark (biodegradation); and (iii) the difference between the two treatments which was taken as the rate of photic processes.

The rates of DOC degradation were also calculated for the very first hour of the experiments, and for the whole days and nights in the experiments, to give the initial rates and the day/night rates. These rates were then analysed using ANOVA and ANCOVA as for DOC concentrations.

3.2.5. Priming effect

One aspect of the DOC and POC degradation not extensively studied is "priming", that is the extent to which a treatment causes a greater capacity to respond to a second stimulus (Bianchi, 2011). DOC priming has been studied under elevated CO_2 conditions in peat cores, where the microbial breakdown of labile soil carbon led to the production of "priming compounds" that are rapidly cycled by microbes causing more carbon to be lost as CO_2 (Freeman et al., 2004). In this chapter, it is hypothesized that "priming" could be expected to lead to increased rate of breakdown of DOC and POC during the night as a result of exposure to daylight during the day. This was tested by comparing the night time rates for both treatments and if the night time rate of degradation for light treatment was significantly greater than the night time degradation rate for the dark treatment then this implies a "priming" effect.

3.3. Results and Discussion

It was possible to generate a sample size of 301 DOC concentrations, and a summary of the water chemistry at the two sites at the extremes of the experiment catchments (in the light treatment) is given in Table 3.2. These two extremes of the catchments bracket the water chemistry of the other sites used within this chapter.

All of the covariates showed the same pattern between t_0 and t_{30} at both of the two sites shown in Table 3.2. The suspended sediment, chloride, fluoride, nitrate and phosphate concentrations decreased, and the conductivity, pH, E4:E6, bromide and sulphate concentrations increased. The absorbance at 400 nm did not change at either site. Of these changes, only two were significantly different between t_0 and t_{30} across both sites: the E4:E6 increase at PEN ($p=0.0181$) and the phosphate decrease, also at PEN ($p=0.0318$).

The concentrations of the conservative ions (bromide and chloride) were investigated further to show how much natural variability there was in the dataset. An ANOVA on the whole dataset showed there were no significant differences between the Site, Time and Treatment for either ion.

As mentioned in the previous chapter, the changes in anion concentrations, pH and conductivity suggest that some of the water was likely to have evaporated, increasing the concentrations, or that some of the anions were utilised in the production of biomass, decreasing the concentrations. However, there were no significant changes in the conservative ions (bromide and chloride) between the beginning and end of the experiments, suggesting that there was little evaporation of the water, and the other changes were likely due to natural variability. The changes in the average E4:E6 ratio suggest that the DOC underwent some compositional changes during the experiment.

Table 3.2. The average and coefficient of variation (CV - %) of the 12 variables measured from the smallest and largest sites (PEN and ASH), averaged across all four sampling months. The table shows the initial (t_0) and end (t_{30}) concentrations for each variable from the light treatment.

	PEN				ASH			
	t_0		t_{30}		t_0		t_{30}	
Determinant	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Suspended sediment (mg/l)	102.58	101.86	25.02	34.74	28.46	92.01	9.18	41.19
Conductivity (µS/cm)	48.30	15.91	54.33	11.35	81.08	10.39	90.76	8.81
pH	4.19	5.27	4.89	14.79	6.35	7.43	6.68	2.73
DOC (mg C/l)	92.01	71.21	19.18	68.15	51.14	75.82	5.56	108.65
Abs_{400}	0.25	49.46	0.25	51.5	0.03	35.53	0.03	25.43
E4:E6	3.86	27.91	5.11	16.22	3.71	30.73	4.43	70.22
Bromide (mg/l)	0.33	68.16	0.43	67.3	0.17	25.52	0.33	110.77
Chloride (mg/l)	24.08	121.59	19.47	171.76	8.42	25.56	6.86	41.15
Fluoride (mg/l)	0.55	67.44	0.34	20.67	0.50	81.76	0.31	17.78
Nitrate (mg/l)	0.39	200.00	0.33	184.05	1.47	45.88	0.99	87.85
Phosphate (mg/l)	0.72	60.40	0.23	86.11	0.45	115.48	0.18	120.41
Sulphate (mg/l)	8.36	93.61	15.01	81.51	17.58	19.88	18.43	42.11

3.3.1. DOC concentrations

For every season of measurement the DOC concentration in both light and dark treatments decreased over the time in the experiment. The average DOC concentration over time, over all sites, showed three different stages of decline: a steep initial decline in the first 10 hours, followed by a slower, almost negligible, decline between 10 and 20 hours, and a shallow decline between 20 and 30 hours (Figure 3.2). For the daylight treatment, the average decline in DOC concentration across all seasons and all sites was from 54.5 to 8.56 mg C/l after 30 hours; when concentrations were judged relative to the DOC_0 (the initial DOC concentration) at each site then the average decline over 30 hours was 85.5%. For experiments only in the dark, the average decline over a 30 hour period was 55.4%. The average difference across all sites and all times between samples in light and dark was 14.52 mg C/l; DOC_{30} concentrations of samples that were kept in the light were on average 30% lower than those kept in the dark when judged relative to the DOC concentration at t_0. Of all the experiments run, no samples had a higher DOC concentration at t_{30} than t_0.

The most interesting result observed was the strong diurnal cycle of DOC decline. There is very little change in the DOC concentrations in either light or dark treatment between 10 and 20 hours, i.e. during the hours of darkness.

An ANOVA on the relative DOC concentrations (Table 3.3) found that Time was the factor that explained the largest proportion of the variance (31%), with Treatment explaining the second largest proportion (13%). All four single factors were significant, along with three interactions (Treatment*Time, Treatment*Season and Site*Treatment*Season). Adding covariates to the model found two covariates were significant: the DOC_0 concentration and the sulphate concentration of the water. Analysis including covariates showed that Treatment became the most important factor, explaining 19% of the variance, with Time and the interaction between Treatment*Season being significant also.

Figure 3.2. Average relative DOC at each site over time for the two treatments. Error bars are the standard errors.

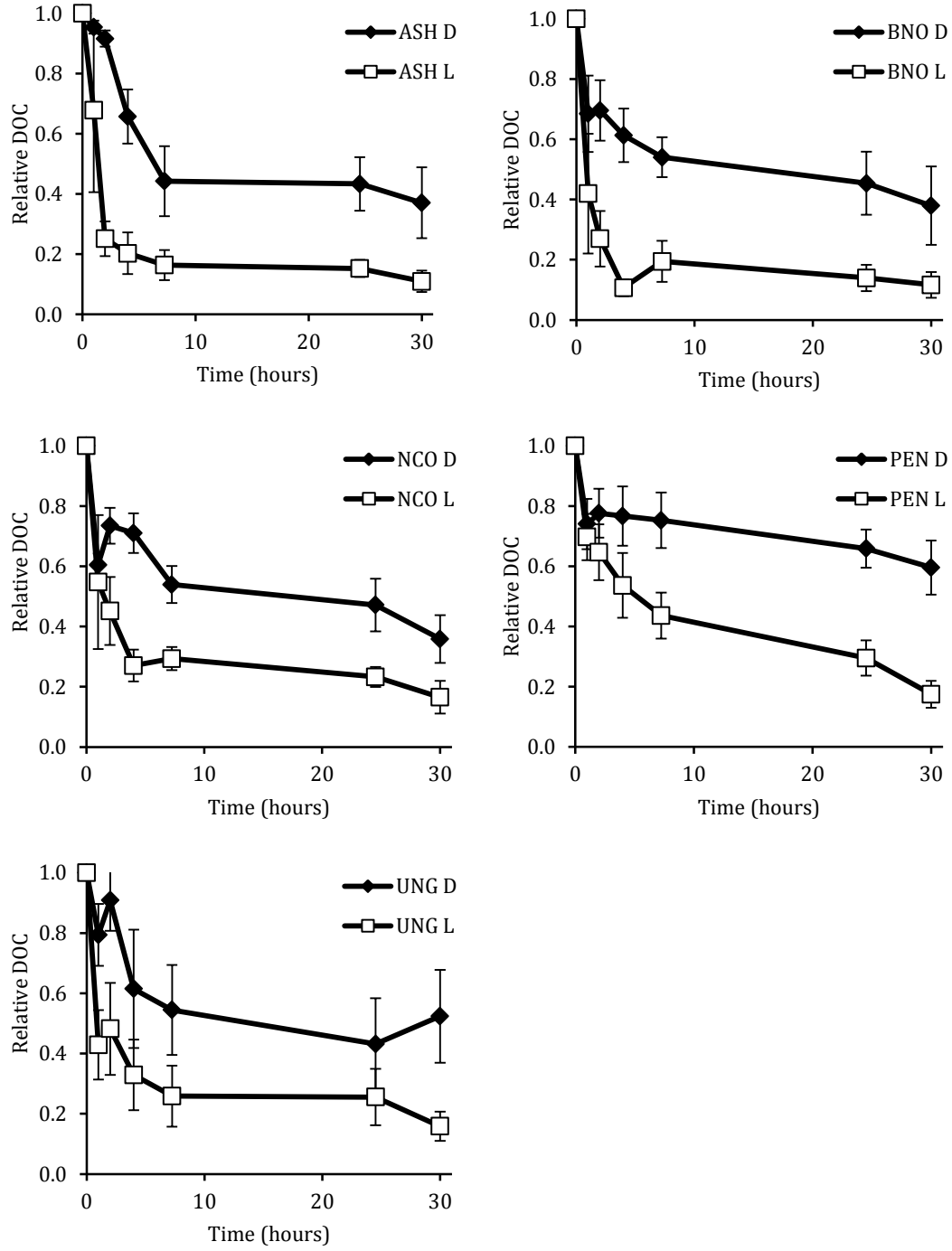


Table 3.3. Results of ANOVA for relative DOC concentrations for all experiments across both daylight and dark treatments.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	0.0417	0.49
Sulphate	na	-	0.0180	0.71
Site	<0.0001	4.29	ns	-
Treatment	<0.0001	13.26	<0.0001	18.70
Time	<0.0001	30.75	0.0050	1.87
Season	0.0464	0.47	ns	-
Treatment*Time	<0.0001	3.10	ns	-
Treatment*Season	<0.0001	5.10	<0.0001	6.43
Site*Treatment*Season	<0.0001	6.57	ns	-
Error		13.36		36.18

3.3.2. Photo-induced degradation of DOC

The difference between light and dark DOC concentrations is described as the photo-induced degradation of DOC. The loss of DOC due to photo-induced degradation ranged from -110.6 to 12.1 mg C/l, where positive numbers indicate that the light DOC was higher than the dark DOC (this happened in only 13 of 154 cases).

An ANOVA on the photo-induced degradation concentrations (Table 3.4) found all three single factors were significant (there was no Treatment factor, as photo-induced DOC is the difference between the treatments); however it was the interaction of Site*Season that explained the largest proportion of variance (23%). Once covariates were added to the model, DOC_0 concentration was again significant and explained almost half the variance (47%). This shows that the initial concentration of the DOC significantly affects the photo-induced DOC concentration throughout the rest of the experiment, much more than any other factor or covariate.

Table 3.4. Results of ANOVA on the photo-induced degradation.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	<0.0001	47.28
Site	<0.0001	6.42	ns	-
Time	<0.0001	8.28	ns	-
Season	<0.0001	3.42	ns	-
Site*Time	<0.0001	12.20	ns	-
Site*Season	<0.0001	23.17	ns	-
Time*Season	<0.0001	8.30	0.0021	9.47
Site*Time*Season	<0.0001	14.50	ns	-
Error		23.32		9.91

3.3.3. Rates of light DOC degradation

The rate of DOC degradation in the light treatment ranged from 73.6 to -2.8 mg C/l/hour, where negative rate means that the DOC increased over the hour (this happened in only 15 of 134 cases). The mean rate was 5.01 mg C/l/hour.

An ANOVA on the rates of degradation in the light treatment (Table 3.5) showed that Time and the interaction of Time*Season were the only significant factors, explaining 34% and 13% of the variance respectively. Adding covariates to the model increased the r^2 from 0.57 to 0.64, with DOC_0 being significant and explaining 6% of the variance. Time still explains the majority of the variance, with the interaction of Time*Season explaining the second largest part.

Table 3.5. Results of ANOVA on the rates of light DOC degradation.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	<0.0001	6.31
Time	<0.0001	34.41	<0.0001	34.52
Time*Season	0.0004	13.00	<0.0001	14.14
Error		9.46		8.58

3.3.4. Rates of dark DOC degradation

The rate of DOC degradation in the dark treatment ranged from 44.1 to -7.4 mg C/l/hour, where negative rate means that the DOC increased over the time (this happened in 25 of 133 cases). The mean dark rate was 2.73 mg C/l/hour.

An ANOVA on the rates of degradation in the dark treatment (Table 3.6) showed that Time and the interactions of Site*Time and Site*Season were the only significant factors, explaining 40%, 15% and 7% of the variance respectively. Adding covariates to the model once again found that the only significant covariate was the DOC_0 concentration and explained 6% of the variance.

Table 3.6. Results of ANOVA on the rates of dark DOC degradation.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	<0.0001	6.07
Time	<0.0001	40.45	<0.0001	39.48
Site*Time	<0.0001	15.48	<0.0001	13.94
Site*Season	0.0029	6.84	ns	-
Error		12.01		10.51

3.3.5. Rate of photo-induced degradation

The difference between the light and dark rates of degradation is described as the rate of photo-induced DOC degradation. The rates ranged from 67.1 to -17.7 mg C/l/hour, where negative rate means that the dark rate is greater than the light rate (this happened in 58 of 132 cases). The mean rate was 2.27 mg C/l/hour.

An ANOVA on the rates of photo-induced degradation (Table 3.7) showed that Time and the interaction of Time*Season were the only significant factors, explaining 18% and 17% of the variance respectively. Adding covariates to the model increased the r^2 from 0.46 to 0.48, with DOC_0 concentration as the only significant covariate.

Table 3.7. Results of ANOVA on the rates of photo-induced DOC degradation.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	0.0428	1.52
Time	<0.0001	18.01	<0.0001	18.07
Time*Season	0.0002	17.21	0.0001	17.42
Error		11.24		11.46

3.3.6. Day versus night rates of degradation

The average rate of DOC degradation for each site, in the dark and light treatments, for the main three stages (day 1, night 1, day 2) of the experiments are shown in Table 3.8. The average rates across all sites are shown in Figure 3.3.

An ANOVA on these data (Table 3.9) showed that there were significant differences between the rates of degradation in the two different treatments for the first day, but no significant difference between the night time rates. Other factors were found to be significant in the ANOVA: Site, and the interaction of Season*Site. The factor explaining the largest proportion of the variance in the ANOVA was the Stage, which explained 33% of the variation.

Table 3.8. The average rate (mg C/l/hour) of DOC degradation in the dark and light treatments for the main three stages of the experiments.

	Light treatment			Dark treatment		
Site	Day 1	Night 1	Day 2	Day 1	Night 1	Day 2
ASH	5.98	-0.10	0.41	4.65	0.01	0.65
BNO	7.14	0.09	0.73	4.26	0.85	1.65
NCO	3.62	0.04	0.51	2.26	0.16	0.95
PEN	7.84	0.36	2.02	3.07	3.04	1.55
UNG	3.15	0.06	0.26	2.28	-0.21	0.21

Figure 3.3. Average rates of degradation for all sites across the first day, first night and second day.

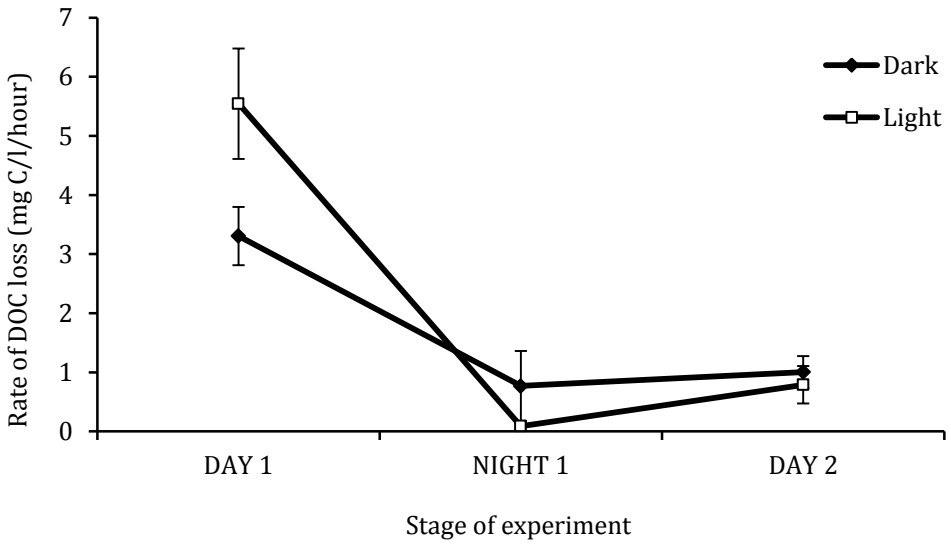


Table 3.9. Results of ANOVA on the rates of degradation during the day and night stages.

	Without covariates	
Factor	p	ω^2
Site	0.0060	3.97
Stage	<0.0001	33.18
Season*Site	<0.0001	15.00
Stage*Treatment	0.0045	3.79
Error		9.08

The lack of significant differences between the night time rates in the light and dark treatments suggest that there is no "priming" effect, i.e. no more DOC is likely to degrade overnight due to exposure to light during the day than in the dark treatment.

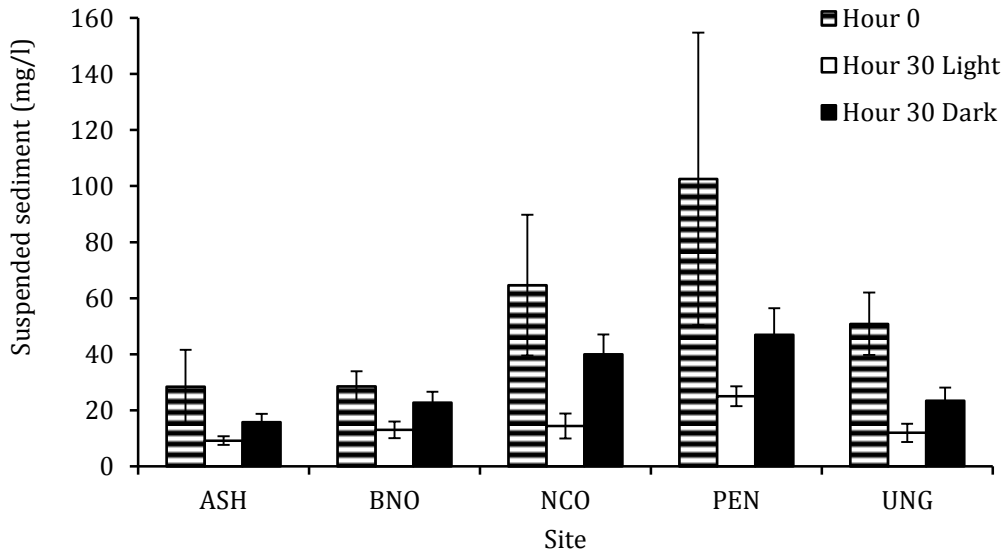
3.3.7. Suspended sediment concentrations

The suspended sediment (SS) concentrations were measured at the beginning and end of each season's experiment: t_0 and t_{30}. This resulted in a smaller dataset for analysis than the DOC data. There were 95 SS concentration measurements.

For every season of measurement the SS concentration in both treatments decreased (Figure 3.4). For samples in the light treatment, the average change in SS concentration across all seasons and all sites was from 55.0 to 14.7 mg C/l after 30 hours; this is a decrease of 73.3%. For experiments only in the dark the average decline over a 30-hour period was 45.9%.

As four of the five sites have are in entirely peat catchments (Table 3.1), the suspended sediment concentrations can be used to estimate the POC concentrations. For PEN, BNO, UNG and NCO, the average loss of POC was 74% in the light and 48% in the dark. Calculating these as rates results in losses of POC of 0.68 mg C/l/hour in the light and 0.45 mg C/l/hour in the dark. The ASH catchment in not entirely peat, and therefore this method could be applied for that site.

Figure 3.4. Average suspended sediment concentrations for the five sites.



3.3.8. Discussion

This chapter found an average DOC loss of 86% over 30 hours in the light, compared with the 76% loss over 10 days in Chapter 2. If this loss was constant over the 30 hours, and extrapolated to 10 days, it would have resulted in a 600% loss of DOC. Chapter 2 found 76% loss of DOC in 10 days; in that experiment there would have been 9.5% loss after 30 hours. The average DOC loss in Chapter 2 includes data from a site with a catchment areas of over 800 km^2, whereas all the sites used for this chapter have comparatively very small catchment areas (all less than 12 km^2), which may account for the differences in the average loss values, as the DOC from the larger catchments is older, and therefore more recalcitrant than the fresher DOC from the smaller catchments.

The covariates found to be significant in the ANOVA and ANCOVA on the change in relative DOC concentrations across all sites and treatments were the DOC_0 and the sulphate concentration. The same ANCOVA on Chapter 2 data also found the initial DOC to be significant. DOC_0 was found to be a significant covariate in every statistical test that found covariates to be significant at all. In fact, the only other covariate found to be significant was the sulphate concentration in the ANCOVA mentioned above. This suggests that regardless of the site catchment scale and time-scale of the experiment, the initial DOC is significant in determining the change in DOC concentration and the rate of DOC decline. This also suggests that knowing the initial DOC concentration of a river, it should be possible to estimate the potential net change in the DOC concentration due to photo- and bio-degradation.

There were significant changes in the E4:E6 ratio at PEN over the 30 hours of the experiment, suggesting that the composition of the DOC may play a role in controlling the rate or magnitude of DOC loss in a river.

Assumptions were made in the calculations of the POC concentrations from the suspended sediment concentrations, and so the resulting values may not be accurate reflections of the POC concentrations of the sites. However, data from the literature gives similar concentrations, suggesting that the values in this chapter are at least the correct order of magnitude (Pawson et al., 2008).

3.4. Conclusions

The main findings of this chapter are outlined in Table 3.10. The most interesting result of these experiments is the significant differences in the rate of loss of DOC between the day and night phases, indicating a diurnal cycle of DOC degradation. The high rates of loss during the first few hours of the experiments suggest that the DOC is initially highly labile and degradable by both photo- and bio-degradation. The reduction in degradation rates overnight and during the second day shows that the highly degradable fraction of the DOC has been depleted, and that the remaining DOC is more refractory and less bioavailable. The Teesdale chapter (Chapter 2) did not pick up on a diurnal cycle due to the daily sampling regime, and so the role of diurnal cycles should be investigated further on the Tees using an hourly sampling regime.

There were significant differences in the loss of DOC between the sites, and the initial DOC was often a significant covariate in the analyses, showing that the site differences in DOC type and concentration play a large role in determining the DOC degradation rates. The rate of DOC degradation declined over time, suggesting a decrease in the 'degradability', or an increase in the recalcitrance, of DOC.

The high rate of decline of DOC even in the dark suggests that it is not only photo-induced degradation that takes place, but also bio-degradation plays a major part in the degradation of DOC. Temperature was never a significant covariate in the models, whereas treatment was significant; so the observed differences are therefore unlikely to be due to temperature differences between the day and night. There was no PAR data for this experiment, so carrying out further experiments with PAR data will allow for a more robust analysis of the rates of degradation in the light.

The decrease in particulate carbon as well as in dissolved carbon show that there is no net production of POC from; it is leaving the catchment, predominantly as CO_2.

The diurnal cycle requires further investigation to better quantify the rates of degradation during the different phases of the experiment.

Table 3.10. The main findings of Chapter 3.

Sites studied	Peak District: PEN, BNO, UNG, NCO, ASH
Range of catchment areas (km^2)	0.005 – 11
Range of percentage peat cover (%)	Unknown – 100
Duration of experiments	Seasonally, for 30 hours
Sample size	301
Net DOC loss in the light treatment (%)	86
Net DOC loss in the dark treatment (%)	55
Range of rate of DOC loss in the light treatment (mg C/l/hour)	73.6 – -2.8
Range of rate of DOC loss in the dark treatment (mg C/l/hour)	44.1 – -7.4
Significant covariates:	
Change in DOC concentrations	Initial DOC, sulphate concentration
Light rate	Initial DOC
Dark rate	Initial DOC
What to investigate next?	Diurnal cycles of DOC degradation over a longer time, maybe up to four days, on a river with a known residence time, such as the Tees.

Chapter 4:
Diurnal cycles in the degradation of fluvial carbon from a peat headwater stream

4.1. Introduction and Aims

Chapters 2 and 3 have shown that both photo and bio-degradation reduce the concentration of DOC in headwater rivers with significant differences between the DOC concentrations in the water from the light and dark treatments. Chapter 3 showed a strong diurnal cycle in the rate of DOC loss, with most of the degradation being focussed in the first day, and a negligible loss of carbon during the night time darkness and a renewed increase in the rate of DOC loss on the second day. A review of the existing literature on diurnal cycles in outlined in Section 1.2.8 in Chapter 1.

The aim of this chapter is to investigate further and quantify the rates of loss of DOC, especially to focus upon the initial hours when degradation was observed to be occurring at its fastest rate., and to discover if the pattern of low losses of DOC overnight holds for a second and third nights, with the intervening days having higher rates of loss. This chapter also aimed to show that there was a relationship between the initial composition and concentration of the DOC and the rate of DOC loss, using two sites with different DOC concentrations and that are likely to have different DOC compositions. Sections 1.2.9 and 1.2.10 in Chapter 1 outline the literature review on the different types and the composition of DOC.

4.2. Approach and Methodology

This chapter adapts the method of Moody et al. (2013) and Chapters 2 and 3, to conduct in-situ degradation measurements of DOC from the headwater and former tidal limit of the River Tees in North-East England over periods of time up to 70 hours.

4.2.1. Field sites

This chapter used two of the four sites used in Moody et al. (2013) and Chapter 2: the source water, Cottage Hill Sike within Moor House NNR, and former tidal limit site, Broken Scar near Darlington (CHS and DBS; Figure 2.1, Table 2.1).

4.2.2. Degradation measurements

The degradation measurements were made as in Chapters 2 and 3, outside of the laboratory in ambient light and temperature conditions (rather than indoors under artificially controlled conditions).

Water samples were taken on a monthly basis from the two sites in the River Tees. January samples were obtained only from the downstream site as poor weather conditions prevented the site within the Moor House NNR from being visited. Each degradation experiment spanned approximately 70 hours with sacrificial sampling taking place at hour 0, 1, 2, 8, and then at dawn and dusk on days 2, 3 and 4, with light and dark treatments for each site on each month. Fixed numbers of hours since the start of the experiment were not used in the experiment because change in day length would mean that samples in daylight one month may be in darkness in a subsequent month, and thus samples were taken relative to dawn and dusk for each period of experimentation each month. Replicates were included within each degradation experiment and, over the course of the year, each combination of factors (see section 4.2.4) was replicated. No hour 0 (t_0) samples were replicated, but 47% of all other measurements were replicated (362 of 772 samples). As in Chapter 2, a data logger with a PAR (photosynthetically active radiation) meter and thermocouple recorded the radiation levels and air temperature at 15-minute intervals throughout the 70-hour period of each month's experiment. Radiation and temperature conditions were summarised as the average conditions over the period for each sample and PAR measurements were summed to give the total radiation experienced by any one sample. The radiation measurements were treated in this way because a sample after 70 hours may have experienced the same average radiation as a sample after 24 hours but will have received a larger total radiation dose.

The first day of the experiment was conducted at the field sites so the samples were exposed to the same light and temperature conditions as the river. At dusk all tubes were taken to the laboratory and placed outside so they would

continue to experience natural light and temperatures with ongoing monitoring of these conditions.

4.2.3. Sample analysis

Samples were analysed for DOC as in Chapter 3, and for absorbance at 400, 465 and 665 nm, E4:E6, pH, water temperature, conductivity, ion chromatography and acidity/alkalinity as in Chapters 2 and 3.

Suspended sediment (SS) concentration in each monthly experiment was measured in samples at the beginning, in the middle and at the end of each experiment. Samples were filtered through pre-weighed, 0.45 μm, glass fibre filters; dried to 105 °C and the filter paper re-weighed to give the concentration of suspended sediment. The filter papers were then put in a furnace for 4 hours at 550 °C, and then re-weighed. The mass lost in the furnace equates to the mass of particulate organic matter (POM), and 47.5% of this was assumed to be particulate organic carbon (Moody et al., 2013; Worrall et al., 2003). This value was chosen as the literature values range from 45 to 50%.

4.2.4. Statistical methodology

The design of the experiment incorporates four factors: Month, Time, Site and Treatment. Each factor has a number of levels: Month has 12 levels (one for each calendar month); Time has 10 levels (average hours after start of experiment as: 0, 1, 2, 4.37, 9, 21.96, 30.96, 45.09, 54.48, 68.87); Site has two levels (CHS and DBS); and Treatment has two levels (light and dark). The sample times were the averaged values (each has a standard error) that represent the samples taken on the first day (hours 0, 1, 2, 4.37, 9), dawn and dusk on day 2 (hours 21.96 and 30.96), dawn and dusk on day 3 (hours 45.09 and 54.48) and dawn on day 4 (hour 68.87, called 't_{70}' in this chapter). A similar analysis progression to Moody et al. (2013) was used, as the experimental design was similar and this allowed comparisons to be made between that study and this chapter. Tests for normality (Anderson-Darling tests), ANOVA, ANCOVA and regressions were carried out as in Chapters 2 and 3.

The concentrations of DOC were analysed in both absolute and relative terms where the relative value for each sample in an experiment was expressed as the ratio of the measured value to measurement at t_0 for the same site on that

experimental run. The magnitude of the effects and interactions, using generalized ω^2 (Olejnik and Algina, 2003), of each significant factor and interaction were calculated. For some analyses, the two sites were analysed separately due to the differences between them; often this resulted in better r^2. Main effects plots were used to visualise the data as they show the overall effect of the factor once all other factors and covariates have been taken in to account.

As in Chapter 3, the rates of DOC degradation were also calculated for the very first hour of the experiments, and for the whole days and nights in the first 48 hours of the experiments, to give the initial rates and the day/night rates. These rates then underwent the same ANOVA, ANCOVA and regression process as the DOC concentrations. The effects of priming were also analysed, as in Chapter 3.

As in Chapter 2, the apparent quantum yields (AQY) and activation energies were estimated where the PAR or temperature variables were significant in ANCOVA or regression equations.

4.3. Results and Discussion

In total 772 individual experiments with complete covariate information and within the context of the factorial design were conducted and analysed. Summary of the water chemistry at the two sites over the 70 hours of the experiment period in light conditions is given in Table 4.1.

Some of the covariates showed the same pattern between t_0 and t_{70}, at both of the two sites shown in Table 4.1. The conductivity, pH, E4:E6, bromide, nitrate, phosphate and sulphate concentrations increased, and the chloride concentration decreased. The absorbance at 400 nm and the fluoride concentration both increased in the CHS water and decreased in the DBS water. Of these changes, only five were significantly different between t_0 and t_{70} across both sites: conductivity increase at CHS ($p=0.0041$), the pH increase at CHS ($p<0.0001$) and DBS ($p<0.0001$), the absorbance at 400 nm decrease at DBS ($p=0.0498$) and the chloride decrease at CHS ($p=0.0027$).

The concentrations of the conservative ions (bromide and chloride) were investigated further to show how much natural variability there was in the dataset. An ANOVA on the whole dataset showed there were no significant differences between the Site, Time and Treatment for bromide, however there were significant

differences for the chloride, which was all due to the differences between the concentrations at the two sites, with DBS having significantly higher chloride concentrations than CHS. The changes in the average absorbance at 400 nm and the E4:E6 ratio suggest that the DOC underwent some compositional changes during the experiment.

4.3.1. DOC concentrations

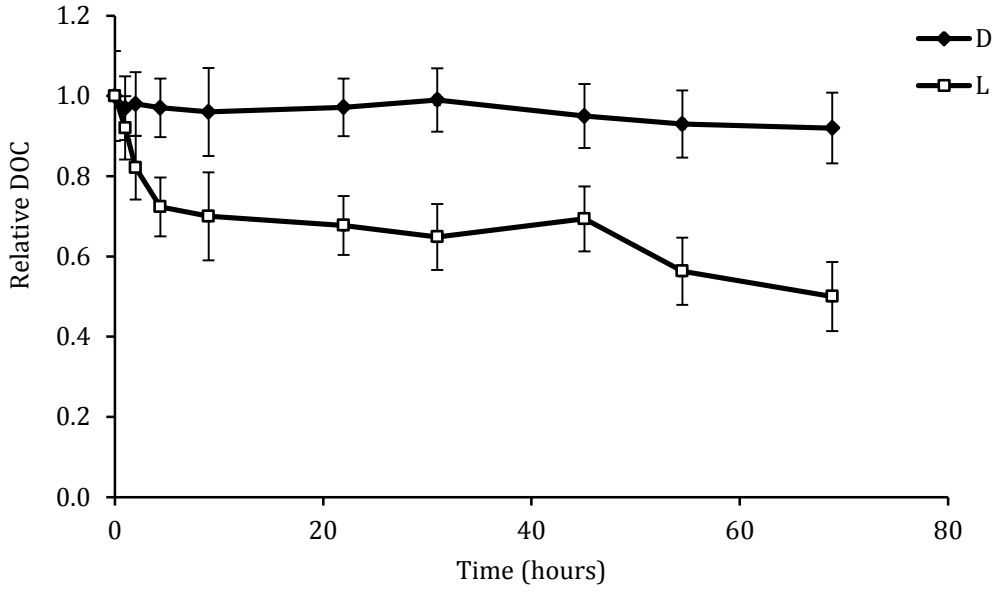
For nearly every month of measurement the DOC concentration in both treatments decreased. The average DOC concentration over time over all sites showed a steep initial decline, although the rate of decline was still not zero even after 70 hours (Figure 4.1). The average decline in DOC concentration across all months for all sites for samples in daylight was from 35 to 19 mg C/l after 70 hours: when concentrations were judged relative to the DOC_0 concentration at each site then the average decline over 70 hours was 35%. For experiments only in the dark the average decline over a 70-hour period was 3%. The average difference across all sites and all times between samples in light and dark was 10.7 mg C/l with DOC_{70} concentrations (DOC concentration at t_{70}) of samples kept in the light being on average 32% lower than those kept in the dark when judged relative to the DOC concentration at t_0.

Table 4.1. The average and coefficient of variation (CV - %) of the 12 variables measured from the two sites (CHS and DBS), averaged across all 12 sampling months. The table shows the initial (t_0) and end (t_{70}) concentrations for each variable from the light treatment.

	Cottage Hill Sike (CHS)				Broken Scar (DBS)			
	t_0		t_{70}		t_0		t_{70}	
Determinant	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
POC (mg C/l)	2.86	31	3.23	14	2.96	29	3.14	19
Conductivity (µS/cm)	35.87	25	78.23	61	573.58	51	609.49	46
pH	4.57	14	6.34	5	6.76	3	7.61	3
DOC (mg C/l)	41.75	30	16.52	85	29.21	68	21.98	89
Abs_{400}	0.16	39	0.17	45	0.07	43	0.05	46
E4:E6	4.54	41	4.94	31	4.78	61	6.40	44
Bromide (mg/l)	0.18	154	0.32	103	0.78	166	0.86	128
Chloride (mg/l)	21.54	85	7.15	106	66.13	85	65.58	74
Fluoride (mg/l)	0.36	80	0.40	90	0.47	53	0.44	61
Nitrate (mg/l)	1.01	64	0.80	84	13.38	81	13.69	102
Phosphate (mg/l)	0.56	80	1.31	210	0.60	78	4.23	383
Sulphate (mg/l)	5.49	71	10.19	74	66.78	55	83.16	50

Of all the experiments run, there were 178 samples (out of a total of 772 samples) where an increase in DOC concentration was observed relative to the initial DOC concentration. In 15 of the cases there was a higher DOC_{70} concentration than DOC_0. Given that no raw water samples were filtered prior to inclusion in the experiment it was possible that particles or the microbial population within the sample generated DOC over the course of the experiments. Samples where there was an increase in DOC over the course of the experiment were not removed from the analysis, as the experiment was interested in the conversion of POC to DOC.

Figure 4.1. The main effects plot of relative DOC concentration change for light (L) and dark (D) for all sites, over the course of the experiment. Error bars are standard errors.



The Anderson–Darling test showed that neither the distribution of DOC concentration nor relative DOC concentration for the experiments conducted in the light, nor those in the dark, met the condition of normality; therefore all subsequent ANOVA were performed on log-transformed data as that data did meet the test conditions, and the Anderson-Darling test showed that no further transformation was necessary.

When the relative concentration data for both treatments (light and dark) were considered without covariates, all single factors were found to be significant

(Table 4.2). The least important single factor was Time (explaining only 2.77% of the variance in the original dataset). The most important factor was Treatment, explaining 14.53% of the original variance.

Table 4.2. Results of ANOVA for relative DOC concentrations for all experiments across both daylight and dark treatments.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
pH	na	-	<0.0001	1.36
Abs_{400}	na	-	<0.0001	2.93
DOC_0	na	-	<0.0001	4.25
ΣPAR	na	-	<0.0001	2.62
Site	<0.0001	5.04	ns	-
Treatment	<0.0001	14.53	<0.0001	3.12
Time	<0.0001	2.77	ns	-
Month	<0.0001	4.08	<0.0001	5.40
Site*Treatment	<0.0001	2.36	<0.0001	2.40
Site*Time	0.0033	0.82	0.0002	1.45
Site*Month	<0.0001	8.50	ns	-
Treatment*Time	<0.0001	3.77	ns	-
Treatment*Month	<0.0001	5.92	<0.0001	6.75
Time*Month	<0.0001	4.34	<0.0001	5.21
Error		13.09		25.57

One of the reasons for using relative DOC concentration was to minimize the difference between sites and months. To show that this has been effective, the same ANOVA was carried out on the raw DOC values, and this found that the variance explained by the Month factor was substantially smaller when the relative concentrations were used. The variance explained by the Site factor was also smaller, though the relative difference was not as great as for the Month factor.

Even using the relative DOC concentrations there was still a significant effect due to Month, but this may reflect the importance of the t_0 DOC concentration for the degradation rate (with faster degradation rates associated with higher initial concentrations) rather than a seasonal cycle in degradation behaviour *per se*, which also explains the significant interactions between the Month factor and the sample

Time and the Treatment factors. Overall the ANOVA of the relative DOC concentration explained 65.22% of the variance in the original data. The error term represented 13.09% of the variance. This error term represents the unexplained variance in the model, which was not only due to sampling or measurement error but also variables, factors or their interactions that were not or could not be included in the ANOVA, such as the river discharge at the start of each experiment.

Including covariates in the ANOVA (ANCOVA) showed the most important covariate was the DOC_0 concentration, where relative concentration declined faster over time with increasing DOC_0. This suggests that degradation rate was concentration dependent. Three other covariates were significant: pH, absorbance at 400 nm and ΣPAR.

As the ΣPAR was significant, the apparent quantum yield (AQY) was estimated for the 305 light treatment samples and it was found to vary between 0 and 82 mmol C/mol photons. The average values for CHS and DBS samples were 7.31 and 3.87 mmol C/mol photons respectively, and the AQYs were much higher for the first few hours of the experiment compared with the later hours. For CHS, the AQY in the first hour of the experiment was 26.95 mmol C/mol photons, which decreased to 7.92 mmol C/mol photons by t_4. The DBS samples for the same time period decrease from 11.59 to 5.08 mmol C/mol photons. The AQYs for the final samples (t_{70}) were 0.73 and 0.39 mmol C/ mol photons for CHS and DBS respectively. This suggested that the DOC was more susceptible to loss during the first few hours of the experiment, and the susceptibility decreased towards the end of the first day, staying low for the remainder of the experiment.

An ANOVA on the AQYs data found that they were significantly different between Site, Month and Time, and the three interactions of Site*Month, Site*Time and Month*Time. The Month, Time and Month*Time explained approximately 20% of the variation each. The significance of Month suggests that some months were associated with proportionally more DOC loss than others. The Time factor showed a clear pattern, as mentioned above, with the first few hours being significantly higher than the AQY of times after t_{10}. The factor that explained the smallest proportion of the variation was Site (2.83%), followed closely by the interactions of Site*Time (3.40%) and Site*Month (4.21%).

Guided by the results of the DOC ANOVA and ANCOVA it was possible to give the best-fit equation for the change in the DOC concentration (ΔDOC) in light conditions:

$$ln\Delta DOC = \underset{(0.05)}{-0.15pH} + \underset{(0.08)}{0.67lnDOC_0} - \underset{(0.05)}{0.03}\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + \underset{(0.00)}{0.02t} + \underset{(0.52)}{1.88}$$

n=364, r^2=0.38 *(Eq. 4.1)*

where pH is the pH of the water, DOC_0 is the DOC concentration on time zero of each experiment (mg C/l), t is the time since the start of the experiment (hours) and m is the month number (January = 1 to December = 12). Only variables that were found to be significantly different from zero at least at a probability of 95% were included. The values in brackets give the standard errors on the coefficients and the constant term.

This regression analysis shows that the pH, DOC_0, time and month were significant in determining the change in the DOC over the course of the experiment. An ANOVA on the pH values showed that they were significantly different between the two sites ($p<0.0001$) and that Site explained 67% of the variance in the pH values. The ANCOVA on DOC showed that pH was a significant covariate, and Site was no longer a significant factor (Table 4.2). The significance of the pH in that ANCOVA and the ANOVA on the pH values suggests that once covariates such as pH were included they explained the variance that was attributed to the site factor in the first ANOVA on the DOC concentrations, and as such, pH is a proxy for the Site factor. This means that the chemical differences between the two sites can explain the differences in DOC between the two sites.

When the regression analysis was carried out for the two sites separately, it was found that the r^2 value for CHS was lower than for the two sites together, and for DBS was higher:

CHS:

$$ln\Delta DOC = \underset{(0.82)}{-2.69Abs_{400}} + \underset{(0.19)}{0.99lnDOC_0} + \underset{(0.008)}{0.017Cl^-} + \underset{(0.002)}{0.01t} - \underset{(0.72)}{0.80}$$

n=177, r^2=0.24 *(Eq. 4.2)*

DBS:

$$ln\Delta DOC = \underset{(2.74)}{11.47}Abs_{400} + \underset{(0.09)}{0.52}lnDOC_0 - \underset{(0.001)}{0.004}Cl^- + \underset{(0.005)}{0.02}NO_3^- + \underset{(0.003)}{0.02}t - \underset{(0.34)}{0.92}$$

n=160, r^2=0.50 *(Eq. 4.3)*

where Abs_{400} is the absorbance at 400 nm, Cl^- is the chloride ion concentration (mg/l), NO_3^- is the nitrate ion concentration (mg/l) and all other terms are as described above. As above, these equations show that the absorbance at 400 nm and the anion concentrations are significant in determining the change in DOC. The significance of the absorbance at 400 nm suggests that there is an effect of the composition of the DOC on the photo-induced degradation. The chloride concentration significance is harder to explain, as there is unlikely to be a direct mechanistic interaction between the DOC and chloride. The nitrate concentration may reflect the importance of nutrients in the biodegradation of DOC.

In Chapter 2 the equation for the change in DOC (*ln*ΔDOC – Eq. 2.2) found the DOC_0 concentration, time since the start of the experiment and the month of the experiment to be significant, although that equation was derived from all four sites used in that chapter. The equation using both sites in this chapter (Eq. 4.1) found the same factors to be significant, as well as pH, showing that these factors are consistent across different time scales and in two separate experiments.

The r^2 in Chapter 2 was 0.76, whereas the r^2 in this chapter was lower, 0.38. The r^2 increased for DBS when the two sites were analysed separately, but decreased for CHS, suggesting that the change in DOC concentration is harder to model for the CHS samples. This may be because the regression analysis is trying to fit a single straight line through the data, when CHS especially may benefit from using two lines, one for the initial rapid decrease during the first day, and one for the remaining time of the experiment.

Analysing the change in DOC concentrations for these two sections separately for CHS samples found an r^2 of 0.49 for the first 10 hours (Eq. 4.4), and 0.19 for the last 60 hours of the experiment (Eq. 4.5). Different parameters were

found to be significant in the two equations, with absorbance at 400 nm and chloride concentration being significant during the first 10 hours but not significant during the last 60 hours. The equations had two factors in common: the initial DOC concentration and the ΣPAR, however the parameter estimates suggest that both of these were more influential in the first 10 hours. The inverse of the cumulative temperature was significant in the final 60 hours of the experiment. It is interesting to note that neither equation found time of the experiment, or month of the year to be a significant parameter, however both the ΣPAR and cumulative temperature factors will reflect changes in both time and month.

CHS, between t_0 and t_{10}:

$$ln\Delta DOC = \underset{(1.41)}{-6.24Abs_{400}} + \underset{(0.34)}{2.18lnDOC_0} + \underset{(0.002)}{0.008\sum PAR} + \underset{(0.01)}{0.02Cl^-} \underset{(1.28)}{- 5.12}$$

n=65, r^2=0.49 *(Eq. 4.4)*

CHS, between t_{10} and t_{70}:

$$ln\Delta DOC = \underset{(0.21)}{0.52lnDOC_0} + \underset{(0.0005)}{0.002\sum PAR} + \underset{(204)}{\frac{802}{T}} \underset{(0.98)}{- 0.99}$$

n=95, r^2=0.19 *(Eq. 4.5)*

where ΣPAR is the cumulative photosynthetically active radiation experienced by the sample (W/m^2), T is the cumulative temperature (Kelvin) and all other terms are as described above.

The average AQY for CHS between t_0 and t_{10} was 7.31 and between t_{10} and t_{70} was 2.58 mmol C/mol photons, showing that, as described above, the DOC was more susceptible to light in the first few hours of the experiment. As the reciprocal of absolute temperature was significant in the regression equation (Eq. 4.5), it was possible to estimate the activation energy of the degradation of DOC in the CHS samples after t_{10} using the same values for the universal gas constant in Chapter 2

(0.692 J/K/g C). The activation energy was estimated to be 0.56 ± 0.14 kJ/g C. This was quite a bit smaller than the values obtained in Chapter 2, and by Alperin et al. (1994).

4.3.2. Photo-induced degradation

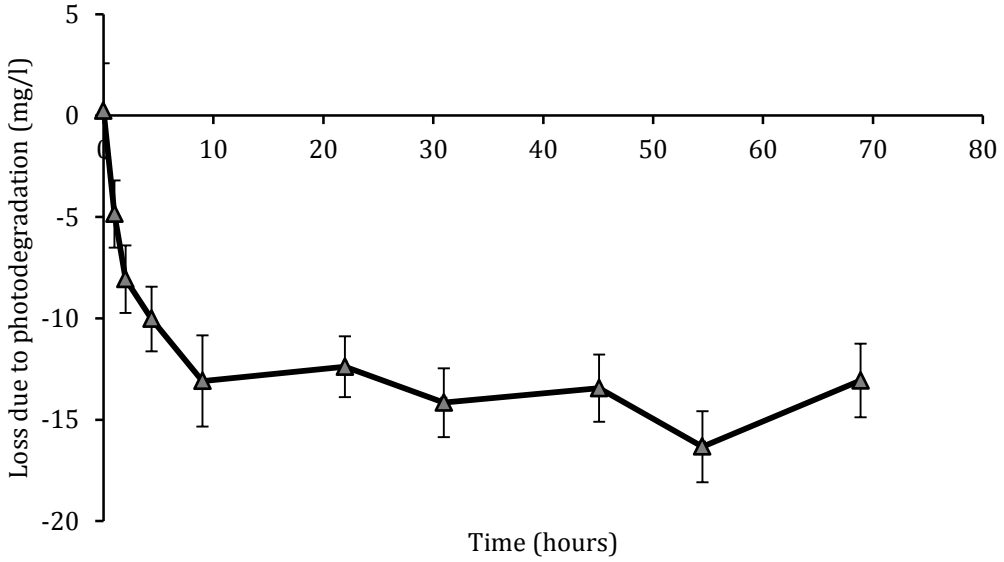
The difference between the dark and light concentrations in each experiment was taken as the estimate of the impact of photic processes (Figure 4.2). The extent of photo-induced degradation could be estimated in 421 cases and the loss due to photo-induced degradation varied from 48 mg C/l to -48 mg C/l (i.e. similar to the above there were 69 occasions where the DOC concentration was observed to increase, implying photo-induced production). Of the 69 occasions where an increase was observed, only 11 were higher than 10 mg C/l, showing the majority of cases have higher dark DOC than light DOC, or a very small difference between the two. The average difference in DOC concentration that can be ascribed to photo-induced degradation for CHS over the 70 hours was -15.23 mg C/l.

The ANOVA shows that all single factors and all interactions were significant (Table 4.3). Only one covariate (the initial pH of the water), and no others, was found to be a significant. The Month factor, although significant and explaining the highest proportions of the variance in both the ANOVA and ANCOVA (23.04% and 32.27% respectively), shows no clear seasonal cycle, which may imply that hydroclimatic conditions on the day of sampling (e.g. river flow) were more important than the season of the year or that DOC_0 concentration represents the seasonal differences. The other significant factors, Site and Time, and the three significant interactions (Site*Time, Site*Month, Time*Month) all explain small proportions of the variance in the ANOVA. Unlike the raw DOC concentrations ANCOVA, the Site factor is still significant in the ANCOVA even with the inclusion of the pH_0 (initial pH) values as a significant covariate.

Table 4.3. Results of ANOVA for the difference in DOC concentrations between light and dark treatments.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
pH_0	na	-	<0.0001	3.20
Site	<0.0001	6.40	<0.0001	6.21
Time	<0.0001	7.14	ns	-
Month	<0.0001	23.04	<0.0001	32.27
Site*Time	0.0302	1.00	ns	-
Site*Month	<0.0001	3.14	ns	-
Time*Month	0.0002	7.26	<0.0001	11.07
Error		22.47		14.15

Figure 4.2. The main effects plot of the change in loss due to photo-induced degradation over the course of the experiment. Error bars are the standard errors.



Given the results of the ANOVA it was possible to identify the best-fit equation for the loss due to photo-induced degradation:

$$\Delta DOC_{photo} = -1.60\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) - 0.11t - 0.16DOC_0 + 2.94pH_0 - 20.20$$

(0.68) (0.03) (0.04) (0.59) (4.50)

n=398, r^2=0.21 *(Eq. 4.6)*

where ΔDOC_{photo} is the change in the difference between the dark and light DOC concentrations (mg C/l), pH_0 is the initial pH and all other terms are as described above. It should be noted that neither temperature nor PAR variables were found to be significant covariates in any of the above approaches. However, for the purposes of comparison with Chapter 2, the AQYs were estimated for the photo-induced DOC loss in 362 experiments. It was found to vary between 92 and -82 mmol C/mol photons; this range is much larger than the range found in Chapter 2 of 9.6 to -1.7 mmol C/mol photons. The average AQY for this chapter was 3.57 mmol C/mol photons, which is the range of Chapter 2, and the literature values cited therein (Osburn et al., 2009). The ANOVA on the AQYs found that there were significant differences between the Site, Month and Time factors, and the interactions of Site*Month, Site*Time and Month*Time. The seasonal cycle exhibited a similar pattern to that described in Chapter 2, with a peak in December and a minimum between February and June, showing the DOC in December was more photodegradable than the DOC in June.

The regression analysis on ΔDOC_{photo} (Eq. 4.6) showed that the DOC loss due to photo-induced degradation could be calculated from the seasonal cycle, sample time, DOC_0 and pH_0; all variables that can be easily measured, and therefore the equation is easily physically interpretable and easy to apply to other data sets.

When the regression analysis was carried out for the two sites separately, it was found that the r^2 values for both were higher than for the two sites together. For CHS, ten variables were significant in the regression, so the equation is rather complicated:

CHS:

$$\Delta DOC_{photo} = 12.4\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + 0.14t + 0.08DOC_0 + \frac{8406}{T} - 14.6pH_0$$
$$+ 3.13cond_0 + 596Abs_0 + 20E4{:}E6_0 + 1.79Cl^- - 19.3NO_3^- - 290$$
$$(2.04) \qquad (0.07) \quad (0.06) \quad (1912) \quad (2.57)$$
$$(0.31) \quad (54) \quad (1.75) \quad (0.19) \quad (1.73) \quad (26)$$

n=191, r^2=0.59 *(Eq. 4.7)*

where $cond_0$ is the hour 0 conductivity, Abs_0 is the hour 0 absorbance at 400 nm, $E4{:}E6_0$ is the hour 0 E4:E6 ratio, Cl^- and NO_3^- are the hour 0 chloride and nitrate ion concentrations (mg/l) and all other terms are as described above. From this equation (Eq. 4.7) onwards, the anion variables were always the hour 0 concentrations.

As the reciprocal of absolute temperature was significant in the regression equation (Eq. 4.7), it was possible to estimate the activation energy of the degradation using the same values for the universal gas constant in Chapter 2 (0.692 J/K/g C). The activation energy was estimated to be 5.82 ± 1.32 kJ/g C. This was the same order of magnitude as the values estimated in Chapter 2, and by Alperin et al. (1994), but quite a bit higher than the value estimated for the change in the DOC concentration the last 60 hours of the CHS experiments, in section 4.3.1, which was 0.56 ± 0.14 kJ/g C.

The regression equation for DBS was slightly less complicated than for CHS, but still included six variables:

DBS:

$$\Delta DOC_{photo} = 3.59\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) - 0.07t - 0.31DOC_0 + 7.48pH_0$$
$$+ 147Abs_0 + 0.09Cl^- - 64$$
$$(1.68) \qquad (0.03) \quad (0.05) \quad (2.91)$$
$$(47) \quad (0.02) \quad (22)$$

n=207, r^2=0.30 *(Eq. 4.8)*

where all terms are as described above.

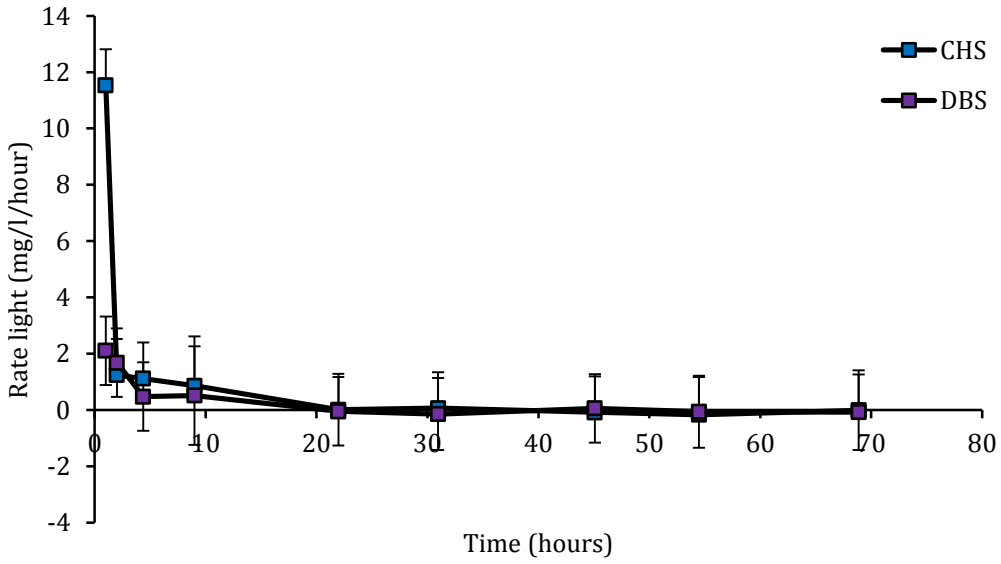
These models are much more complicated than the equation for both sites together, and so lose some of the benefits, as they are harder to physically interpret, so the model for both sites may be better than the separate sites even though the r^2 is lower. Both models found the absorbance at 400 nm to be significant, indicating that the composition, as well as the initial concentration, of the DOC is important in the degradation of DOC.

Comparing these equations to that derived in Chapter 2 (Eq. 2.3) shows that there are few factors in common, as the equation in Chapter 2 found that the DOC concentration and absorbance at 400 nm were significant in modelling the change in photo-induced DOC, and resulted in a much simpler equation. The equation derived using data from both sites in this chapter is simpler than the equations for the sites separately, but results in a much lower r^2. The three equations in this section have a few factors in common: DOC_0 and pH_0 were significant in all three. The water chemistry variables that are significant in the equations above (Eq. 4.7 and 4.8) include the absorption at 400 nm, which is a measure of the colour of the water that is sometimes used as a proxy for DOC, and so reflects the DOC concentration. The water chemistry variables in the CHS equation (all parameters except month, DOC_0 and time) collectively explain a larger proportion of the variation than the sinusoidal function; these parameters will all vary in a seasonal cycle, and so are significant in determining the photo-induced changes in DOC concentration.

4.3.3. Rate of degradation in the light

For samples in the light, the degradation rate varied from 37 mg C/l/hour to -18 mg C/l/hour (Figure 4.3); i.e. increases or no change in DOC concentrations were observed in 62 cases out of 189. Again, out of the 62 cases that observed increases, only 7 were more than 10 mg C/l/hour, showing that the majority of cases have a positive rate of degradation. The average rate of degradation in the light for samples from CHS only was 1.73 mg C/l/hour.

Figure 4.3. Main effects plot of rate of DOC loss in light conditions over time in the experiment. Error bars give the standard error.



The ANOVA of the rate of degradation for samples in the light showed that all factors were significant, but the interaction of Site*Month was not significant (Table 4.4). No Treatment factor was included because only experiments in the light were being considered. When included, no covariates were found to be significant, which means that the rate of degradation was not dependent on anything other than Time, Site and the Month of sampling.

Table 4.4. The results of ANOVA of the degradation rate of DOC in the light.

	Without covariates	
Factor	p	ω^2
Site	0.0271	0.95
Time	<0.0001	16.78
Month	0.0226	3.07
Site*Time	<0.0001	7.46
Time*Month	<0.0001	26.01
Error		26.72

Guided by the results of the ANOVA, the best fit equation for degradation rate in daylight was best represented by two equations, one for each site:

CHS:

$$lnrate_{light} = \underset{(0.8)}{0.08} - \underset{(0.1)}{0.79lnt} + \underset{(228)}{\frac{277}{T}} + \underset{(0.0005)}{0.00024}\sum PAR$$

n=141, r^2=0.57 *(Eq. 4.9)*

DBS:

$$lnrate_{light} = \underset{(1.3)}{4.79} - \underset{(0.1)}{1.65lnt} - \underset{(357)}{\frac{914}{T}} - \underset{(0.001)}{0.00018}\sum PAR$$

n=90, r^2=0.69 *(Eq. 4.10)*

where $rate_{light}$ in the rate of DOC change in the light treatment and all other terms are as defined above.

The regression analysis showed that the cumulative light exposure and inverse temperature, along with the time since the start of the experiment, were significant in determining the rate of DOC degradation at both sites, suggesting that the DOC degradation was influenced by environmental factors, such as the temperature and weather during the experiments.

Chapter 2 found the rate of degradation in the light to be dependent on the DOC_0, time since the start of the experiment and the inverse temperature (Eq. 2.4). This shows that the temperature and time since the start of the experiment are consistently significant in modelling the rate of DOC degradation in the light over the two time scales considered by this chapter and Chapter 2.

As the ΣPAR was significant in the regression equations (Eq. 4.9 and 4.10), the apparent quantum yield (AQY) was estimated for the 173 light rate data. It was found to vary between -37 and 81 mmol C/mol photons. The negative AQYs occurred when the DOC increased rather than decreased during the time. The

average values for CHS and DBS samples were 3.26 and 0.92 mmol C/mol photons respectively, and the AQYs were much higher for the first few hours of the experiment compared with the later hours. For CHS, the AQY in the first hour of the experiment was 22.26 mmol C/mol photons, which decreased to 1.74 mmol C/mol photons by t_4. The DBS samples for the same time period decreased from 2.86 to 1.08 mmol C/mol photons. The AQYs for the final samples (t_{70}) were 0.15 and -0.10 mmol C/mol photons for CHS and DBS respectively.

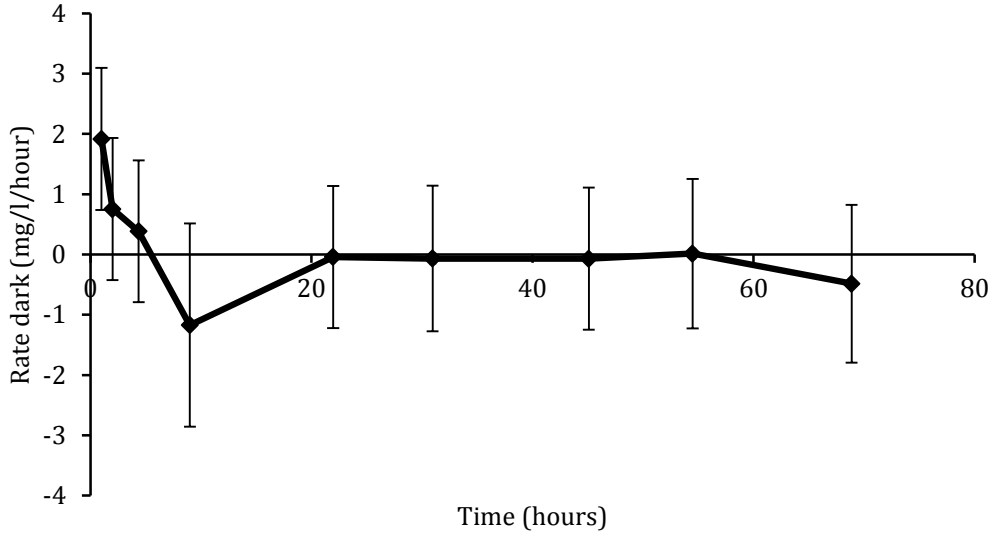
An ANOVA on the AQY data found that they were significantly different between Month and Time, and two interactions of Site*Time and Month*Time. The Time and Month*Time explained approximately 15% of the variation each.

As the reciprocal of absolute temperature was significant in the regression equations (Eq. 4.9 and 4.10), it was possible to estimate the activation energy of the degradation for CHS and DBS to be 0.19 ± 0.16 and 0.63 ± 0.25 kJ/g C, respectively. This shows that the DBS DOC had higher activation energy than the CHS DOC.

4.3.4. Rate of degradation in the dark

It was possible to calculate the rate of degradation in the dark in 258 experiments, which ranged from a decrease of 43 mg C/l/hour to -36 mg C/l/hour (in 108 cases, an increase or no change in DOC concentration was observed), however, only 4 values fell outside the -10 to 10 mg C/l/hour range. The median values for the rates of dark degradation at CHS and DBS were 0.005 and 0.001 mg C/l/hour respectively, i.e. the majority of the rates were negligible (Figure 4.4). A negative rate occurred at t_{10}, indicating that DOC was produced rather than degraded during the preceding few hours. However, for the rate of degradation in the dark, the ANOVA and ANCOVA show that no factors or covariates were significant; even so regression was attempted, but no significant variables were found for either site. There were no significant differences between the rates at different times during the experiment. Chapter 2 found that the rate of degradation in the dark could be modelled from the DOC_0, time since the start of the experiment, month of the experiment and inverse temperature, (Eq. 2.5) but applying that equation to the data in this chapter found none of the same variables to be significant.

Figure 4.4. Main effects plot of rate of DOC loss in the dark over time of the experiment. Error bars are the standard errors.



4.3.5. The rate of photo-induced degradation

The rate of the photo-induced degradation could be calculated from 190 experiments and varied from 33 mg C/l/hour to -47 mg C/l/hour (in 112 cases an increase or no change was observed). Of all the cases where an increase was observed, only 8 cases were the increases greater than 10 mg C/l/hour, showing that the majority of cases showed a decrease or a small increase (Figure 4.5). The average rate of photo-induced degradation for samples from CHS was 1.25 mg C/l/hour, and the median values for both sites were 0.07 mg C/l/hour for CHS and 0.02 mg C/l/hour for DBS. Time and Month, and the interaction between them were found to be significant (Table 4.5) in an ANOVA.

Figure 4.5. Main effects plot of rate of photo-induced DOC loss over time of the experiment. Error bars are the standard errors.

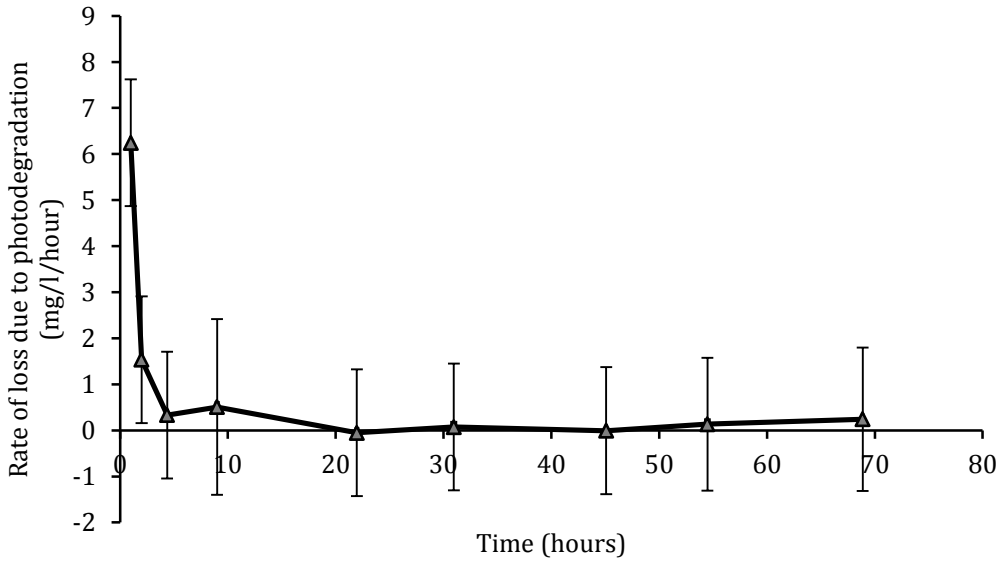


Table 4.5. The results of ANOVA of the photo-induced degradation rate of DOC.

	Without covariates	
Factor	p	ω^2
Time	0.0007	5.69
Month	0.0029	5.16
Time*Month	<0.0001	40.05
Error		26.06

The interaction was the most important component of the ANOVA, explaining 40% of the variation, and when included no covariates were found to be significant. Again, the regressions were carried out for the two sites individually:

CHS:

$$lnrate_{photo} = 1.8 - 1.12lnt$$
$$(0.2) \quad (0.1)$$

n=59, r^2=0.7 *(Eq. 4.11)*

DBS:

$$lnrate_{photo} = 2.0 - 1.2lnt$$
$$(0.4) \quad (0.1)$$

n= 53, r^2=0.63 *(Eq. 4.12)*

where $rate_{photo}$ is the rate of photo-induced degradation (mg C/l/hour) and t is the time in hours since the beginning of the experiment.

The regression shows that the only factor affecting the rate of photo-induced degradation is the time since the start of the experiment. The same equation in Chapter 2 found that DOC_0, time since the start of the experiment, month of the experiment and cumulative PAR to be significant (Eq. 2.6), making those more complicated than the equations found in this section. Also the equation in Chapter 2 has a much lower r^2 than these equations, once again showing the benefit of analysing the sites separately.

4.3.6. Rate of degradation during each day and night

The rates in each stage varied from 10 mg C/l/hour at CHS in the light during day 1 (between t_0 and dusk on day 1) to -4.92 mg C/l/hour at DBS in the dark during night 1 (between dusk on day 1 and dawn on day 2).

An ANOVA on the rates of degradation during each stage of the experiment had four factors, Treatment, Site, Stage and Month, with the "Stage" factor having four levels: day 1, night 1, day 2 and night 2. The ANOVA found all four factors significant (Table 4.6), as well as four interactions: Treatment*Stage, Treatment*Month, Site*Stage and Stage*Month. Stage explains the largest proportion of the variance (11.31%) closely followed by the interaction of

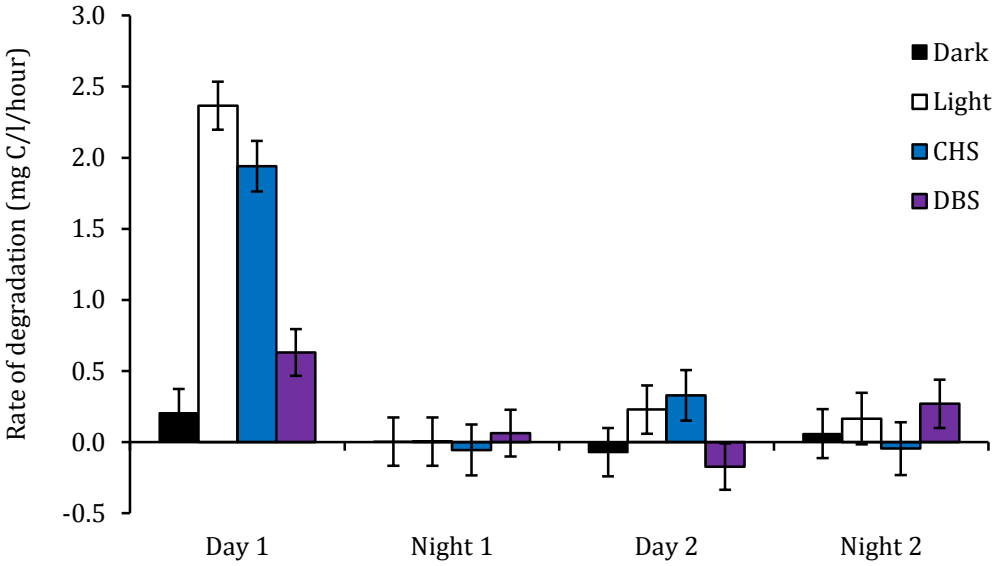
Stage*Month (10.54%), showing that the rates of DOC degradation differ significantly between the four stages of the experiment and between months. However, there was no clear seasonal cycle to the rates during each stage. The relationship between Treatment, Site and Stage showed the significant differences between the average rates per stage for both treatments and sites, with the night rates being not significantly different from zero (Figure 4.6). There were no significant covariates.

Table 4.6. The results of the ANOVA on the rates of degradation in each stage.

	Without covariates	
Factor	p	ω^2
Treatment	<0.0001	4.13
Site	0.0051	1.08
Stage	<0.0001	11.31
Month	0.0150	2.02
Treatment*Stage	<0.0001	8.07
Treatment*Month	0.0315	1.64
Site*Stage	<0.0001	3.70
Stage*Month	<0.0001	10.54
Error		11.69

The rates of degradation for CHS in the light treatment during the first two days and nights were modelled using ANOVA, and it was found that the stage of the experiment was significant, and no month factor or DOC_0 concentration was significant, i.e. it would be reasonable to use single zero-order rates for day 1, day 2, night 1 and night 2 without correction and that would account for 45% of the original variance. This is a large proportion of the variation accounted for by the rate at each stage, comparable to the results of the more sophisticated ANCOVA above and the corresponding ANCOVA in Chapter 3. The rates of degradation at CHS are the most interesting as they represent the rate of change in the newest, freshest material in the river system.

Figure 4.6. The main effects plot of average rates of DOC degradation per stage of the experiment for both treatments and both sites. Error bars are the standard errors.



Chapter 2 did not have the temporal resolution for this type of analysis, whereas Chapter 3 did, but not to the same extent as this chapter, only having data for Day 1, Night 1 and Day 2 rates. Chapter 3 found the same main result as this chapter, that the Day 1 rate was far greater than the Night 1 or Day 2 rates in both light and dark treatments. The ANOVA in Chapter 3 (Table 3.9) found that stage explained the largest proportion of the variation, as it did in this chapter.

4.3.7. Initial rates of degradation

The average initial rates of DOC degradation (during the first hour of the experiment) varied from 11.57 ± 2.89 mg C/l/hour in CHS in the light to 0.21 ± 0.52 mg C/l/hour in DBS in the dark.

An ANOVA on the rates of degradation during the first hour of the experiment had three factors, Treatment, Site and Month. The ANOVA found all three factors were significant (Table 4.7), as well as two interactions: Treatment*Month and Site*Month. The interaction of Treatment*Month explains the largest proportion of the variance (24.62%), closely followed by the Month factor, showing that the initial rates of DOC degradation differ significantly between

the treatments and between months. Again, there was no clear seasonal cycle to the monthly initial rates by site or treatment. Once covariates were added, the DOC_0, sulphate and nitrate concentrations were significant and the Site factor was no longer significant. This shows that the initial rate of DOC degradation was dependent in the initial concentration of DOC and two anions, as well as varying with treatment and month. The significance of the anions may reflect the importance of nutrients in the biodegradation of DOC.

Table 4.7. The results of the ANOVA on the rates of degradation in the first hour.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	0.0099	2.02
$Nitrate_0$	na	-	0.0005	4.22
$Sulphate_0$	na	-	0.0044	2.57
Treatment	<0.0001	8.54	<0.0001	8.50
Site	<0.0001	6.82	ns	-
Month	<0.0001	23.57	<0.0001	13.11
Treatment*Month	<0.0001	24.62	<0.0001	24.58
Site*Month	0.0031	6.82	ns	-
Error		12.41		24.36

Guided by the results of the ANCOVA, the following rate equation could be derived for the light treatment at CHS:

$$lnrate_0 = \underset{(0.7)}{2.3lnDOC_0} + \underset{(0.3)}{0.6cos\left(\frac{\pi m}{6}\right)} - \underset{(2.6)}{6.3}$$

n= 18, r^2=0.5 *(Eq. 4.13)*

where $rate_0$ is the initial rate of DOC change (mg C/l/hour), DOC_0 is the initial DOC concentration and m is month number (1 = January, 12 = December).

This regression shows that the factors affecting the initial rate are the initial DOC concentration and a seasonal factor. A significant regression for DBS could not be calculated, and the r^2 value was much lower when the two sites were used

together. This method of analysis would suggest that for CHS in the light, the initial important reaction is of the order 2.3 ± 0.7 which is not significantly different from second or third order. However it is most likely to be fractional or mixed order because of the number of processes contributing.

4.3.8. Priming

One measure of priming was considered; that if there were priming then there should be difference between the night time rates measured in samples that have been exposed to light from the night time rate for those samples that has always been in the dark. The four site and treatment combinations had the following average rate of degradation during the first night (mg C/l/hour ± standard error):
CHS D: -0.2 ± 0.13, CHS L: 0.1 ± 0.07, DBS D: 0.19 ± 0.13, DBS L: -0.07 ± 0.24

An ANOVA based on the night time rates, using Treatment, Site and Month as factors, found no significant differences in the rate of degradation.

Also, the ratio of the day to night rates should not be significantly different from 1. Firstly, a single sample t-test was used which showed that the mean ratio was 2.15 (95% confidence interval = 0.31 – 3.98) i.e. not significantly different from 1. Equally, an ANOVA based upon the ratio, Site and Month factors, and the t_0 concentration was applied but found not to be significant different between samples that had or had not experienced light conditions. Therefore, it was concluded that there was no priming effect.

4.3.9. POC concentrations

The suspended sediment concentrations were measured in each of the 12 months at the beginning, middle and end of the experiments. Six months of these suspended sediment measurements were analysed further to calculate the particulate organic matter (POM) concentrations, resulting in 126 POM measurements. Extrapolating from the six months of data, the percentage of POM, and therefore POC, for each site was calculated, and applied to the whole suspended sediment data set, resulting in a year of calculated POC concentrations.

The average change in POC concentration across all months for all sites for samples in the daylight was from 7.43 to 6.39 mg C/l after 70 hours; this is a decrease of 14%. For CHS, samples kept in the dark is the only combination in

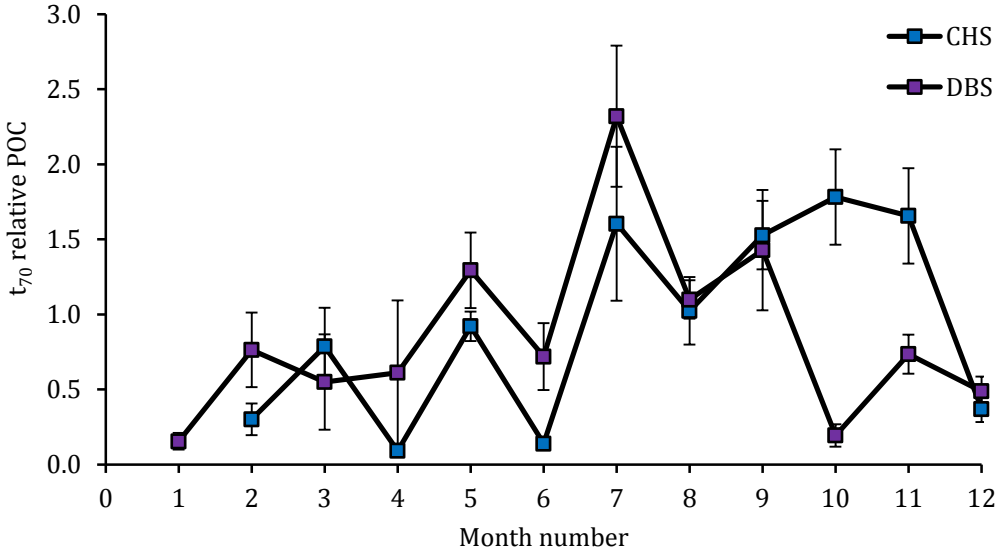
which the POC concentration increased between t_0 and t_{70} (increase of 45%); all other treatment and site combinations decreased; CHS in the light decreased by 15%. Again, the change at CHS in the light is the most interesting number as the POC at CHS will be the newest material into the river and so the change in its concentration treatment represents the most realistic scenario.

The Anderson-Darling test showed that the distribution of POC concentration did not meet the conditions of normality, and so the data was log transformed. An ANOVA on POC concentrations found that Time and Month were significant single factors, as was the interaction between them (Table 4.8). The interaction of Site*Month was also significant (Figure 4.7). Month explained the highest proportion of the original variance (49.8%). When included, no covariates were found to be significant. Carrying out the same ANOVA on the relative POC found that Month was still significant and explained the largest proportion of the variation (19.6%). The main effects plot of the POC_{70} concentration over the months of the experiment showed that there were greater increases in POC concentration in the water during the late summer, autumn and winter months, possibly to coincide with the end of the growing season (Figure 4.7).

Table 4.8. The results of ANOVA of the POC concentrations.

	Without covariates	
Factor	p	ω^2
Time	<0.0001	3.19
Month	<0.0001	49.8
Site*Month	<0.0001	7.76
Time*Month	<0.0001	9.89
Error		5.41

Figure 4.7. The main effects plot of the relative POC_{70} concentration over the months and sites during the experiments. Error bars are standard errors.



Guided by the results of the ANOVA and ANCOVA it was possible to give the best-fit equation for the change in the POC concentration between t_0 and t_{70}:

$$ln\Delta POC = 0.68\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + 0.08POC_0 + 0.28E4{:}E6_0 + 0.02Cl^- + 0.07SO_4^{2-} - 0.01cond_0 - 1.2$$

(0.18) (0.02) (0.04) (0.006) (0.02) (0.003) (0.3)

n=35, r^2=0.71 *(Eq. 4.14)*

where POC_0 is the hour 0 POC concentration (mg/l), SO_4^{2-} is the hour 0 sulphate ion concentration and all other terms are as described above.

The regression analysis showed that the sinusoidal month, POC_0 and $E4{:}E6_0$ ratio were significant in determining the POC concentration during the experiments. The significance of the E4:E6 ratio in this regression is interesting as this is a measure of DOC composition, with higher ratios indicating a greater degree of humification. The negative relationship between the $E4{:}E6_0$ ratio and the POC concentration suggests that less humified DOC and higher concentrations of POC are

linked. Also significant were the chloride and sulphate ions, and the conductivity of the water, all factors that were significantly different between the two sites, with CHS being significantly lower than DBS for all three variables.

4.4. Discussion

Moody et al. (2013), and Chapter 2, found 73% DOC removal over 10 days. If this rate of loss were constant, it would relate to a 21% loss in 70 hours. This is a lower estimate than this chapter (35%), although that experiment was conducted over 10 days rather than 70 hours, and presuming a constant rate of loss is unrealistic, especially as the majority of the decline occurred in the first two days of the experiments. Ten days is much longer than the residence times of most British upland rivers, and so will not provide a reliable estimate of the in-river loss of DOC. The more frequent sampling of this chapter enabled sub-daily rates to be calculated, and therefore the day/night rates could be compared. This led to the diurnal cycle that would not be observed in experiments where samples were only taken daily which could lead to over/under estimates of DOC losses though degradation.

For Chapter 2, the rates of loss in the light and dark in the first day were calculated as 72 mg C/l/day and 49 mg C/l/day respectively. However, this was the total loss of DOC between the beginning of the experiment and day 1 (approximately 24 hours), whereas in this chapter, the value was for the first stage of light of the experiment, between the beginning of the experiment and dusk on day 1. The rate of loss in the first hour for Chapter 2 was calculated by dividing the rate for the whole first day by 24, resulting in a loss of 3 mg/l/hour in the light and 2 mg /l/hour in the dark. This method for calculating the rates had certain drawbacks, as it assumed a constant rate of loss over the 24 hours and resulted in initial rates much lower than those measured in this chapter (12 mg C/l/hour in the light and 4 mg C/l/hour in the dark). It could be assumed that of the first 24 hours, 12 of them were the hours of darkness, when the rate of DOC decline in the light treatment was negligible in this chapter, and so the total DOC loss in Chapter 2 actually took place in the 12 hours of daylight, resulting in the rate in the light being 6 mg C/l/hour, more comparable rate to this chapter. The rate of DOC decline in the dark treatment would not be as affected by the change between daylight and darkness, and so the

estimate for the decline in the first hour may be fairly accurate, as it is similar to the value for the rate in the dark from this chapter.

Chapter 3 had the temporal resolution required for the rate comparison, and included peat dominated catchments, so produced data comparable to the CHS data from this chapter. The Chapter 3 average rate of DOC decline between the beginning of the experiment and dusk on day 1 in the light was 6 mg C/l/hour, slightly higher than the rates found for CHS in this chapter. The average rate of DOC degradation during the first hour of the experiment in the light and dark were 23 and 14 mg C/l/hour respectively. These rates are much higher than those in Chapters 2, and this chapter, and as nearly all regression equations found the DOC_0 concentration to be a significant parameter, these probably reflect the higher initial DOC concentrations (55 mg C/l) found in Chapter 3.

Other studies have measured rates of loss of DOC in similar environments to this chapter. Gennings et al., (2001) states that 40-70% of annual inputs of organic carbon into boreal lakes is evaded into the atmosphere. In rivers, Cole et al. (2007) estimated that at a global scale 1.9 Pg C/yr enters rivers of which 0.8 Pg C/yr (42% of the input) is returned to the atmosphere. Battin et al. (2009a) suggested a lower removal rate of 21%. The estimates of loss provided by this chapter and by Chapter 2 are the same order of magnitude as these other studies. Chapter 3 found a much higher rate of loss (85% in 30 hours), but that was an average of five sites, all of which were closer to the headwater and had small catchment areas; they also had higher initial DOC concentrations, which would be fresher and more readily degraded.

Various studies have estimated the global riverine flux of organic carbon to be between 100-1000 Tg C/year (Billett et al., 2004; Brunet et al., 2005; Wallage et al., 2006). More specific to the UK, Worrall et al. (2012) estimated the flux of DOC to be 555-1263 Gg C/yr. Applying the 35% loss of DOC to this would suggest the DOC flux at the source would have been 854-1943 Gg C/yr. Rates of loss of DOC to the atmosphere would be 299-680 Gg C/yr, or 1096-2493 Gg CO_{2eq}/yr.

This chapter shows the importance of the diurnal cycle in flux calculations. Previous estimates of flux that do not account for the diurnal cycle of in-stream processing are prone to under/over estimation, due to the times of day at which the majority of samples are taken. Residence times of rivers are rarely an exact multiple of 24, and so estimates of fluxes based on measurements during the day and extrapolated to represent the whole 24 hours will overestimate the flux, as the night

time flux is unlikely to be the same as the flux during daylight. Worrall et al. (2013a) developed a 'correction factor' dependent on the residence time of the water body and the day:night ratio of the biogeochemical process being investigated. They applied their model to the flux on the River Tees and found that fluxes could have been overestimated by between 5 and 25%.

The regression analyses of this chapter shows that the most consistent predictors of DOC concentration or rate of DOC decline are the month of the year, the time since the start of the experiment and the initial DOC or POC concentration (and these are therefore included in the most regression analyses). Other variables that were significant in more than one ANCOVA were the pH (or initial pH) and the environmental variables (inverse temperature and cumulative PAR). Several of the regressions found the absorbance at 400 nm, the specific absorbance or the E4:E6 ratio to be significant, showing that the composition of the DOC is an important factor in determining the rate of DOC degradation or the total loss of DOC.

The main findings were that the DOC concentration declined by 35% in the light and only 3% in the dark over 70 hours, and the greatest loss of DOC occurred during the first 10 hours of the experiment. The rates of loss were practically zero in the dark treatment, and differed significantly during the different stages of the experiment, with no significant differences between treatments and sites over night. The initial rates of loss were greatest in the headwater light treatment, where they were as much as 12 mg C/l/hour. The analysis of initial rates would suggest that for CHS in the light, the initial reaction is of the order 2.3 ± 0.7 which is not significantly different from second or third order. However it is most likely to be fractional or mixed order because of the number of processes contributing. Multiple rate laws may be a more accurate way to describe the day and night processes.

4.5. Conclusion

The main findings of this chapter are outlined in Table 4.9. This chapter investigated the loss of organic carbon from a peat-sourced headwater of the river Tees, and found the average loss of DOC in light conditions was 35% over 70 hours with the majority of the loss occurring within the first 10 hours of daylight. The chapter found a strong diurnal cycle, with the average rates of headwater DOC degradation during the daylight being approximately 30 times higher than those

during the night for the same treatment. The analysis of the initial rates of DOC degradation in the light found that that a 2nd order, or a mixed order reaction best explains the process. The results also suggested that the composition of the DOC may affect the rate of the degradation.

As in Chapters 2 and 3, there were significant differences in the loss of DOC between the sites, and the initial DOC was often a significant covariate in the analyses, showing that the site differences in DOC type and concentration play a large role in determining the DOC degradation rates. The rate of DOC degradation declined over time, suggesting a decrease in the 'degradability', or an increase in the recalcitrance, of DOC.

In this chapter, as in Chapters 2 and 3 the DOC concentration does not become zero during the experiment, suggesting that something other than time is limiting the DOC degradation. A factor that could be limiting the degradation is the nutrient concentration of the river water, as anion concentration has been found to be significant in various ANCOVAs and regressions.

Both this chapter and Chapters 2 and 3 have looked at rivers with upland, peat-dominated headwater catchments, and therefore it would be incorrect to apply these rates of loss of DOC to all rivers in the UK without first investigating the rates of degradation on a lowland river system, which generally have higher nutrient concentrations than upland rivers, especially if they pass through agricultural land.

Table 4.9. The main findings of Chapter 4.

Sites studied	Teesdale: CHS, DBS
Range of catchment areas (km^2)	0.2 – 818.4
Range of percentage peat cover (%)	33.9 – 100
Duration of experiments	Monthly, for 70 hours
Sample size	772
Net DOC loss in the light treatment (%)	35
Net DOC loss in the dark treatment (%)	3
Range of rate of DOC loss in the light treatment (mg C/l/hour)	37 – -18
Range of rate of DOC loss in the dark treatment (mg C/l/hour)	43 – -36
Initial rate of DOC loss in the light treatment (mg C/l)	11.6 ± 2.9
Initial rate of DOC loss in the dark treatment (mg C/l)	0.2 ±0.5
Significant covariates:	
Change in DOC concentrations	Initial DOC, pH, absorbance at 400 nm, PAR
Light rate	Inverse temperature, PAR
Dark rate	-
What to investigate next?	The effect of the DOC composition on the rate of degradation, due to the significance of the absorbance and E4:E6 ratio in earlier analyses. The effect of nutrients on the bio-degradation of DOC.

Chapter 5:
The composition of dissolved solids and other organic solids from Moor House and Teesdale

5.1. Introduction and Aims

This chapter has three aims. Firstly, to investigate the differences in composition of the dissolved solid in river water from three sites (an upland headwater and tidal limit, and a lowland river) to show if the loss of DOC found in the previous chapters was due to a change in the composition of the material as it progresses through the fluvial system.

The second aim was to compare the dissolved solid to 'source' materials from the headwater catchment, such as the peat, vegetation and litter layer, and to the suspended solids in the water, to trace the carbon through the various sinks/sources of the organic matter.

The third and most important aim was to compare the composition of the dissolved solid to the rates of degradation of DOC observed in the water, and to the initial DOC concentration of the water, to discover if the composition, structure, complexity and seasonality of the dissolved solid control the rate or extent of degradation. Chapters 2 and 4 have both shown that the variables that are proxies for compositional information about the DOC (the absorbance at 400 nm, the specific absorbance and the E4:E6 ratio) have been significant covariates in both ANCOVAs and regression models, suggesting that the composition may affect the rate of the DOC degradation.

Chapters 2, 3 and 4 have shown there were significant differences in the loss of DOC between the sites, and the initial DOC was often a significant covariate in the analyses, showing that the site differences in DOC type and concentration played a large role in determining the DOC degradation rates. The rate of DOC degradation declined over time, suggesting a decrease in the 'degradability', or an increase in the recalcitrance, of DOC.

An interesting 'side-effect' of this chapter was the analysis of the same matter by several different methods, allowing assessment to be made as to whether

the compositional data obtained was consistent across the different techniques (Poirier et al., 2005).

5.2. Approach and Methodology

5.2.1. Field sites

Samples were collected from four sites in the river Tees catchment – two upstream and two downstream sites. Two of these sites were within Moor House National Nature Reserve (Figure 2.1, Table 2.1): Hard Hill (National grid ref. NY 756 326) and Cottage Hill Sike catchment (CHS; Table 2.1). The two sites downriver of Moor House were Broken Scar, Darlington, on the upland river Tees (DBS; Table 2.1) and Coatham Mundeville, on the lowland river Skerne (MUN; National grid ref. NZ 291 207). The river Skerne is a tributary of the lower Tees and flows into the Tees downstream of Darlington. The Coatham Mundeville site was chosen for its part in a subsequent experiment described in Chapter 7.

5.2.2. Sample collection

5.2.2.1. Total Dissolved Solid (TDS)

Water from CHS, DBS and MUN was sampled over a two year period. Samples of at least 20 litres were collected on the same day from each site and allowed to settle in the lab, but were not filtered, and the supernatant was tapped off for evaporation to dryness at 80 °C. The water was not filtered so as not to impose a 'size' constraint on the TDS. The suspended material that sank to the bottom was excluded by not adding the last couple of litres of water to the evaporating dish. The residue of the evaporation was scraped out of the evaporation dish and collected. Water was taken from CHS from October 2011 until September 2013, resulting in 21 solid samples (some months were not sampled due to the site being inaccessible). Water was taken from DBS from December 2011 until November 2012, resulting in 14 solid samples, as March and July were sampled twice. Water was taken from MUN from December 2012 until September 2013, resulting in 10 solid samples (Table 5.1).

The three different sites had very different TDS; the CHS solid was very dark brown/black, and there was usually a very small amount (approximately 1g),

whereas the solid from the other two sites was much lighter in colour, and there was generally a much larger amount (approximately 5g).

The organic fraction of these samples was dissolved organic matter (DOM), and the proportion of DOM that was carbon was dissolved organic carbon (DOC).

5.2.3. Source samples for comparison

Four different types of 'source' samples were collected from sites within Moor House NNR for comparison to the Total Dissolved Solid samples.

5.2.3.1. Litter (LIT)

Three litter samples were taken from the CHS catchment, dried in an oven at 105 °C before being crushed in a pestle and mortar. The samples were considered as duplicates of each other (Table 5.1).

5.2.3.2. Peat (PEAT)

Two cores, 100 cm deep, were taken from Hard Hill within Moor House NNR using a Dutch Auger. Cores were taken from unfenced, unburnt (since 1954) plots used in Clay et al. (2009). The cores were divided into 17 sections by depth in the field – the top 20 cm was divided into ten 2 cm sections, the depths between 20 and 50 cm were divided into six 5 cm sections, and the final section was the deepest 5 cm of the core, from 95-100 cm. The separate sections were transported to the laboratory in plastic sample bags and stored at 4 °C until they were prepared for analysis. All sections were dried in an oven at 105 °C before being crushed and having any roots removed. This resulted in 17 depths and two replicate cores (A6 and B6 - Table 5.1).

5.2.3.3. Total Suspended Sediment (TSS)

A large quantity of water was taken from CHS and the suspended sediment collected by filtration through 0.45 µm filter papers, the residues on the filter papers were then washed into a glass petri dish using DI water. The water was then evaporated off and the residual matter collected. This was carried out twice, in July and September 2013 (Table 5.1).

The organic fraction of these samples was particulate organic matter (POM), and the proportion of POM that was carbon was particulate organic carbon (POC).

5.2.3.4. Vegetation (VEG)

Three pairs of randomized 50x50 cm quadrats within the CHS catchment at Moor House NNR were sampled. All aboveground biomass in the first of each pair was sampled, regardless of species. In the second of the pair, the vegetation was divided into its three main functional groups – heather (*Calluna vulgaris*), cotton grass (*Eriophorum spp.)* and mosses (including but not exclusively *Sphagnum spp.*). Belowground biomass was sampled from both quadrats in the pair. The samples were amalgamated across the three pairs of quadrats, resulting in five samples (Table 5.1): aboveground (AGB), belowground (BGB), heather (HEA), sedge (SED) and moss (MOS). The plants were dried in an oven at 105 °C before being crushed.

5.2.3.5. Standards (STD)

Three standards were analysed for comparison to the field samples (Table 5.1):

- Cellulose (Whatman 90 mm filter papers, GE Healthcare UK Ltd, Buckinghamshire, UK)
- Humic acid (humic acid crystalline powder, Alfa Aesar A Johnson Matthey Company, Massachusetts, USA)
- Lignin (SIGMA-ALDRICH, Steinheim, Germany)

5.2.4. Water samples

Samples of the source water of the TDS samples were taken to look at the changes in the water chemistry over the duration of the evaporation. When water was taken from each site, initial samples were taken and analysed as in Chapter 4; one sample was 'fixed' with H_2SO_4 and one sample filtered for covariate analysis. Another sample was taken and used to calculate the suspended sediment concentration in the water. The same was done at the end of each evaporation as the evaporation of each monthly water sample could take several days. Initial samples were CHS_0, DBS_0 and MUN_0, and end samples were CHS_1, DBS_1 and MUN_1. Initial samples were taken from October 2011, and end samples were taken from May 2012 to September 2013 (Table 5.1).

Table 5.1. The samples and sites used in this chapter, with the numbers of samples collected.

Water or solid	Type of sample	Site/name of sample	Site location/description	Number of samples
Solid	TDS	CHS	CHS, Moor House	21
	TDS	DBS	DBS, River Tees	14
	TDS	MUN	MUN, River Skerne	10
Solid	LIT	LIT	CHS catchment, Moor House	3
	PEAT	A6	Hard Hill, Moor House	17
	PEAT	B6	Hard Hill, Moor House	17
	TSS	TSS	CHS, Moor House	2
	VEG	AGB	CHS catchment, Moor House	1
	VEG	BGB	CHS catchment, Moor House	1
	VEG	SED	CHS catchment, Moor House	1
	VEG	HEA	CHS catchment, Moor House	1
	VEG	MOS	CHS catchment, Moor House	1
Water	Water	CHS_0	CHS initial water	25
		CHS_1	CHS end water	15
		DBS_0	DBS initial water	15
		DBS_1	DBS end water	8
		MUN_0	MUN initial water	15
		MUN_1	MUN end water	14
Standard	Standard	Cellulose		1
	Standard	Humic acid		1
	Standard	Lignin		1

5.2.5. Solid samples analysis

5.2.5.1. $\delta^{13}C$ content

The samples were analysed for $\delta^{13}C$ content, and the ratio of ^{13}C to ^{12}C, in order to make some observations about the source of the organic matter. Stable-isotope measurements were performed at Durham University using a Costech Elemental Analyser (ECS 4010) coupled to a ThermoFinnigan Delta V Advantage. Carbon-isotope ratios were corrected for ^{17}O contribution and reported in standard delta (δ) notation in per mil (‰) relative to the VPDB scale. Data accuracy is monitored through routine analyses of in-house standards, which are calibrated against

international standards (e.g., USGS 40, USGS 24, IAEA 600, IAEA CH6): this provides a linear range in $\delta^{13}C$ between +2‰ and –47‰. Analytical uncertainty for $\delta^{13}C_{org}$ is typically ± 0.1‰ for replicate analyses of the international standards and typically <0.2‰ on replicate sample analysis (D. Gröcke, pers. comm.). $\delta^{13}C$ content was calculated for 14 CHS samples only, from October 2011 to January 2013.

5.2.5.2. Bomb Calorimetry

The bomb calorimeter calculates the energy content of the samples by measuring the temperature change in the water surrounding the 'bomb'. Energy contents can be used to infer information about the structure of the samples. The bomb calorimetry was carried out using a Parr 6200 calorimeter with an 1108 Oxygen Bomb, with a Parr 6510 water handling system, which is precise to 0.1%. Approximately 250 mg of each solid was used. Benzoic acid standards were used to calibrate the machine. When the sample was small (typically less than 0.2 g available for bomb calorimetry analysis) then a known mass of benzoic acid was used as a 'spike' to increase the mass to approximately 1 g. All peat, vegetation, litter, suspended sediment and CHS samples were analysed, but DBS and MUN samples weren't, as they contain more inorganic matter that would burn satisfactorily, even with a benzoic acid spike. Humic acid, cellulose and lignin were also analysed for comparison to the samples.

For each sample, the temperature rise (°C) and gross heat (megajoules/kg) was recorded.

5.2.5.3. Elemental Analysis (EA)

The elemental analysis analysed the samples for their carbon, hydrogen, nitrogen and oxygen composition, information that is useful in calculating the stoichiometry, carbon oxidation state and oxidative ratio of the samples. The elemental analysis was carried out using a Costech elemental combustion system with pneumatic autosampler. Between 1.5 and 2.5 mg of each sample was used, packed in 5x3.5 mm tin capsules. The samples were analysed in triplicate for carbon, hydrogen and nitrogen, and separately for oxygen. Samples with a relative standard error of more than five were reanalysed. Standards of acetanilide (C_8H_9NO) were used to calibrate the machine. Only calibrations with a linear regression r^2 of 0.999 were used in calculations.

For the CHN combustion analysis, the reaction column contained chromium (III) oxide, copper wires and silvered cobaltous-cobaltic oxide and was heated to 1000 °C. The carrier gas was helium at a flow rate of 140 ml/min; the oxygen flow rate was 40 ml/min. The oxygen gas reacted with the tin capsule and sample at high temperatures (1700-1800 °C) and the sample broke down in to its elemental components. The GC column (at 60 °C) was stainless steel, 2 m in length, packed with porapak QS used to separate the gases. A thermal conductivity detector (TCD) was used to calculate the signal of each sample.

For the oxygen pyrolysis analysis, the reaction column contained 20% nickelised carbon and silica chips and was heated to 1060 °C. The carrier gas was helium at a flow rate of 140 ml/min. A water trap of magnesium perchlorate ($Mg(ClO_4)_2$) was used to remove any water from the system. The GC column (at 60 °C) was a stainless steel, 1 m in length, molecular sieve column. The samples were doped with chloropentane ($C_5H_{11}Cl$) to aid combustion and enhance the catalytic effect of the nickelised carbon (by removing the nickel as nickel chloride and exposing a renewed nickel/carbon interface to the pyrolysed gases).

Once the samples had been analysed, the data were corrected to take the unmeasured elements into account. The sum of the CHNO percentages, plus a 2% allowance for S and P, was subtracted from 100%. The proportions of the four measured elements were then calculated using the adjusted sum. Once all the elements had been adjusted for all samples, the molar concentrations were calculated by dividing the proportion by the relative atomic mass of each element. These molar concentrations were then used to calculate the carbon oxidation state, the oxidative ratio and the C:N, O:C and H:C ratio of the samples.

The carbon oxidation state (C_{ox}) is a measure of the degree of oxidation of organic carbon. It can vary from -4 for CH_4, which is fully reduced, to +4 for CO_2, which is fully oxidised. It is calculated using the following equation, from Masiello et al. (2008):

$$C_{ox} = \frac{2[O]-[H]+3[N]}{[C]} \qquad (Eq.\ 5.1)$$

where [O], [H], [N] and [C] are the molar concentrations of those elements.

The oxidative ratio (OR) is the molar ratio of O_2 produced to CO_2 sequestered by a biome. It is calculated using the molar concentrations of C and N, and the C_{ox}, using the following equation (Masiello et al., 2008):

$$OR = 1 - \frac{C_{ox}}{4} + \frac{3[N]}{4[C]} \qquad \textit{(Eq. 5.2)}$$

5.2.5.4. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectroscopy was used to analyse the bonds present in the samples by measuring the bends and stretches of the bonds at different IR frequencies. The transmission FTIR spectroscopy was carried out using a Perkin Elmer 1600 series FTIR, scanning over the frequency range 4000 to 400 cm^{-1} at a resolution of 8 cm^{-1}. The spectrum for each sample was calculated from the average of 64 scans. Seven CHS samples were analysed by FTIR.

5.2.5.5. ^{13}C solid-state Nuclear Magnetic Resonance (^{13}C-NMR)

The ^{13}C solid-state NMR was used to identify the main functional groups of the samples. The ^{13}C solid-state NMR data were obtained at the EPSRC UK National Solid-state NMR Service at Durham University. Solid-state ^{13}C spectra were recorded at 100.56 MHz using a Varian VNMRS spectrometer and a 4 mm magic-angle spinning probe. They were obtained using cross-polarisation with a 0.5 s recycle delay, 1 ms contact time, at ambient probe temperature (~25 °C) and at a sample spin-rate of 14 kHz. Between 50000 and 100000 repetitions were accumulated. Spectral referencing was with respect to an external sample of neat tetramethylsilane (carried out by setting the high-frequency signal from adamantane to 38.5 ppm). Between 50 and 60 mg of each sample was used. Nine DBS samples and 11 CHS samples were analysed by NMR.

The chemical shift ranges used were taken from Chadwick et al. (2004 – Table 5.2). The maximum peak height in each range was divided by the DOC concentration of the corresponding water sample, to get a relative peak height for each type of carbon observed.

Table 5.2. The ranges of chemical shifts for ^{13}C NMR, from Chadwick et al. (2004).

Chemical shift (ppm)	Types of carbon
0-45	alkyl C
45-65	N-alkyl and methoxyl C
65-95	O-alkyl C
95-110	di-O-alkyl C
110-145	aromatic/unsaturated C
145-160	phenolic C
160-190	amide/carboxyl C
190-220	aldehyde/ketone C

The O-alkyl C and phenolic C ranges have been used directly to estimate the carbohydrate and lignin concentration of samples, respectively (Poirier et al., 2005).

5.2.5.6. Thermogravimetric Analysis (TGA)

The thermogravimetric analysis provided information about the composition of the samples by comparing the main ranges of weight loss. The thermogravimetric analysis was carried out using an STA i TGH 1200, with an inert atmosphere of nitrogen. Approximately 200 mg of solid was used for each analysis. The balance in the TGA recorded the exact starting weight; weight loss was reported as a percentage of the starting weight. The starting temperature was 25 °C, and it was ramped up 20 °C a minute to 1000 °C.

For each sample, the total weight loss, weight at 550 °C, and weight loss in each 50 °C increment from 0 °C to 1000 °C were recorded. Several papers (e.g. Lopez-Capel et al., 2008) use the weight loss in three set temperature ranges (200-400, 400-500, 500-650 °C) to compare samples, and so the weight losses for those ranges were also calculated. These ranges were used for comparison with the energy data from DTA, and are called EXO 1, 2 and 3 in the literature.

5.2.5.7. Differential Thermal Analysis (DTA)

This analysis was carried out using the same equipment as the TGA, but set to DTA mode rather than TGA. Simultaneous thermal analysis/differential thermal analysis (STA/DTA) is similar to TGA in that it heats a sample in order to calculate weight change as a function of time. It also detects changes in the energy output from the

sample compared with an inert reference and plots these against the temperature of the sample, so providing data on the transformations that occur such as glass transitions, crystallisation, melting and sublimation. Approximately 50 mg of solid was used for each analysis. The balance in the DTA recorded the exact starting weight, and weight loss was reported as a percentage of the starting weight. The starting temperature was 25 °C, and it was ramped up 20 °C a minute to 1000 °C.

For each sample, the weight loss in each 50 °C increment from 0 °C to 1000 °C was recorded. For the 50 °C range with the greatest weight loss, the energy change that occurred during the same temperature range was calculated. The energy change in the EXO 1, EXO 2 and EXO 3 ranges were calculated for comparison to the TGA data.

5.2.5.8. Pyrolysis Gas Chromatography Mass Spectrometry (Pyrolysis GC/MS)

Pyrolysis GC/MS was used to provide structural information about the different types of organic matter. This analysis was carried out using a CDS Pyroprobe 5150 pyrolyzer, with a Finnigan Trace GC Ultra and a Finnigan Trace DSQ. The samples were packed into quartz sample tubes with glass wool and flash pyrolysed at 750 °C for 10 seconds in a Helium flow (10 ml/min). The GC oven was programmed from 40 °C for 2 minutes, then ramped at 10 °C/min to 300 °C and held for 20 minutes. The spectrometer range was set to 35-550 m/z. The GC column used was a 30-metre 5-phenyl methylpolysiloxane column.

Twelve samples were analysed by Pyrolysis GC/MS: a top, middle and bottom sample from the peat profile, belowground and aboveground vegetation biomass, one litter sample, one suspended sediment sample, and five TDS samples: three from CHS, one from DBS and one from MUN. The CHS samples analysed were taken at regular intervals from the year: April, August and November.

Peaks were identified using seven papers: Arranz et al. (2009), Calvelo Pereira et al. (2011), Christy et al. (1999), Kracht and Gleixner (2000), McClymont et al. (2011), Ralph and Hatfield (1991) and Reiche et al. (2010), and the Xcalibur software library. Only peaks that appeared in at least one of the papers and the library were used for comparison to the results.

5.2.6. Water sample analysis

The water samples taken were analysed as in Chapter 4 for DOC concentration, absorbance at 400, 465 and 665 nm, E4:E6, pH, conductivity, water temperature, suspended sediment and POC concentration. Further analysis of the changes in water chemistry was carried out on the difference between the t_0 (beginning) and t_1 (end) samples for the same month.

5.2.7. Statistical methodology

The data from each analytical technique were analysed, usually by two-way ANOVA, to look for differences between sites, the types of plant for the vegetation data, months for the TDS data and depths for the peat profile data.

The water chemistry data were analysed, relative to their initial concentrations, to look for changes in the organic carbon concentrations and composition proxies (absorbance at 400 nm and E4:E6 ratio) between the beginning and end of each evaporation.

To answer the three main questions of this chapter, the results were analysed in three ways. To investigate the differences in composition of the dissolved solid, the various analytical results were compared for the three sites (CHS, DBS and MUN), particularly looking for differences between CHS and DBS, as they are on the same river. To compare the dissolved solid to 'source' materials, the CHS dissolved solid was compared to the other samples from Moor House, the peat, vegetation and litter layers, and to the suspended solids in the water. To see how the composition of the dissolved solid compared to the rates of degradation of DOC, the initial rates were calculated (change of DOC during the first hour of the experiment) from the corresponding months' degradation experiments, described in Chapters 2 and 4. Data for the degradation rates were also used from future chapters, Chapter 6 for CHS rates, and Chapter 7 for MUN rates. For the TDS samples that had corresponding degradation experiments carried out over days rather than hours, the rate of decline was calculated for the whole first day then divided by 24 to get the hourly rate.

These comparisons were carried out using data that were analysed for homogeneity of variance and normality using the Levene and Anderson & Darling tests respectively. The data were log-transformed if the conditions of the tests were not met, and then correlation, ANOVA, ANCOVA and regression analyses were used

to look for differences and trends in the data – no further transformations were found necessary. The magnitude of the effects, in this case generalized ω^2 (Olejnik and Algina, 2003), of each significant factor and interaction were calculated. Post-hoc testing of the results was made using the Tukey test, for pairwise comparisons between factor levels to assess where significant differences lay.

Principal component analyses (PCAs) were performed on the dataset to extract information about the similarity between samples and variations in the composition of the organic matter. PCA was used to determine the factors affecting the composition, and to show which organic matter was likely to be the main source of the TDS. Only principal components with an eigenvalue of more than 1, and the first with an eigenvalue of less than 1, were considered in the analysis (Worrall et al., 2012).

5.3. Results and Discussion

5.3.1. Results summary

5.3.1.1. $\delta^{13}C$ content

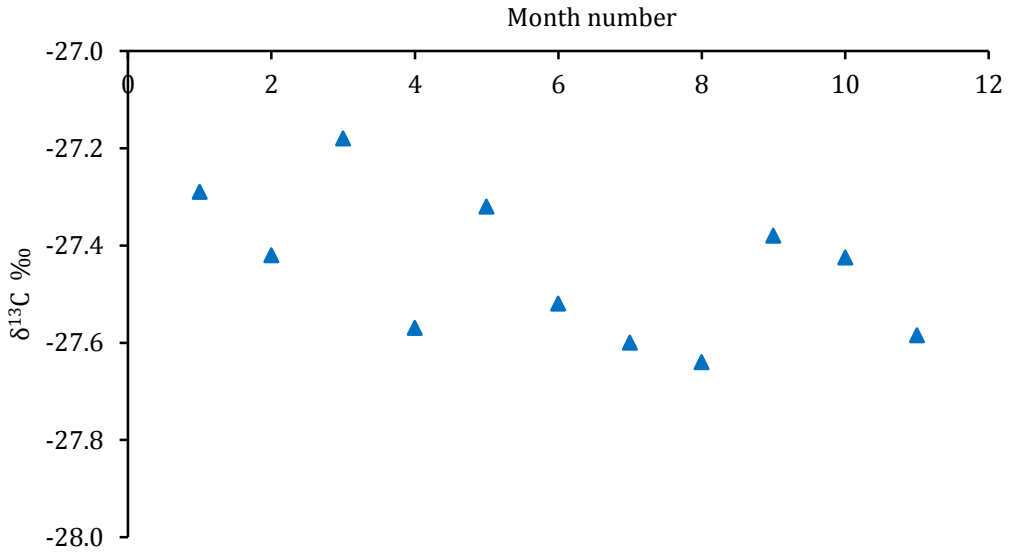
The average $\delta^{13}C$ content of CHS was -27.47 ± 0.05‰ (standard error), from 14 samples. This is comparable with $\delta^{13}C$ data from studies on DOC, POC and peat: Charman et al. (1999) found the $\delta^{13}C$ of DOC, extracted from various depths, to be between -27.3 and -25.2‰, varying slightly with depth; Raymond and Bauer (2001b) found it to be between -32.4 and -25.5‰. For peat, Charman et al. (1999) found it to be between -26.8 and -25.8‰, while Hardie et al. (2011) found values between -28.2 and -26.7‰. Raymond and Bauer (2001b) found the $\delta^{13}C$ of POC to be between -33.7 and -22.3‰. The $\delta^{13}C$ of fulvic acid from streams and rivers was found to be between -26.9 and -25.9‰ by Thurman (1985), and Kracht and Gleixner (2000) found moss to have a $\delta^{13}C$ of 26.3‰. All of these values are similar to those found for CHS, and fall within the expected range for terrestrial organic matter. Marine organic matter tends to have higher (closer to zero) $\delta^{13}C$ contents (Abdulla et al., 2010a).

Wang et al. (2002) states that the two main photosynthetic pathways in plants (C3 and C4) lead to differences in the $\delta^{13}C$, as 'C3 plants discriminate against $^{13}CO_2$ during photosynthesis to a greater extent than C4 plants'. C4 plants therefore have $\delta^{13}C$ values closer to zero (range: -9 to -17‰), compared to C3 plants (range:

-23 to -34‰). The values found for vegetation-sourced materials in this chapter would suggest that peatland plants are C3 plants.

The $\delta^{13}C$ of CHS varied slightly monthly, but there was no clear seasonal cycle (Figure 5.1). A one-way ANOVA on the data showed there were no significant differences between the months (p=0.274). Given the lack of significant variation in the data this analysis was not pursued further.

Figure 5.1. The $\delta^{13}C$ of the TDS from CHS for the different months.



5.3.1.2. Bomb Calorimetry

The average gross heat output from CHS was 9.51 ± 1.66 MJ/kg. This is much lower than the values for the other source materials (Table 5.3), which range from 15.56 MJ/kg for TSS to 20.57 MJ/kg for heather. The only sample with a gross heat similar to CHS is the humic acid standard, which had a value of 12.57 MJ/kg. Both cellulose and lignin had higher gross heat values than CHS. The low values for CHS would be expected to be indicative of highly oxidised organic matter.

The gross heat data for the peat and vegetation are similar to values reported in literature. Values for peat range from 4.90 (Lähdesmäki and Piispanen, 1988) to 23.10 MJ/kg (Bergner and Albano, 1993). For vegetation, there are various gross heat values reported for different species and parts of the plant. Values range from 17.77 (leaves (Golley, 1961)) to 23.82 MJ/kg (*Empetrum* stems (Forrest,

1971)). More specifically, reported values for heather are very similar to those found in this chapter, 21.18-22.61 MJ/kg (Forrest, 1971). Reported values for roots (below ground biomass) range from 19.83-23.61 MJ/kg (Currie, 2003; Golley, 1961), and reported values for sedge range from 19.37 to 20.00 MJ/kg (Forrest, 1971). Data for litter from the literature are from various ecosystems, including pine and oak forests, and range from 13.1 to 23.66 MJ/kg (Currie, 2003; Lähdesmäki and Piispanen, 1988). The gross heats of the standards, cellulose and lignin, were 16.62 and 25.53 MJ/kg respectively. The majority of vegetation is composed of a mixture of cellulose and lignin, and so it was expected, and found, that the vegetation values recorded fell between these two standards. Similarly, peat is derived from vegetation, and so the recorded values for the two peat cores fell between the cellulose and lignin standards also.

The gross heat from the peat varied slightly with depth, increasing from 17 MJ/kg at the surface of the peat, to 20 MJ/kg at 1 metre deep (Figure 5.2). An ANOVA on the data showed there were no significant differences between the depths ($p=0.4044$). A peat profile from the literature shows a similar trend of increasing gross heat with depth, although the increase between the surface and deep peat is more marked, from 4.9 to 21.6 MJ/kg (Lähdesmäki and Piispanen, 1988). The lower values of the shallower peats was closer to the value of cellulose than lignin, suggesting that the surface peats were more oxidised, and comprised of more cellulose-like compounds than lignified compounds, whereas the deeper peats were less oxidised and more lignified.

The intermediate gross heat values found for TSS (15.56 MJ/kg) suggests that this material is a mixture of matter from various sources, including the vegetation and peat, especially moss and the surface peat, which has a lower gross heat output than the deeper peat (Figure 5.2). Cellulose had a similar gross heat to the TSS samples, showing that these had a similar degree of oxidation.

The monthly gross heat values varied for CHS, but there was no clear seasonal cycle (Figure 5.3). An ANOVA on the data showed there was a significant difference between the months ($p=0.049$).

Table 5.3. The average gross heat and number of samples analysed for each type of sample.

Type	Site	Number of samples	Gross Heat (MJ/kg)
TDS	CHS	21	9.51
LITTER	LIT	3	18.90
PEAT	A6	12	19.78
PEAT	B6	17	19.20
TSS	SUS	2	15.56
VEG	AGB	1	18.95
VEG	BGB	1	19.55
VEG	SED	1	18.58
VEG	HEA	1	20.57
VEG	MOS	1	17.91
STD	Cellulose	1	16.62
STD	Humic acid	1	12.57
STD	Lignin	1	25.53

Figure 5.2. The gross heat for the peat profiles. Error bars show the standard errors.

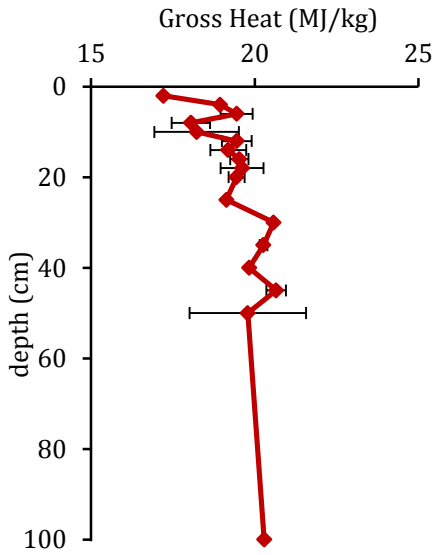
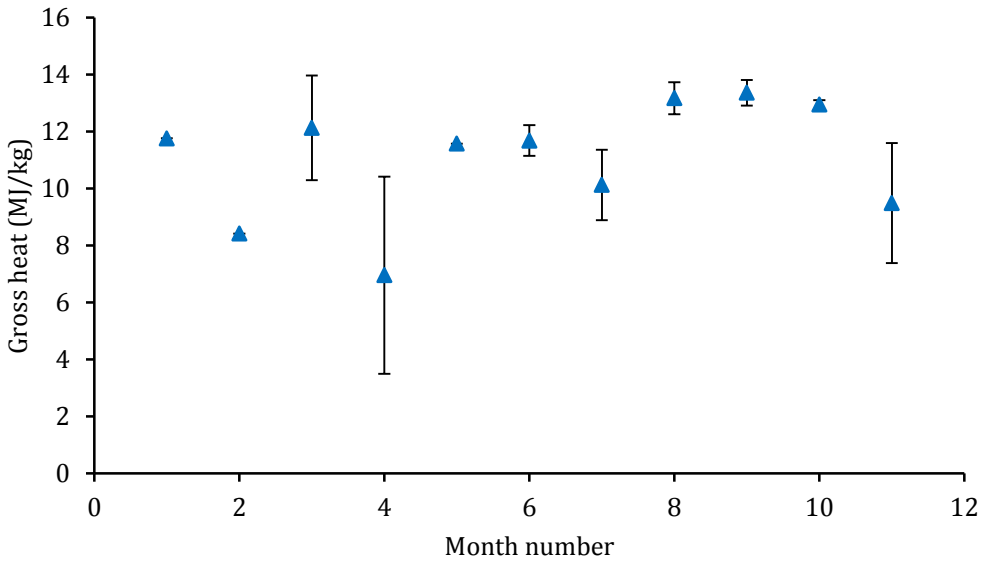


Figure 5.3. The monthly gross heat values for the TDS from CHS. Error bars show the standard errors.



5.3.1.3. Elemental Analysis (EA)

The molar concentrations for the different types of organic matter analysed are in Table 5.4. The average molecular formulas were calculated, proportionate to 100 moles of carbon, and the other elements were adjusted accordingly: CHS was calculated to be $C_{100}H_{126}O_{79}N_4$, DBS was $C_{100}H_{161}O_{243}N_{12}$ and MUN was $C_{100}H_{272}O_{361}N_{17}$. The average formula of the vegetation samples was $C_{100}H_{152}O_{62}N_2$, and for the peat it was $C_{100}H_{134}O_{58}N_2$. The litter and suspended sediment had very similar molecular formulas: Litter: $C_{100}H_{141}O_{61}N_4$, TSS: $C_{100}H_{158}O_{62}N_4$. Proportionately, DBS and MUN had much higher oxygen and nitrogen concentrations than the more organic samples.

Molar concentrations for various components of organic matter have been reported in Baldock et al. (2004), including lipids ($C_{100}H_{194}O_{24}$), lignin ($C_{100}H_{124}O_{43}$), carbohydrates ($C_{100}H_{167}O_{83}$) and proteins ($C_{100}H_{110}O_{16}N_{27}$). Lou et al. (2006) reported the molar concentrations of two 'standards' of humic and fulvic acids, from Suwannee River, South-East USA, as $C_{100}H_{98}O_{60}N_2$ and $C_{100}H_{99}O_{62}N_1$ respectively. Comparing these and the data for the samples in this chapter, CHS has a similar H content to lignin and peat, a similar N content to peat, vegetation, litter, suspended sediment, fulvic acid and humic acid, and a similar O content to carbohydrates,

suspended sediment, vegetation, litter, fulvic acid and humic acid. DBS has high H, similar to carbohydrates, and high N and O, similar to MUN.

Many studies report the elemental composition as percentages, rather than molar concentrations. Data from literature was compared to the data in this chapter, according to the type of organic matter and element analysed. It was found that all elemental percentages for each type of organic matter fell either within, or very close to, the reported ranges.

The range of C_{ox} is from 4 (CO_2) to -4 (CH_4); the values for DBS (3.74 ± 0.98) and MUN (5.12 ± 1.67) fall outside of this range, so they have been removed from all further analysis. This error is probably related to their high inorganic content.

Masiello et al. (2008) and Baldock et al. (2004) report ranges of C_{ox} for various biochemicals and components of organic matter, including lipids (-2 to 2), lignin (-0.5 to 0.5), carbohydrates (0), proteins (0.03), amino acids (-2 to 1), soluble phenolics (-0.5 to 1) and organic acids (0 to 3). Chadwick et al. (2004) estimated the C_{ox} for natural organic matter, including marine biomass (-0.55), sinking organics (-0.4), surface sediments (-0.35), deep soil (-0.3), terrestrial biomass (-0.1) and surface soil (-0.03). The measured values for the more organic samples fall between -0.32 and 0.42, well within the expected range for organic matter.

Since the oxidative ratio (OR) is calculated using the C_{ox}, the values for DBS and MUN should be viewed with caution, even though DBS falls within the possible range of 0 to 2. Most literature values OR of organic matter fall close to 1 and have been estimated for the same compounds/components as C_{ox}: lipids (1.25 to 1.5), lignin (1.0 to 1.14), carbohydrates (0.75 to 1.25), proteins (1.13), amino acids (0.75 to 1.5), soluble phenolics (0.75 to 1.13) and organic acids (0.25 to 1.0). Additionally, estimates of OR have been made for cellulose (1.0), DOM (0.87 to 0.95), fulvic acids (0.82 to 1.26), various parts of plants (1.02 to 1.1), humic acids (0.88 to 1.12), forest soil (0.91 to 1.04), lake sediment (0.89 to 0.94) and peat cores (0.92 to 1.08) (Baldock et al., 2004; Chadwick et al., 2004; Davis et al., 1999; Hockaday et al., 2009; Masiello et al., 2008; Randerson et al., 2006; Worrall et al., 2013b). The measured values for the samples in this chapter fall within the ranges expected for that type of organic matter, e.g. the peat cores fall within the 0.92-1.08 range for values reported from the literature. Most importantly, the CHS value (0.92) is within the range expected for carbohydrates, amino acids, soluble phenolics, organic acids, DOM, fulvic acids and humic acids; all materials that are likely to be components of the

TDS from CHS. The relatively oxidised nature of the CHS samples, low OR and high C_{ox}, reflects the low gross heat values observed above.

The peat profiles showed that carbon concentration increased with depth, whereas hydrogen, oxygen and nitrogen decreased (Figure 5.4). This resulted in the C:N ratio increasing, and the C_{ox} and OR changing from 0.02 to -0.07, and from 1.02 to 1.03 respectively. An ANOVA on the peat core data showed that there were no significant differences in carbon, nitrogen, hydrogen or oxygen concentrations with depth. Also, there were no significant differences between the C_{ox}, OR, C:N, H:C or O:C with depth. Some differences may be expected due to the increasing age of the peat with depth, and due to the lower depths being in anoxic conditions, and therefore decaying anaerobically (Hardie et al., 2011). Various studies have found differences in the carbon, hydrogen, nitrogen, oxygen, C_{ox}, OR, C:N, H:C and O:C with depth in peat profiles, although there are few consistencies. Reiche et al. (2010) found that the percentage nitrogen decreased with depth, but the carbon increased for two of their cores and decreased for two. Zaccone et al. (2007) found that the carbon increased with depth, and the oxygen and O:C ratio decreased with depth. Hardie et al. (2011) found that the carbon and nitrogen contents increased with depth, although they only looked at the first 30 cm of a peat profile. Cocozza et al. (2003) also found that the carbon content increased with depth. Klavins et al. (2008) found that in some profiles the content of the four elements increased, whereas in some profiles the contents decreased. Worrall et al. (2013b) found no significant differences in the OR with depth.

The TDS samples with monthly data are shown in Figure 5.5. An ANOVA on the monthly data showed that there were significant differences between the sites for the carbon, nitrogen, hydrogen and oxygen concentrations. The Site factor explained the largest proportion of the variation for carbon and oxygen (80% for carbon, 81% for oxygen). There were significant differences between the months for the nitrogen, hydrogen and oxygen, but there was no obvious seasonal cycle. There were no significant differences between the months for the carbon content. The interaction of Site*Month was significant for the carbon, nitrogen, hydrogen and oxygen, and explained the largest proportion of the variation for nitrogen and hydrogen (47% and 33% respectively). The C:N ratio was significantly different between Site, Month and the interaction between them, with Site explaining 65% of the variation. C_{ox} and OR, for CHS only, were not significantly different between months.

Table 5.4. The number of samples analysed, the average molar concentrations, C:N ratios, C_{ox} and OR of each type of sample.

Type	Site	Number of samples	Carbon	Hydrogen	Oxygen	Nitrogen	C:N	C_{ox}	OR
TDS	CHS	21	3.70	4.65	2.91	0.13	30.30	0.42	0.92
TDS	DBS	14	1.75	2.82	4.25	0.21	9.83	3.74	0.16
TDS	MUN	10	1.26	3.43	4.55	0.21	8.40	5.12	-0.15
LITTER	LIT	3	4.13	5.83	2.51	0.17	23.98	-0.07	1.05
PEAT	A6	17	4.34	5.41	2.43	0.10	41.99	-0.06	1.03
PEAT	B6	17	4.06	6.25	2.61	0.08	49.86	-0.19	1.06
TSS	SUS	2	4.05	6.39	2.50	0.18	22.36	-0.21	1.09
VEG	AGB	1	4.15	6.40	2.55	0.07	59.29	-0.26	1.08
VEG	BGB	1	4.23	6.30	2.48	0.09	49.23	-0.25	1.08
VEG	SED	1	4.04	6.03	2.59	0.12	33.03	-0.19	1.07
VEG	HEA	1	4.29	6.58	2.53	0.05	94.93	-0.32	1.09
VEG	MOS	1	4.03	6.29	2.65	0.07	55.21	-0.19	1.06
STD	Cellulose	1	3.60	6.25	3.21	0.00	-	0.05	0.99
STD	Humic acid	1	2.99	3.68	2.03	0.06	48.52	0.19	0.97
STD	Lignin	1	5.16	6.06	1.81	0.06	87.33	-0.43	1.12

Figure 5.4. The average carbon, hydrogen, oxygen and nitrogen molar concentrations, C:N ratio, C_{ox} and OR for the peat profiles. Error bars are the standard errors.

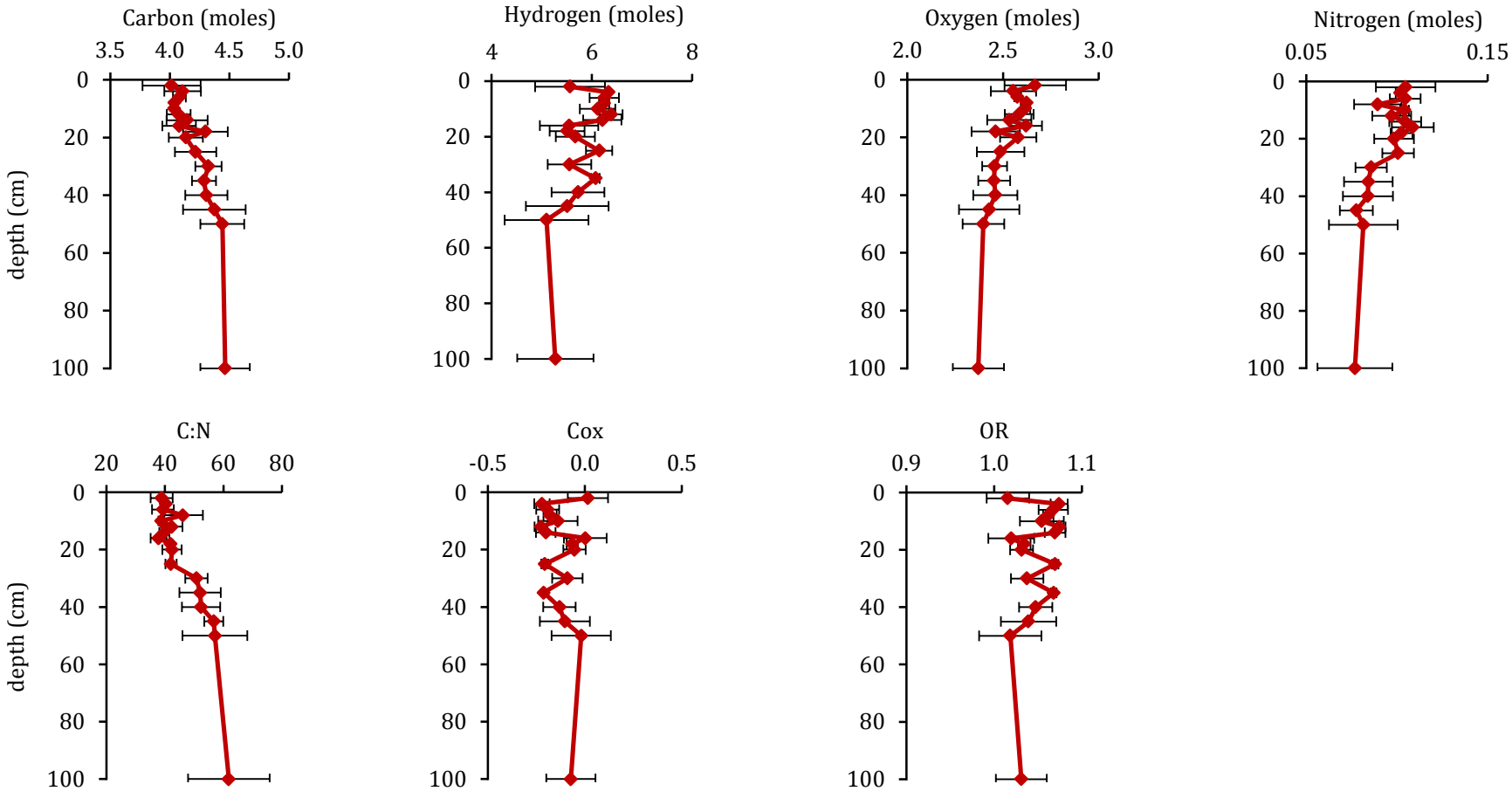
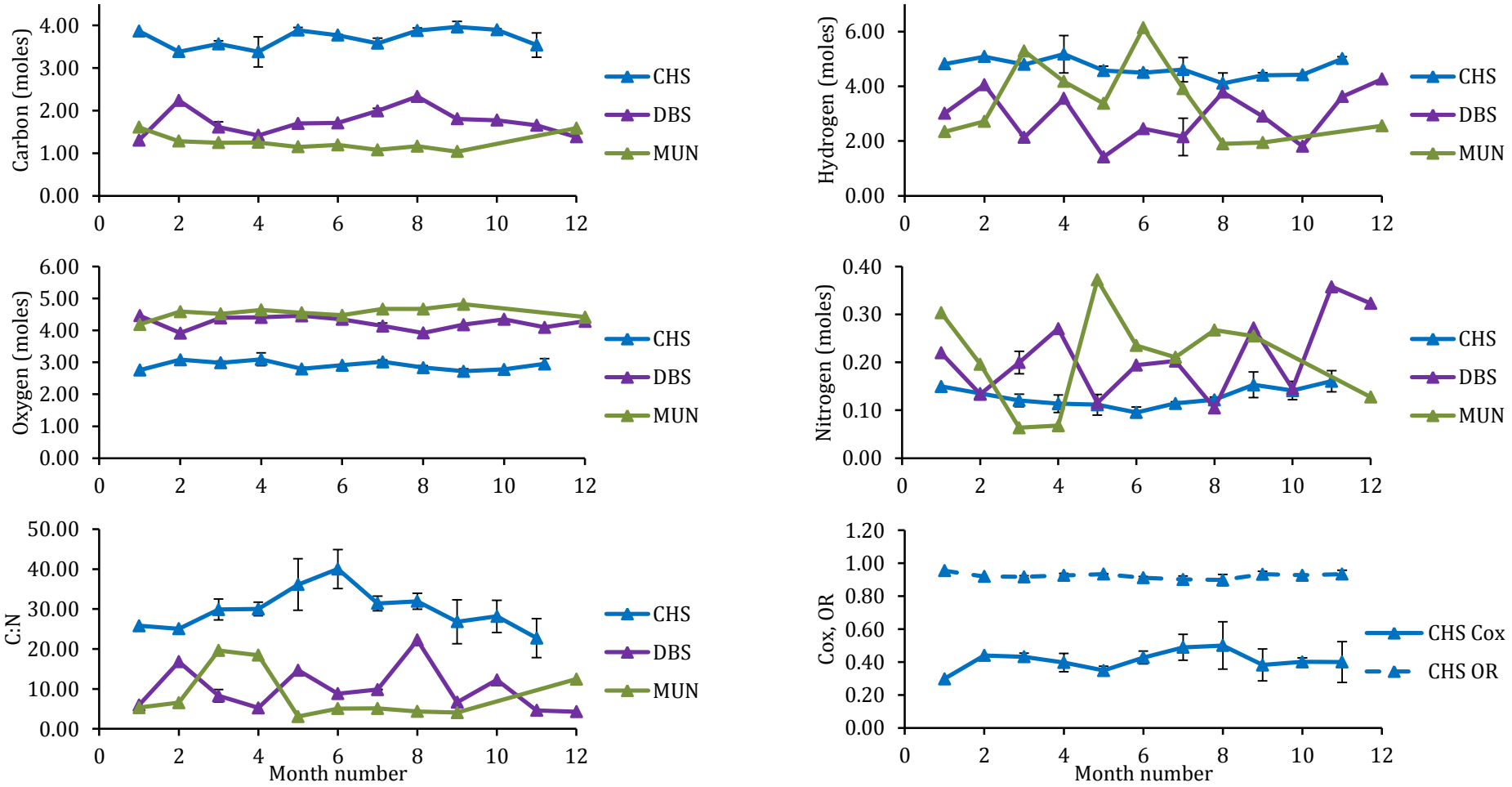


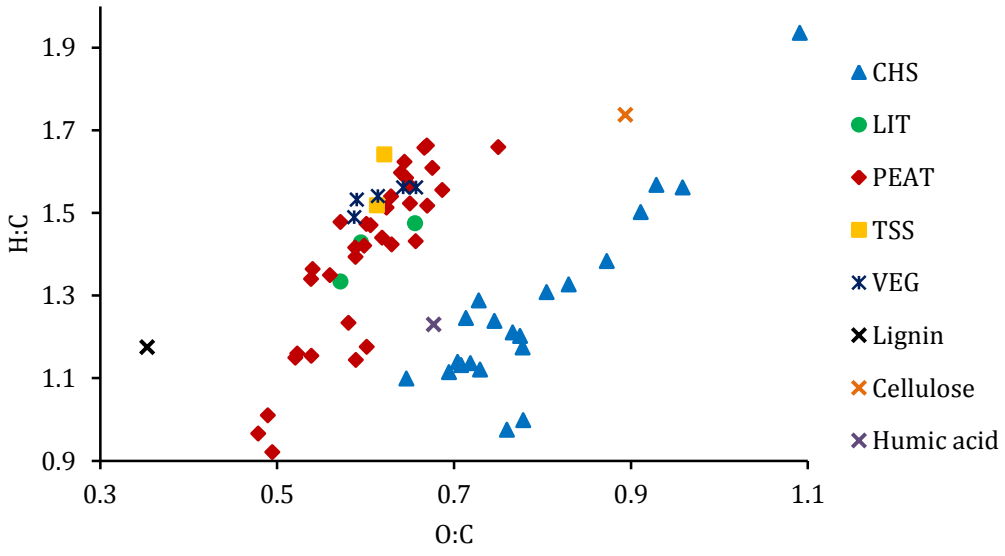
Figure 5.5. The average carbon, hydrogen, oxygen and nitrogen molar concentration, C:N ratio, C_{ox} and OR for the TDS samples per month. Error bars are the standard errors.



Another way of looking at the composition of organic matter is a Van Krevelen plot (Klavins et al., 2008; Kracht and Gleixner, 2000), where the H:C ratio is plotted against the O:C ratio (Figure 5.6). Applying the guidelines from Kracht and Gleixner (2000), the plot shows that the CHS samples are more methanated and have more carboxyl groups than the other sites, which all fall along the dehydration axis, with the peat samples generally more dehydrated than the TSS and litter samples. Humic acid falls close to the CHS samples, which is not surprising as humic acid is a large component of DOC.

An ANOVA on the O:C and H:C ratios found that there were no significant differences between the sites for H:C ($p=0.0676$), but there were significant differences between O:C ratios ($p<0.0001$), with all sites being significantly different from CHS.

Figure 5.6. The Van Krevelen plot for the organic sites, including lignin, cellulose and humic acid standards.

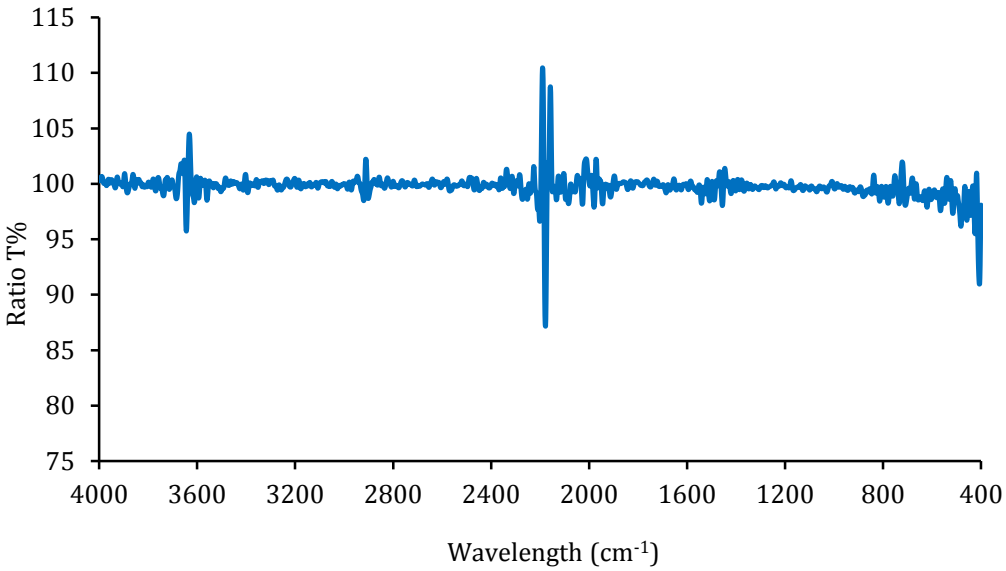


5.3.1.4. Fourier Transform Infrared Spectroscopy (FTIR)

Only seven CHS samples were analysed by FTIR as the spectra did not show many large peaks, possibly due to the complex mixture of compounds in the TDS. An example spectrum is shown in Figure 5.7. The largest peaks are at approximately 2200 cm^{-1}, and can be attributed to a stretch of alkyne bonds (C≡C), although this

bond is unlikely to be present in a high proportion in natural samples. The smaller peak at approximately 3600 cm^{-1} indicates a stretch of O-H bonds of alcohol and phenol functional groups. The other small peaks at 2910 cm^{-1}, 1970 cm^{-1} and 1450 cm^{-1} show alkane CH, CH_2 and CH_3 stretch, alkene C=C stretch and alkane CH_2 and CH_3 bends (Abdulla et al., 2010a; Cocozza et al., 2003; Davis et al., 1999; Mursito et al., 2010; Provenzano and Senesi, 1999; Zaccone et al., 2007).

Figure 5.7. An example FTIR spectrum of CHS (from November 2011).



Davis et al. (1999), investigating the composition of various humic acids, fulvic acids, sediments, DOC and soil samples, found a significant, positive correlation between the O:C atomic ratio and the absorbance at 1713 cm^{-1}, and a significant, negative correlation between the E4:E6 ratio and absorption at 1611 cm^{-1}. These two absorption bands are assigned to the C=O stretch and aromatic C respectively. Using the equation from these correlations, it was possible to estimate the peak heights for these two ranges for the samples in this chapter. Figure 5.8 shows the results of the O:C ratio and 1700 cm^{-1} peak height correlation, and Figure 5.9 shows the E4:E6 ratio and 1600 cm^{-1} peak height, with Davis et al. (1999) data and the sites from this chapter. The peak heights in Davis et al. (1999) are normalised according to the solvent used, and the results are not directly transferable to the measured spectrum above.

The correlation between O:C and the C=O peak indicates that carboxylic acid functional groups are important in determining the O:C ratio of the samples. Applying this to the samples for this chapter shows that the CHS samples have more carboxylic functional groups than the other organic samples as shown in Figure 5.8. The correlation between E4:E6 and the aromatic C peak indicates that the E4:E6 ratio is strongly influenced by the aromatic C content of the sample. Only the TDS samples with accompanying water samples have E4:E6 data, and so only these three sites are correlated with this peak height. The results show that MUN has the highest aromatic C content, DBS has an intermediate content and CHS has the lowest content of aromatic C.

As FTIR analysis was only carried out on seven CHS samples, the data were not used in further analysis.

Figure 5.8. The correlation between O:C ratio and height of the peak at 1700 cm^{-1}, indicating a stretch of the C=O bond. Error bars are the standard errors. The equation and r^2 are for the Davis et al. (1999) data.

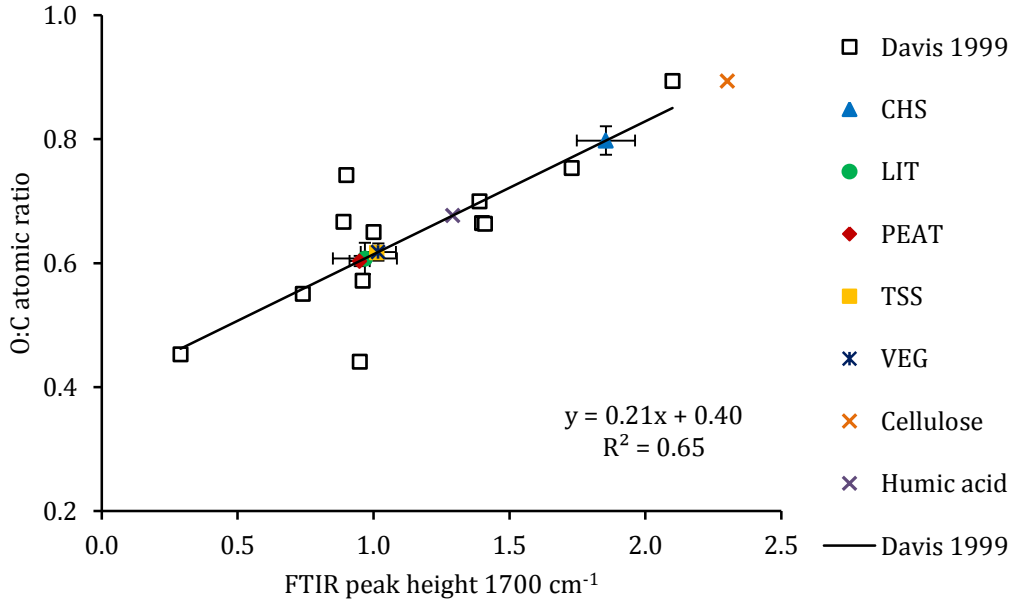
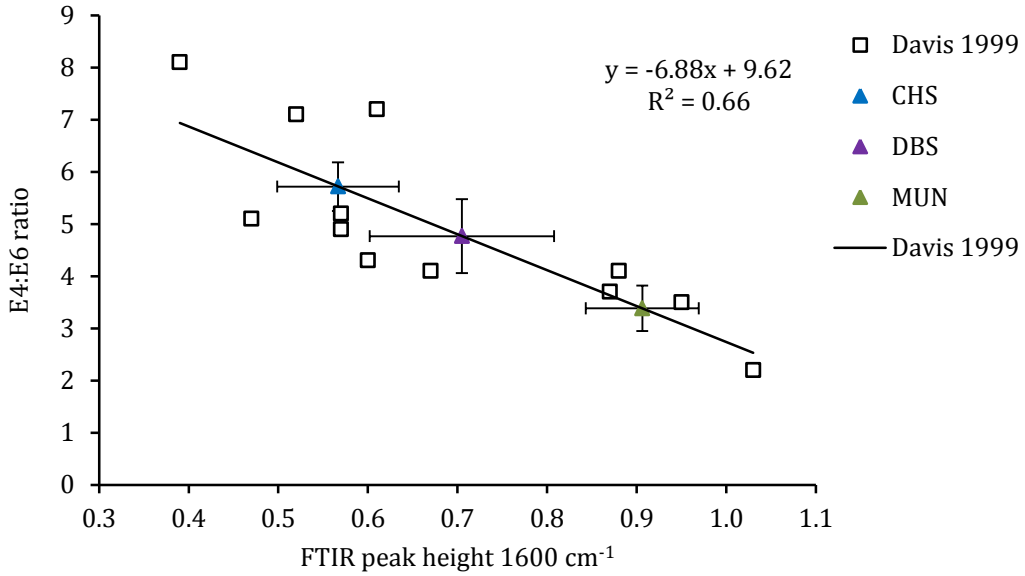


Figure 5.9. The correlation between E4:E6 ratio and height of the peak at 1600 cm^{-1} indicating aromatic C bonds. Error bars are the standard errors. The equation and r^2 are for the Davis et al. (1999) data.



5.3.1.5. ^{13}C solid state Nuclear Magnetic Resonance (^{13}C-NMR)

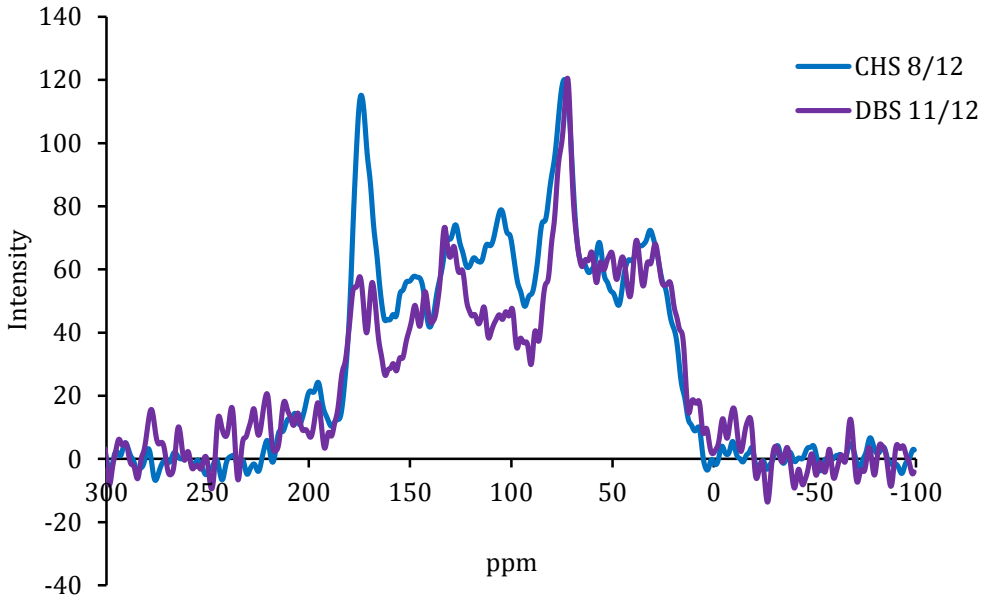
The NMR spectra for the CHS samples showed a variety of results, but due to the low carbon content, the DBS samples did not show many peaks at all. Only samples with peaks with a greater relative intensity than 2 were included in the analysis. This resulted in nine CHS samples and six DBS samples, with no one month having samples from both sites. Using the shift ranges from Chadwick et al. (2004 - Table 5.2) the following notable peaks for two typical samples were identified from the spectra (example shown in Figure 5.10), and outlined in Table 5.5.

For further analysis, the peak height in each range was divided by the sample DOC concentration, to get a relative peak height for each sample. An ANOVA on the data showed no significant differences between sites or months for each of the eight ranges.

Table 5.5. The main peaks and corresponding carbon types for the ^{13}C NMR in Figure 5.10. The numbers are the maximum intensities for the peaks in each range.

Peak (ppm)	Types of carbon	description
30	alkyl C	higher for CHS (72) than DBS (68)
50	N-alkyl and methoxyl C	higher for DBS (65) than CHS (50)
70	O-alkyl C	similar size for both samples, suggesting a similar carbohydrate concentration in both samples (CHS: 120.13, DBS: 120.47)
105	di-O-alkyl C	much larger for CHS (79) than DBS (46)
130	aromatic/unsaturated C	similar size for both samples (CHS: 74, DBS: 73)
150	phenolic C	broader and slightly taller for CHS (58) than DBS (49), suggesting that CHS contains a higher lignin concentration than DBS
175	amide/carboxyl C	much higher for CHS (115) than DBS (58)

Figure 5.10. The ^{13}C NMR spectra for CHS (August 2012) and DBS (November 2012).



5.3.1.6. Thermogravimetric Analysis (TGA)

The average final weight of the CHS samples was 35%, which was higher than the peat, vegetation, litter and suspended sediment samples, but almost half of the final weight for the two other TDS sites, DBS and MUN, which had final weights of 62 and 72%, respectively (Table 5.6). The monthly final weight varied slightly throughout the year (Figure 5.11), but an ANOVA on the TDS monthly samples showed no significant differences between Months for either the final weight ($p=0.0674$) or the weight at 550 °C ($p=0.0605$).

The weight loss in each 50 °C increment from 0 °C to 1000 °C results showed that the weight loss from the more organic samples peaked at approximately 350 °C, and the DBS and MUN samples peaked at 800 °C (Figure 5.12). All samples have a peak between 100 and 200 °C where the physical and chemical water evaporates. The vegetation, litter, peat and suspended sediment lose a larger majority of their weight at 350 °C than CHS, which also peaks at a slightly lower temperature, at 300 °C. DBS loses a large majority of its weight at 800 °C, but the other inorganic site, MUN, doesn't lose much weight at all. The loss of mass at lower temperatures for the CHS samples showed that they were more oxidised, and therefore burned at a lower temperature than the less oxidised samples.

An ANOVA on the weight loss range data showed there were no significant differences between the vegetation types ($p=0.9749$), and so they were grouped as 'VEG' for all further analysis. Similarly, there were no significant differences between the two peat cores ($p=0.9681$) and the different depths ($p=0.0966$), and so these were grouped as 'PEAT' in further analysis.

Various studies have attributed weight loss in slightly different temperature ranges to slightly different components of organic matter (e.g. Ascough et al., 2008; Barros et al., 2007; Kolokassidou et al., 2007; Reiche et al., 2010; Sutcu, 2007), although they tend to agree on the order that the components are lost in.

The weight losses in the ranges for comparison with the energy data from the DTA (EXO 1: 200-400 °C, EXO 2: 400-500 °C, EXO 3: 500-650 °C) are shown in Figure 5.13, and show a similar result to the weight loss ranges data in Figure 5.12. EXO 1 has the largest weight loss for CHS, LIT, PEAT, TSS and VEG, with DBS and MUN having much smaller losses in all three ranges. The EXOtot (total weight loss in the EXO 1 and EXO 2 ranges), proportion of EXOtot attributed to EXO 1 and EXO 2 and the ratio of EXO 1 to EXO 2 for each site and sample type are shown in Table 5.6. The comparison with DTA data is in section 5.3.1.7.

Table 5.6. The average final weight, EXOtot (total weight loss in the EXO 1 and EXO 2 ranges), proportion of EXOtot attributed to EXO 1 and EXO 2 and the ratio of EXO 1 to EXO 2 for each site and sample type. The lignin and cellulose data is from Lopez-Capel et al. (2005).

Type	Site	Final weight (%)	EXOtot (%)	EXO 1 (% of EXOtot)	EXO 2	EXO 1/ EXO 2
TDS	CHS	34.91	32.27	76.40	23.60	3.28
TDS	DBS	62.23	9.28	64.08	35.96	1.86
TDS	MUN	71.54	8.72	70.35	29.65	2.43
LITTER	LIT	22.79	53.90	81.59	18.41	4.51
PEAT	A6	28.07	50.43	75.79	24.21	3.19
PEAT	B6	28.07	49.78	76.08	23.92	3.23
TSS	SUS	20.94	45.82	81.75	18.25	4.52
VEG	AGB	19.64	55.56	82.50	17.50	4.72
VEG	BGB	23.49	53.18	81.49	18.51	4.40
VEG	SED	20.79	58.39	85.16	14.84	5.74
VEG	HEA	21.53	60.88	79.86	20.14	3.96
VEG	MOS	21.53	53.81	83.12	16.88	4.92
Cellulose		3.2	98.8	70.1	29.8	2.35
Lignin		8.6	86.7	20.3	79.6	0.26

Figure 5.11. The monthly final weight for the three TDS sites.

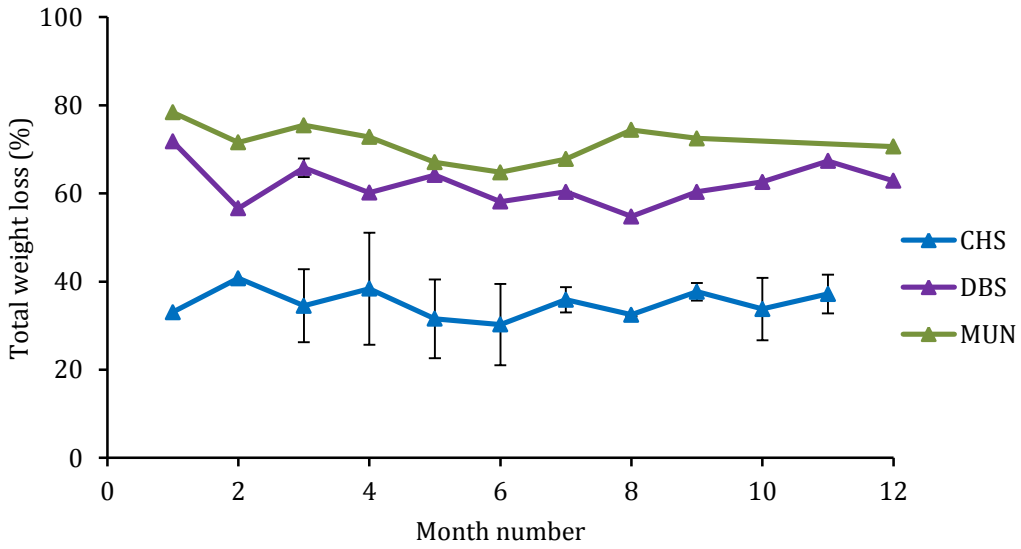


Figure 5.12. The average weight loss in each 50 °C range for each of the sample types.

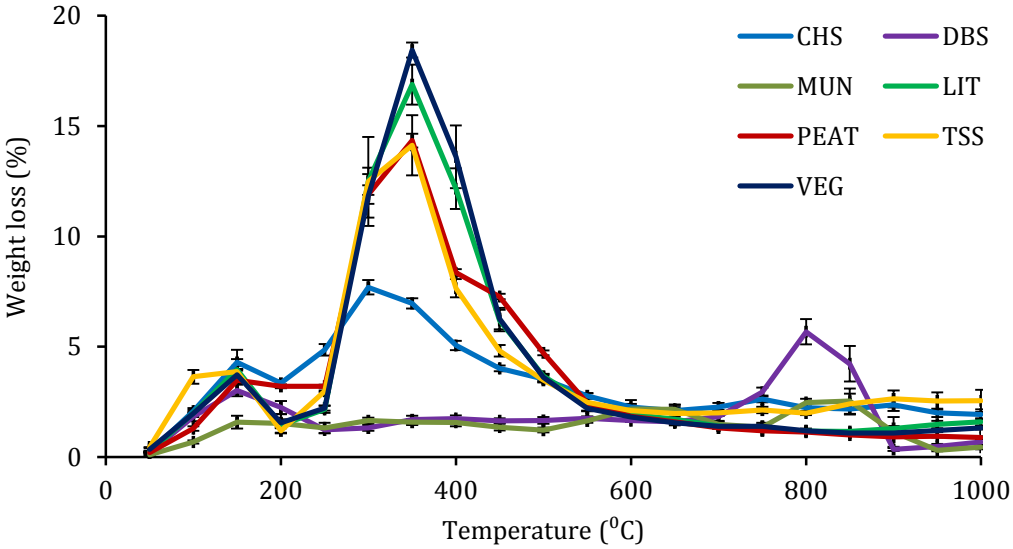
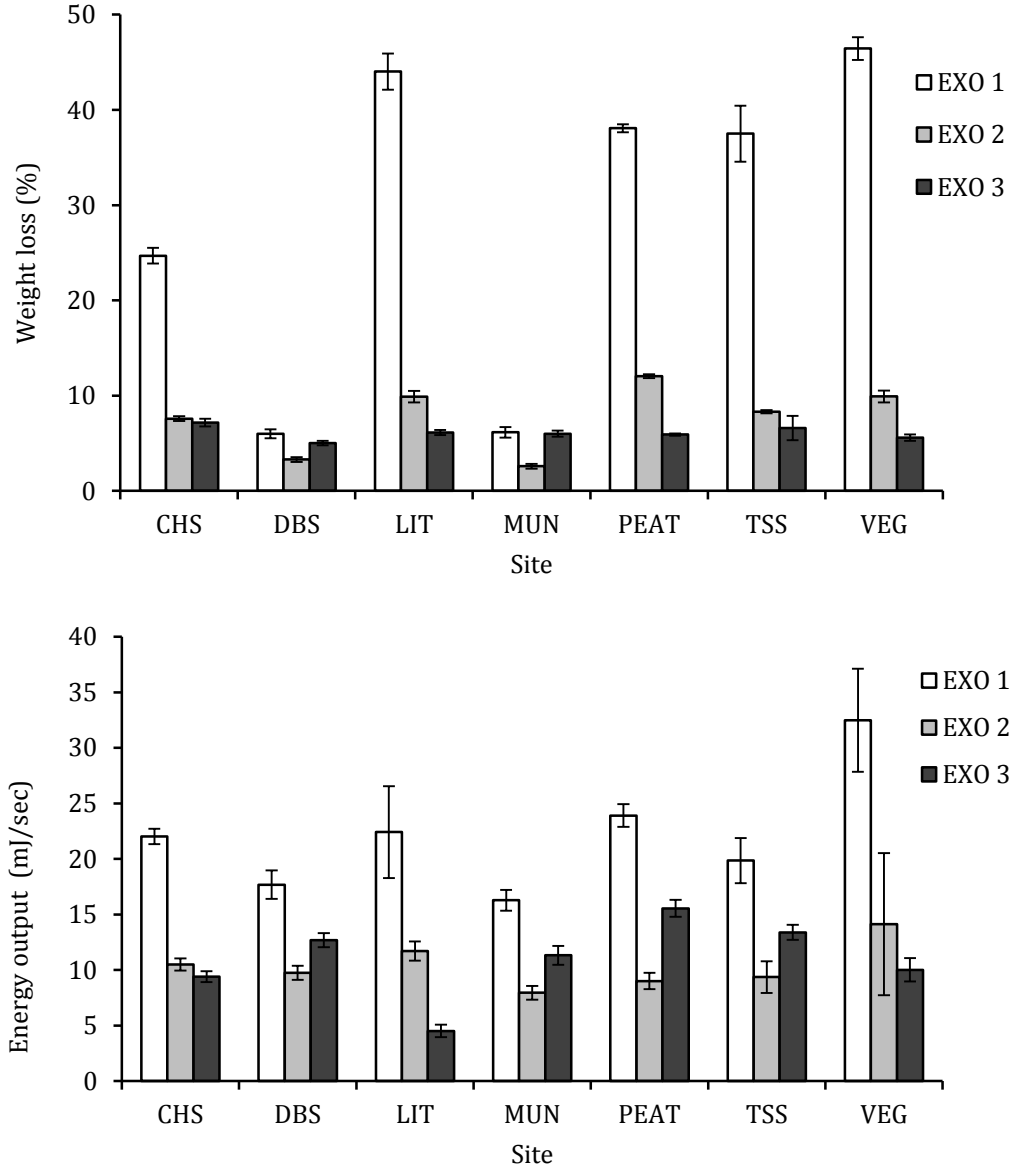


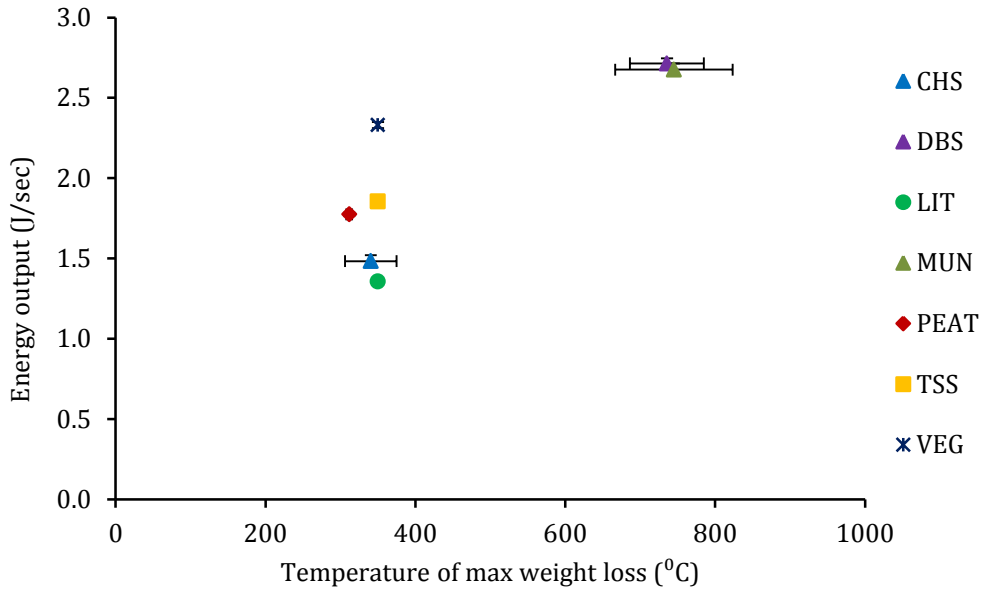
Figure 5.13. The average weight and energy output in each EXO range (EXO 1: 200-400 ℃, EXO 2: 400-500 ℃, EXO 3: 500-650 ℃) for each sample type.



5.3.1.7. Differential Thermal Analysis (DTA)

The average temperature range of maximum weight loss, and the average energy output in the same range is shown in Figure 5.14. As with the TGA results, the more organic samples have a lower temperature of maximum weight loss, at approximately 350 °C, than DBS and MUN. The energy output shows that CHS has a similar output to the litter samples, with the output from the vegetation, suspended sediment and peat being approximately 0.5 J sec^{-1} higher. The higher energy output of DBS and MUN does not correspond well with the gross heat data from the bomb calorimeter, where samples from DBS and MUN wouldn't burn. The higher energy output could be due to the linear increase in the baseline of the heat flow with the increasing temperature, resulting in apparently higher energy output at higher temperatures. For a more accurate comparison with the other sites, the energy outputs for DBS and MUN at 350 °C were 1.65 and 1.60 J sec^{-1}, within the range of the other sites.

Figure 5.14. The average energy losses within the temperature range with the greatest weight loss for each sample type.



The energy output in the ranges for comparison with the weight loss data from the TGA (EXO 1: 200-400 °C, EXO 2: 400-500 °C, EXO 3: 500-650 °C) are shown in Figure 5.13. According to Lopez-Capel et al. (2008), EXO 1 is the exothermic

weight loss corresponding to the labile carbon pool: the aliphatic C and carbohydrates (predominantly as cellulose). Other papers refer to weight loss and energy output in this range as corresponding to 'less humified' components (Plante et al., 2009), the volatisation of –OH and –COOH groups in the compound (Schnitzer and Hoffman, 1965), decomposition of labile aliphatic and carboxylic groups (Barros et al., 2007), the oxidisation of sugars and cellulosic material and an indicator of the degree of humification of the matter (Bergner and Albano, 1993). This correlates well to the cellulose and lignin standards from Lopez-Capel et al. (2005), and this is the range of the greatest weight loss and energy output for the peat, litter, suspended sediment, vegetation and CHS samples, indicating that they contain a large proportion of the labile carbon as defined by Lopez-Capel et al (2008). DBS and MUN have small weight losses in this range, indicating that they do not contain as much labile carbon. The Lopez-Capel et al. (2005) data for cellulose and lignin show that cellulose loses the largest proportion of its weight in EXO 1, as do the samples in this chapter.

Lopez-Capel et al. (2008) have ascribed EXO 2 to the recalcitrant pool, with aromatic-rich C and lignin components; others have suggested it is due to more humified matter (Plante et al., 2009), carbon oxidation (Schnitzer and Hoffman, 1965), acid and cellulose content (Bergner and Albano, 1993) and decarboxylation (Provenzano and Senesi, 1999). For the sites in this chapter there are small weight losses in this range, and again, the more organic sites lose more mass than the inorganic sites. For the energy loss, there are few differences between the sites for the EXO 2 range. The Lopez-Capel et al. (2005) data for cellulose and lignin show that lignin loses that largest proportion of its weight in EXO 2. All samples in this chapter loose a larger proportion of mass in EXO 1, suggesting that they are comprised of more cellulose-like compounds than lignin.

According to the literature, EXO 3 corresponds to the refractory and inert carbon pool, predominantly aromatic C (Lopez-Capel et al., 2008), decomposition of the aromatics (Provenzano and Senesi, 1999) and the polymorphic transformation of quartz (Barros et al., 2007). All the organic sites lose the smallest proportion of their mass in the EXO 3 range (between 5.6 and 7.2%), perhaps indicating a small portion of aromatic material in the samples. DBS and MUN lose a larger proportion of their mass and energy in EXO 3 than in EXO 2, suggesting a larger aromatic, more refractory content of the TDS from these sites.

The ratio of EXO 1 to EXO 2 (Table 5.6) has been suggested to indicate the degree of humification of the solid, with higher numbers having more cellulose-like components than lignin-like components (Lopez-Capel et al., 2005), and being more labile than recalcitrant.

5.3.1.8. Pyrolysis Gas Chromatography Mass Spectrometry (Pyrolysis GC/MS)

The first peak on all the graphs (Figure 5.15) was generally the largest (except in the LIT sample) and was identified as carbon dioxide (CO_2). Further peaks were identified as in Table 5.7.

Table 5.7. The main peaks identified from the samples. The "P, C or L" column indicates if the compound is likely to have derived from phenol (P), cellulose or carbohydrate (C), or lignin (L); this information comes from the literature (Arranz et al., 2009; Calvelo Pereira et al., 2011; Christy et al., 1999; Kracht and Gleixner, 2000; McClymont et al., 2011; Ralph and Hatfield, 1991; Reiche et al., 2010).

Compound	P, C or L	formula	mw	retention time
carbon dioxide		CO_2	44	2.61
1-hexene		C_6H_{12}	84	3.36
acetic acid	C	$C_2H_4O_2$	60	3.82
1-heptene		C_7H_{14}	98	4.22
benzene methyl		C_7H_8	92	5.08
furfural	C	$C_5H_4O_2$	96	5.84
phenylethene		C_8H_8	104	7.03
2 furancarboxaldehyde, 5-methyl	C	$C_6H_6O_2$	110	8.02
phenol	C/L	C_6H_6O	94	8.60
phenol, 3-methyl	P/L	C_7H_8O	108	10.22
benzoic acid		$C_7H_6O_2$	122	11.65
catechol		$C_6H_6O_2$	110	12.10
1,2 benzenediol, 4-methyl	L	$C_7H_8O_2$	124	13.46
4-vinylguaiacol	L	$C_9H_{10}O_2$	150	13.65
4-methylguaiacol	L	$C_8H_{10}O_2$	138	14.90
1,6-anhydro-β-D glucopyranose	C	$C_6H_{10}O_5$	162	18.00
n-hexadecanoic acid		$C_{16}H_{32}O_2$	256	21.40

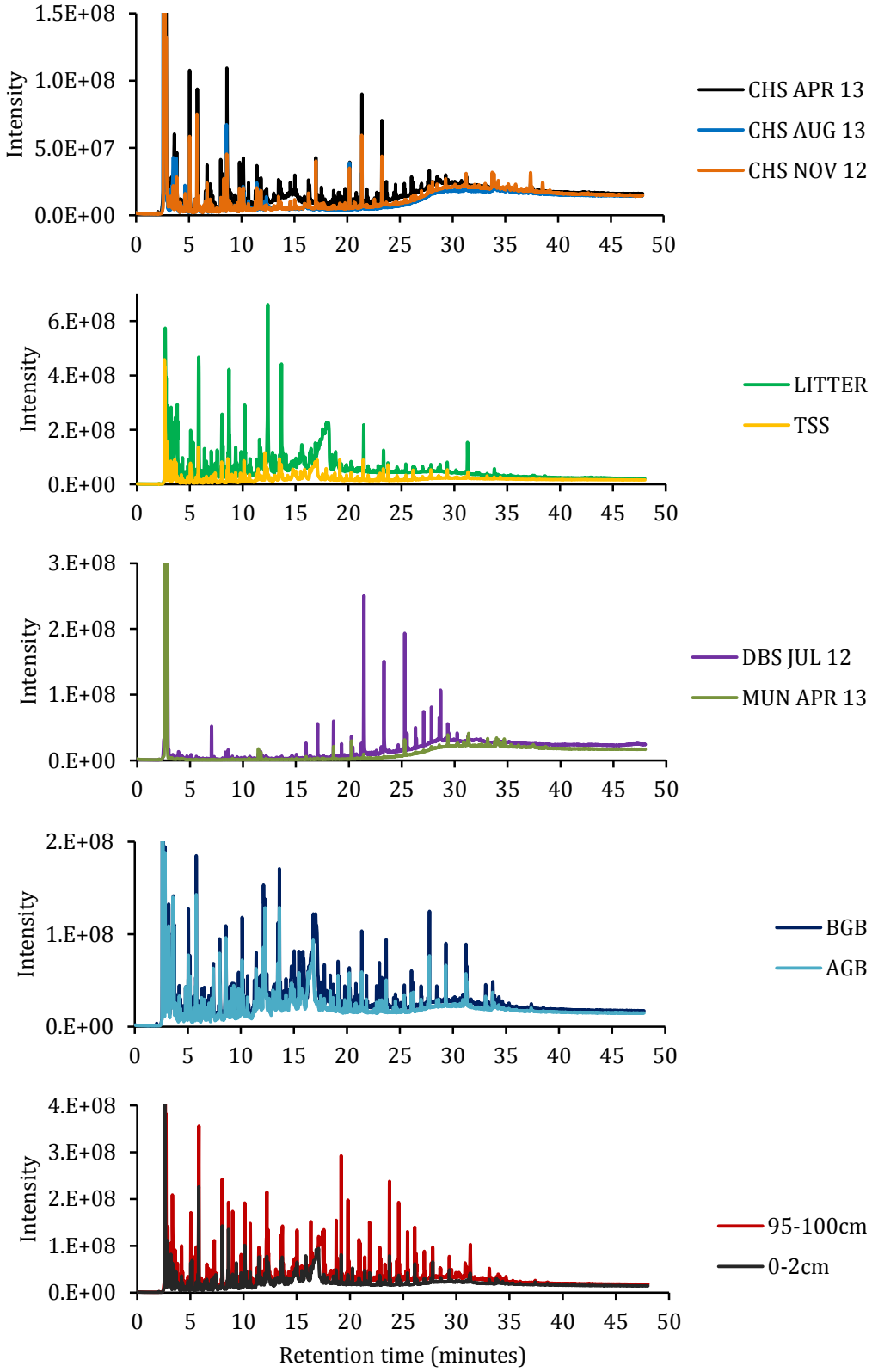
A brief analysis of the data showed that the TDS samples generally had lower abundances of all identified compounds, compared with the other analysed samples. The CHS TDS samples had higher abundances of the identified compounds than the DBS and MUN TDS samples. These TDS samples, DBS especially, had higher abundances of compounds eluted after 20 minutes. The longer retention times of these compounds suggest that they are larger compounds, and their absence from the literature used for referencing compounds suggests that they are not frequently found in organic soils and vegetation samples. The deep peat sample had some of these later peaks, in similar abundances to the DBS sample.

All samples contained lignin-derived compounds (between 13 and 15 minutes retention times); these were present in higher abundances in the vegetation, peat, litter and TSS samples. Within these samples, there was a higher abundance of lignin-derived compounds in the BGB compared with the AGB, in the 95-100 cm peat compared with the 0-2 cm peat, and in the litter compared with the TSS sample.

Variable amounts of levoglucosan (1,6-anhydro-β-D glucopyranose, retention time of 18 minutes) were present in the different samples, with the TDS samples having quite a low abundance of this sugar. The litter sample had the highest abundance, followed by the BGB and 95-100 cm peat, and the AGB and 0-2 cm peat. The TSS sample had quite a low abundance, though it was noticeably higher than the TDS samples.

As this analysis was only carried out on 12 samples, the data were not used in further analysis.

Figure 5.15. The chromatograms from the Pyrolysis GC/MS: CHS, LIT, TSS, DBS, MUN, VEG (AGB, BGB) and PEAT (0-5 cm, 95-100 cm). Note the changing y-axis scale.



5.3.1.9. Water chemistry analysis

The water chemistry variables was analysed relative to their initial concentrations (Table 5.8). The POC concentrations increased in the CHS and MUN water, but decreased in the DBS water. The DOC concentrations increased in the water from all three sites. The composition proxy data showed that the absorbance at 400 nm decreased or stayed almost the same, whereas the E4:E6 ratio increased for all three sites.

Table 5.8. The average relative water chemistry at the end of the evaporations for the three sites, where a value of greater than 1 indicates there was an increase between the beginning and end of the experiment.

Variable	CHS	DBS	MUN
relative POC	1.06	0.81	1.13
relative DOC	1.31	2.10	1.02
relative Abs_{400}	0.95	0.89	1.01
relative E4:E6	1.21	1.25	1.42

A t-test was used to determine whether each variable had increased or decreased over the time of the evaporation, whilst the water samples were kept in the laboratory in fairly constant temperature and light conditions. The DOC concentration of the CHS samples increased significantly between the beginning and end of the experiment, but there were no other significant changes. The POC concentration of the DBS samples decreased significantly, while the DOC concentration increased significantly. There were no significant changes in the MUN water (Table 5.9). The increases in DOC concentration, coupled with a decrease in POC concentration would indicate that the POC was breaking down to release DOC. The increase in DOC concentration that was not accompanied by a decrease in POC concentration, as in the CHS samples, would indicate that there was in-situ production of DOC by microbes in the water. However, the increase of POC could have been due to settling out method used to separate the particulates from the water, so that there were only the smaller fractions of carbon in the evaporating dish. The final water sample was taken from the bottom of the container, and may have therefore had a higher concentration of particulates due to this.

Table 5.9. The results of the t-test on the changes in concentrations of the variables between the beginning and end of the experiment for each site.

Variable	CHS	DBS	MUN
relative POC	0.7984	0.0060	0.5639
relative DOC	<0.0001	0.0401	0.8145
relative Abs_{400}	0.3558	0.5156	0.8805
relative E4:E6	0.2372	0.2267	0.2114

The only significant differences in the relative concentrations between months were the POC concentrations in the CHS and MUN samples, where the April concentrations were significantly higher than all other months. Examining the data, this significant difference between the beginning and end of the experiments seems to be due to a lower than average initial concentration of POC at both sites, rather than abnormally high final concentrations. There were also significant differences in the POC concentrations in the CHS samples between October and June, July and September, July and January, July and June, and March and June.

These results show that there were some changes in the DOC and POC concentrations of the water whilst in was in the laboratory, however there were no significant changes in the composition, as measured by the absorbance at 400 nm and E4:E6 ratio. The aims of this chapter were to consider the composition, rather than total concentrations, and these water chemistry analysis showed that there were no significant compositional changes. These data were not used to calculate the rates of degradation; those were calculated from the degradation experiments in Chapters 2, 4, 6 and 7.

5.3.2. Differences in TDS composition

As stated in section 5.2.2.1, there were differences in colour and amount of the TDS gathered from the three sites. To look further at the differences in the composition of the TDS as it moves through the river system, a PCA was carried out using all the variables consistently measured at the three sites. The variables used were nitrogen, carbon, hydrogen and oxygen molar concentrations, C:N, H:C, O:C ratios, EXO 1 and EXO 2. All these variables were significantly different between the three sites. EXO 3 was not included as the ANOVA showed this was not significantly

different (p=0.5809). C_{ox} and OR were not included as the results were not reliable for DBS and MUN. There were 45 samples and nine variables.

The results of the PCA on the three sites showed that 97% of the variance could be explained by the first three principal components, two with eigenvalues of greater than one, and another one with an eigenvalue of less than 1 (Table 5.10).

Table 5.10. The results of the PCA on the three TDS sites, showing the principal components with eigenvalues > 1 and the first component with an eigenvalue < 1.

Variable	PC1	PC2	PC3
Nitrogen	-0.24	-0.17	0.86
Carbon	0.39	-0.05	0.11
Hydrogen	0.21	0.67	0.21
Oxygen	-0.38	0.00	-0.20
C:N	0.37	0.11	-0.29
H:C	-0.21	0.69	0.09
O:C	-0.37	0.13	-0.09
EXO 1	0.37	0.02	0.18
EXO 2	0.37	-0.05	0.18
Eigenvalue	6.50	1.42	0.77
Cumulative variation explained (%)	72	88	97

The graph of PC1 and PC2 shows very distinct differences in composition between the organic headwater site and the two further downstream sites, reflecting the differences in the physical appearance of the samples (Figure 5.16). The variables that influence the principal components most (with the largest and smallest values) show that the first component is characterised by carbon, oxygen, EXO 1, EXO 2, O:C and nitrogen, of which the nitrogen, oxygen and O:C ratio have negative loadings, and the carbon, EXO 1 and EXO 2 have high positive loadings (Figure 5.16). The CHS samples fall on the positive side, indicating they have high carbon, high EXO 1, high EXO 2, low oxygen, low nitrogen and low O:C ratio. The high carbon content is likely to explain the dark brown/black colour of the solid material. There is quite an overlap between the DBS and MUN samples on the negative side of the x-axis, suggesting fewer differences between these two sites. However, the MUN samples are slightly further left on the x-axis (more negative)

than the DBS samples, suggesting that they have higher nitrogen, oxygen and O:C ratios, with lower EXO 1, EXO 2 and carbon.

The second component is characterised by high positive loadings for H:C, and hydrogen. In Figure 5.16 this is the y-axis, and the positive loadings are attributed to high H:C ratios and hydrogen contents, and the negative loadings are due to high nitrogen and EXO 2 values. There is less distinction between the three sites along this axis; however there are still some differences. The CHS sample with the highest PC2 loading has the highest H:C ratio of all the CHS samples, but it is not as high as the highest values for the MUN sites. The majority of the DBS samples fall on the negative side of this axis, suggesting that they have higher nitrogen contents and higher EXO 2 values than the MUN samples.

The graph of PC2 and PC3 has more overlap of the sites than the PC1 and PC2 graph (Figure 5.17). In this graph, the PC2 axis is horizontal, and exhibits the same loadings as described above, although the main distinction is between the H:C ratios and hydrogen content, rather than the nitrogen and EXO 2 values as in Figure 5.16. The third component, along the y-axis, is predominantly characterised by positive loadings for nitrogen content, and high negative loadings for the ratio of C:N. There is less distinction between the three sites; however the CHS samples are still very grouped and close to the origin, suggesting that they are much more similar to each other than the more variable DBS and MUN samples.

Figure 5.16. PC1 against PC2, showing the three TDS sites.

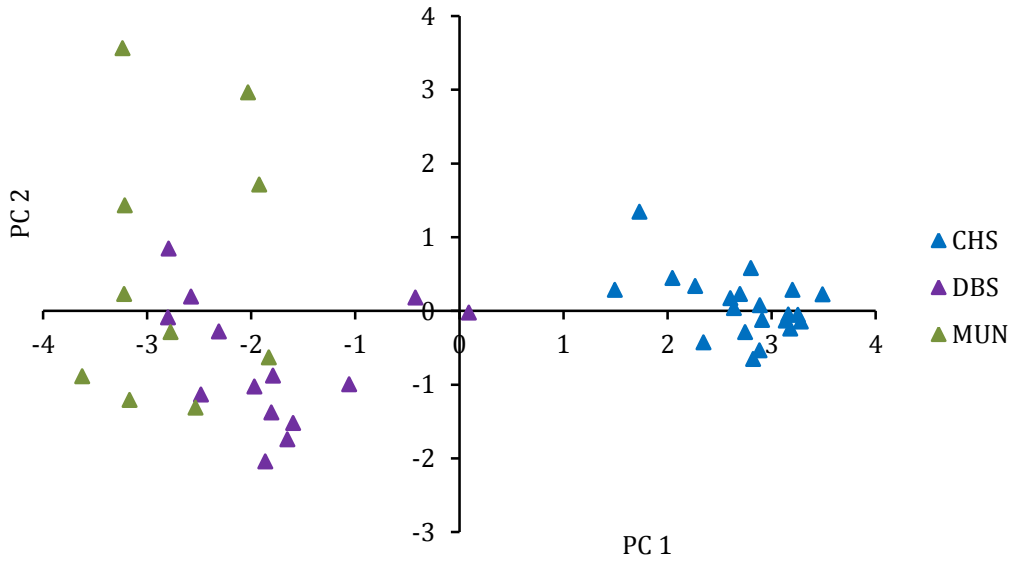
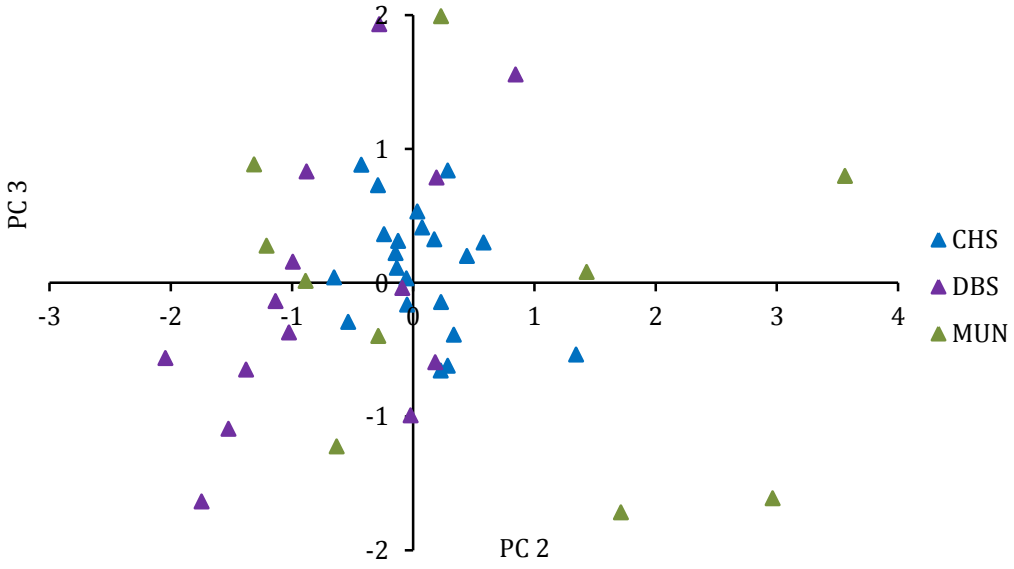


Figure 5.17. PC2 against PC3, showing the three TDS sites.



These results suggest that the composition of the TDS changes significantly downstream, especially the carbon, hydrogen and oxygen concentrations. The carbon and hydrogen decrease, whereas the oxygen increases, suggesting the dark

brown/black material is oxidised as is travels downstream, and becomes lighter in colour.

A second PCA was carried out using the variables measured only at CHS and DBS (the NMR data), but there were no interesting results, and the ANOVA showed no significant differences between the two sites.

5.3.3. TDS and the source materials

To compare the CHS TDS with the source materials, an ANOVA and PCA were carried out on the variables measured for CHS, litter, peat, vegetation and suspended sediment. The variables considered by the ANOVA were the TGA final weight, EXO 1, EXO 2, EXO 3, EXO1:EXO2, gross heat, carbon, nitrogen, hydrogen, oxygen, C:N, H:C, O:C, C_{ox} and OR. The results of the ANOVA showed that the types of matter were significantly different for all variables except H:C ($p=0.0676$). This shows that CHS does not have the same composition as any one of the source materials, and therefore is likely to be composed of a material derived from more than one source, or perhaps it is more degraded than all the source samples, and therefore has compositional differences.

To include the standards in the PCA, the EXO 1 and EXO 2 data from Lopez-Capel et al. (2005 – Table 5.6) were used for cellulose and lignin. There is no data for EXO 3, so this variable was not included in the PCA. The variables included in the PCA were the TGA final weight, EXO 1, EXO 2, gross heat, carbon, nitrogen, hydrogen, oxygen, C:N, H:C, O:C, C_{ox} and OR. There were 67 samples and 13 variables.

The results of the PCA on CHS and source materials showed that 94% of the variance could be explained by the first four principal components, three with eigenvalues of greater than one, and another one with an eigenvalue of less than 1 (Table 5.11).

The graph of PC1 and PC2 shows very distinct differences in composition between the TDS from CHS and the other source sites and standards (Figure 5.18). The CHS samples are separate from the other source materials and standards, indicating that they have a different composition, with higher oxygen, C_{ox} and O:C than the other sites. This shows the clear divide between dissolved matter (TDS) and the solid source materials (all other samples). The vegetation samples fall

almost equidistant between cellulose and lignin, which was expected, as the majority of plants are composed of both cellulose and lignin.

Table 5.11. The results of the PCA on CHS and the source materials, showing the principal components with eigenvalues > 1 and the first component with an eigenvalue < 1.

Variable	PC1	PC2	PC3	PC4
TGA final weight	-0.24	-0.22	0.47	0.21
Nitrogen	-0.15	-0.28	0.40	-0.47
Carbon	0.34	-0.26	-0.08	-0.09
Hydrogen	0.25	0.38	0.31	0.08
Oxygen	-0.33	0.29	-0.10	0.08
C:N	0.26	-0.22	0.18	0.52
H:C	0.01	0.51	0.31	0.19
O:C	-0.33	0.27	-0.04	0.20
C_{ox}	-0.36	-0.14	-0.23	0.01
OR	0.35	0.12	0.29	-0.06
Gross heat	0.36	-0.03	-0.04	-0.15
EXO 1	0.13	0.41	-0.20	-0.46
EXO 2	0.25	-0.02	-0.44	0.35
Eigenvalue	6.68	3.14	1.36	0.99
Cumulative variation explained (%)	51	76	86	94

The variables that influence the principal component (with the largest and smallest values) show that the first component is characterised by carbon, oxygen, O:C, C_{ox}, OR and gross heat, with the positive loadings attributed to high carbon, OR and gross heat values, and the negative due to high oxygen, O:C ratio and C_{ox} values. Along this axis, there are clear differences between the CHS samples and the source materials, as all the CHS samples are on the negative side of the axis. This indicates they have high oxygen, O:C ratios and C_{ox} values; but have low carbon, OR and gross heats. The source materials have higher carbon contents, but the highest carbon content is in the lignin. The litter and TSS samples have lower carbon contents than the vegetation, and the peat samples have very low variation in their carbon contents. The vegetation sample with the highest PC1 loading is the heather, which had high carbon, OR and gross heat. The cellulose standard falls almost exactly in

the middle of the axis, and it has a high oxygen content, a high O:C and a medium C_{ox}, OR and gross heat, but a low carbon.

The second component is characterised by H:C, carbon and C:N, with the positive loadings due to high H:C ratios, and the negative loadings due to the carbon content and C:N ratio (Figure 5.18). The majority of the CHS samples fall on the negative side of this axis, as they have high carbon and C:N ratios. The cellulose standard has the highest positive PC2 loading, indicating it has a high H:C ratio, and low carbon content and C:N ratio. The source materials fall fairly centrally along this axis, in a group with some overlap, with middling carbon contents and C:N ratios. Lignin has a high carbon content and C:N, and a low H:C ratio and has a similar negative loading to the CHS samples.

The graph of PC2 and PC3 has more overlap of the sites than the PC1 and PC2 graph (Figure 5.19). The third component is predominantly characterised by nitrogen concentration and the final weight, with the positive loadings due to both high nitrogen contents and high TGA final weights. The PC2 axis is as described above. There are several interesting features of this graph, one being the diagonal line along which the majority of the samples fall, from negative PC2 and PC3, across the origin, towards positive PC2 and PC3, with very few points falling outside of this general pattern. The cellulose standard is one such 'outlier', having the highest PC2 and smallest PC3 loadings, with high H:C ratio and low carbon content, and low TGA final weight and nitrogen content. The three litter samples all have almost identical PC3 loadings, indicating they have similar TGA final weights and nitrogen contents, but different PC2 loadings, meaning they fall in a straight line with equal distances between the three. The analysis of these samples shows that the litter sample with the negative PC2 loading has the lowest H:C ratio and highest carbon content of the three samples. As in the previous graph, the majority of the CHS samples fall in the same area of the graph, with negative PC2 and PC3 loadings, and the majority of the source materials fall in the positive PC2 and PC3 area of the graph. The two CHS outliers have a-typically high H:C ratios compared with the other CHS samples.

Figure 5.18. PC1 against PC2, showing CHS, cellulose, lignin and the source materials.

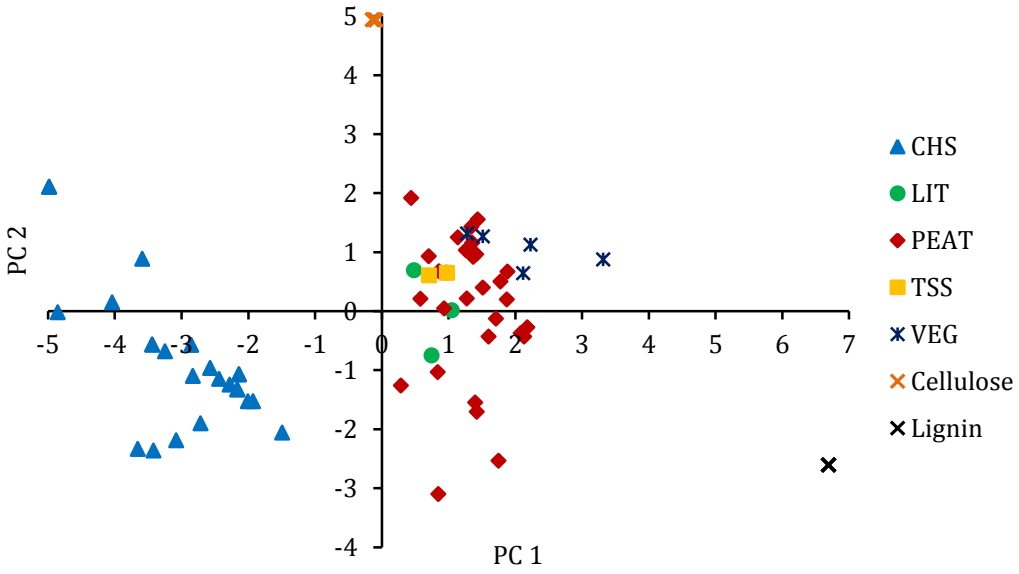
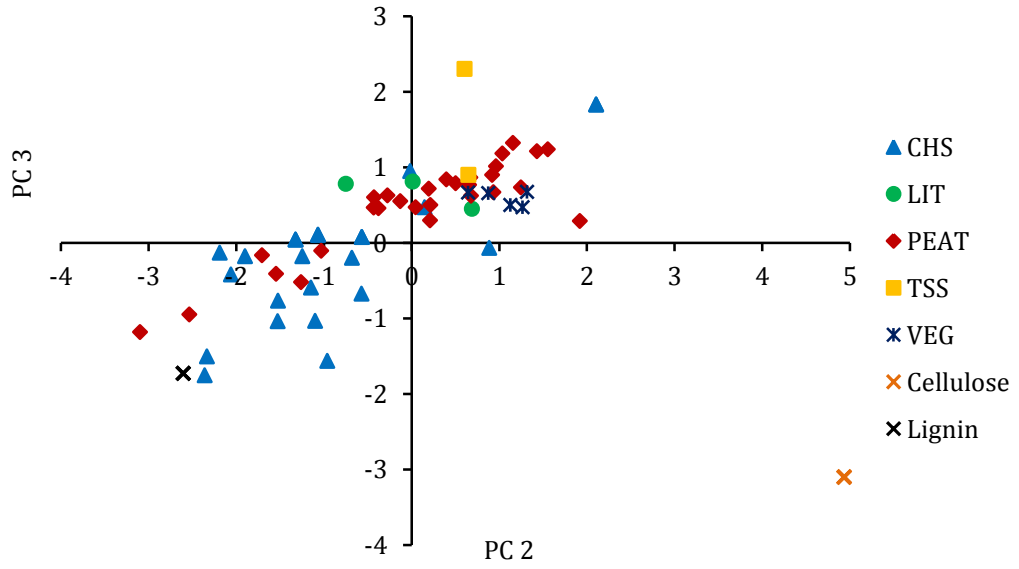


Figure 5.19. PC2 against PC3, showing CHS, cellulose, lignin and the source materials.



Another PCA was carried out on this data using only the elemental composition data, and including the molar composition data for protein ($C_1H_{1.1}O_{0.16}N_{0.27}$) from Baldock et al. (2004) and the measured data for humic acid, as

standards. These could not be included in the previous PCA as they do not have measured TGA data, EXO 1 or EXO 2.

The results of this PCA showed that 97% of the variance could be explained by the first four principal components, three with eigenvalues of greater than one, and another one with an eigenvalue of less than 1 (Table 5.12).

The graph of PC1 and PC2 (Figure 5.20) shows similar results to the previous PCA (Figure 5.18). The variables that influence the principal component (with the largest and smallest values) show that the first component is characterised by OR, hydrogen, C_{ox}, and O:C, with the negative loadings attributed to high OR and hydrogen, and the positive due to high C_{ox} values and O:C ratios. Along this axis, the protein and humic acid samples fall close to the CHS samples on the negative side, while the other source materials generally fall on the positive side.

Table 5.12. The results of the PCA on CHS, source materials, humic acid and protein, showing the principal components with eigenvalues > 1 and the first component with an eigenvalue < 1.

Variable	PC1	PC2	PC3	PC4
Carbon	0.28	0.15	-0.48	0.63
Hydrogen	0.38	0.36	0.18	0.27
Oxygen	-0.17	0.57	-0.07	0.21
Nitrogen	-0.25	-0.33	0.35	0.53
C:N	0.35	0.07	-0.42	-0.40
H:C	0.21	0.35	0.55	-0.15
O:C	-0.29	0.49	0.13	-0.11
C_{ox}	-0.49	0.06	-0.16	-0.07
OR	0.44	-0.21	0.29	0.00
Eigenvalue	3.88	2.61	1.59	0.68
Cumulative variation explained (%)	43	72	90	97

The second component is characterised by nitrogen, OR, oxygen, and O:C, with the positive loadings due to high oxygen and O:C, and the negative loadings due to high nitrogen content and OR (Figure 5.20). The protein sample had the highest values for both nitrogen and OR, and the lowest oxygen content and O:C ratio. The humic acid standard falls close to the CHS samples; this is not surprising as the

elemental composition of the humic acid was most similar to the CHS elemental composition.

The graph of PC2 and PC3 also showed the protein to be separate from the other samples, as it had very low loading (extremely negative) for PC2 (as described above), and high positive loading for PC3 (Figure 5.21). The third component is predominantly characterised by carbon, C:N, H:C and nitrogen content, with the positive loadings due to high nitrogen and H:C ratio, and the negative loadings due to high carbon and high C:N ratio. Protein has low carbon and low C:N ratio, and a high nitrogen content, and so falls separate from the other samples. The humic acid standard falls close to the peat and CHS samples, suggesting a similar elemental composition.

The relationship with oxidation could also be a part of the mechanism for DOC release, and therefore not the result of the difference between the source materials and river C, but the overall cause of the differences.

Figure 5.20. PC1 against PC2 for the PCA on the elemental composition data, including humic acid and protein data.

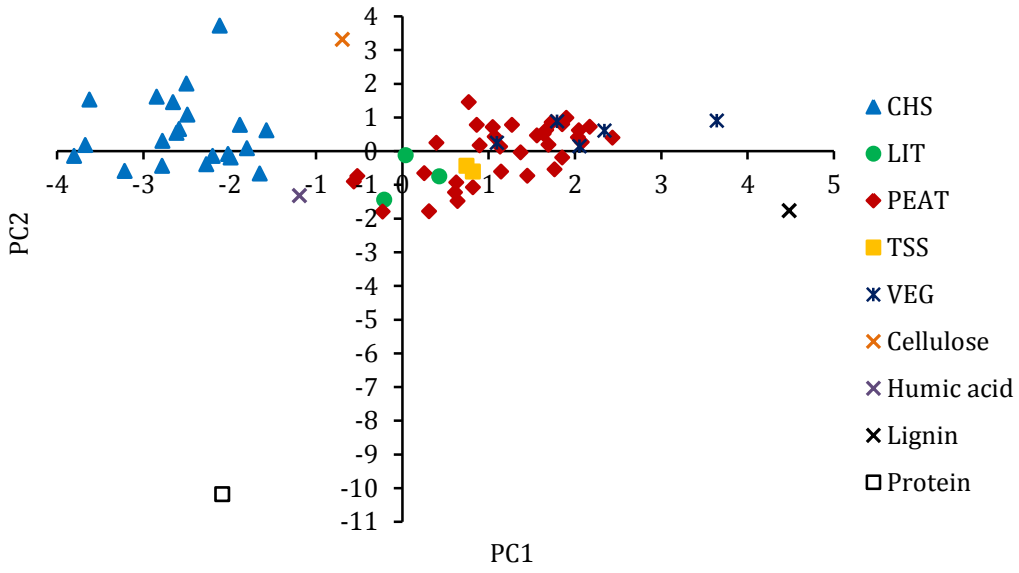
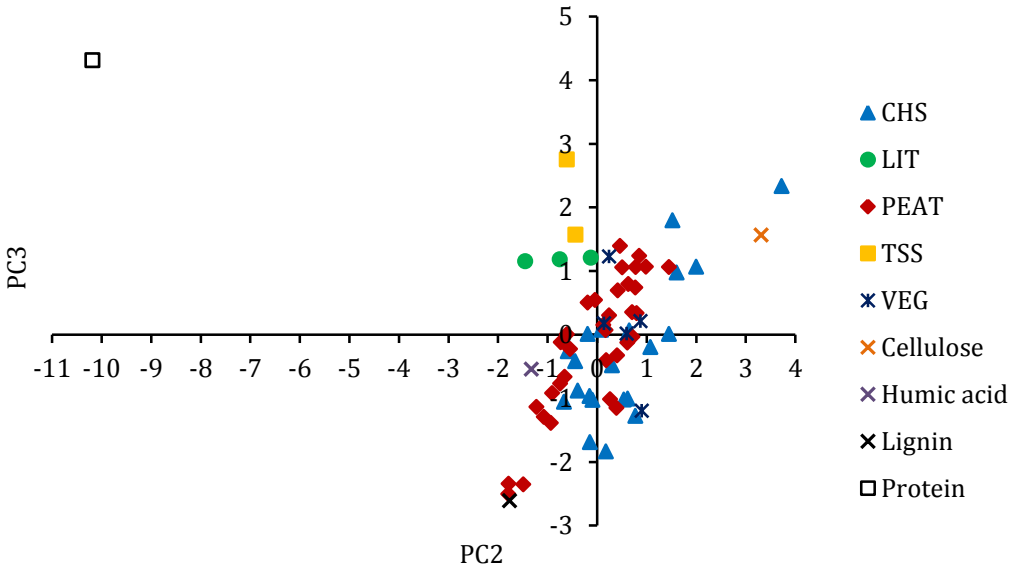


Figure 5.21. PC2 against PC3 for the PCA on the elemental composition data, including humic acid and protein data.



5.3.4. Degradation and composition

The average initial rates of degradation in the light (photo and bio- rate of change of DOC during the first hour of the degradation experiments) for the three TDS sites were 13.74 mg C/l/hour for CHS, 1.45 mg C/l/hour for DBS and 3.16 mg C/l/hour for MUN for the months considered by this experiment. An ANOVA on the data showed that the rates were significantly different between the sites (p=0.0001) and months (p=0.0018), but not the interaction of Site*Month (Table 5.13). Site explained 23% and Month explained 30% of the variation. The rates of degradation were slightly lower in the spring and summer months (from March to September, with the exception of July). An ANCOVA on the rate data, using composition and water data as covariates, found that the molar concentrations of carbon, nitrogen, hydrogen and oxygen, and the ratio of C:N, in addition to the month factor, were significant covariates (Table 5.13) Site was no longer significant, which is not surprising as the five covariates are all significantly different between the sites, and so account for the differences.

Table 5.13. The results of the ANOVA and ANCOVA on initial rates of degradation in the light.

	Without covariates		With covariates	
Factor/covariate	p	ω^2	p	ω^2
Nitrogen	na	-	0.0045	5.40
Carbon	na	-	0.0054	5.11
Hydrogen	na	-	0.0003	5.93
Oxygen	na	-	0.0043	5.46
C:N	na	-	0.0005	9.05
Site	0.0001	22.94	ns	-
Month	0.0018	30.20	0.0001	44.35
Error		12.62		5.35

The regression analyses were performed separately on each site, to take advantage of all the variables measured at each site. The regression equations for the three sites were:

CHS:

$$Rate_0 = 25.05[H] + 235.81OR - 10.51\left(\frac{N-alkyl}{DOC_0}\right) - 306.38$$
$$(10.02) \quad (146.27) \quad (2.42) \quad (150.68)$$

n=11, $r^2 = 0.83$ *(Eq. 5.3)*

DBS:

$$Rate_0 = -5.81[H] - 14.29pH - 35.88[O] + 272.20$$
$$(2.27) \quad (3.24) \quad (8.80) \quad (52.63)$$

n=15, $r^2 = 0.77$ *(Eq. 5.4)*

MUN:

$$Rate_0 = 0.32[Cl^-] - 1.39\left(\frac{[H]}{[C]}\right) - 21.47$$

(0.10) (1.10) (10.87)

n=8, r^2 = 0.79 *(Eq. 5.5)*

where $Rate_0$ is the rate of DOC degradation in mg C/l/hour, *[H]*, *[O]* and *[C]* are the molar concentrations of hydrogen, oxygen and carbon, *OR* is the oxidative ratio, *N-alkyl* is the intensity of the N-alkyl peak, DOC_0 is the t_0 DOC concentration (mg C/l), *pH* and Cl^- are the initial pH and chloride ion concentration of the water. The numbers under each parameter are the standard errors. The small n values were due to few samples having a complete data set, especially for MUN where the degradation rates were only measured every other month in 2013.

For the CHS samples, the initial rate of degradation was higher than the DBS and MUN DOC samples, and was found to be dependent on the hydrogen content, OR, N-alkyl group abundance and the initial DOC concentration (Eq. 5.3). The relationship between the initial rate of DOC degradation in water and the hydrogen content and OR of the TDS was positive, suggesting as these increased, so did the initial rate of DOC degradation. These two parameters were higher in reduced samples. The relationship between the rate and the N-alkyl abundance relative to the initial DOC concentration was negative, which suggests that as the abundance of N-alkyl functional groups increased, the rate of degradation decreased. These relationships pointed to the faster rates of DOC degradation being attributed to TDS samples that were more reduced, and lower rates were attributed to samples that were more oxidised, with lower hydrogen contents and lower OR.

For the DBS samples, the initial rate of degradation was shown to be dependent on the hydrogen and oxygen contents of the TDS, and the pH of the water (Eq. 5.4). The relationship between rate and all of these factors was negative, suggesting that as the hydrogen and oxygen content and pH of the water decreased, the rate increased. The rate of degradation at CHS was higher than at DBS, and the pH at CHS was much lower than at DBS, and so the negative relationship between rate and pH was not unlikely. The relationship between rate and hydrogen content was the opposite of that found for the CHS samples, and showed that the more

reduced samples (higher H content) had lower rates of degradation. However, the relationship between rate and the oxygen content had a larger parameter estimate, and so affected the rate of degradation more than the hydrogen content, which suggested that the more oxidised TDS samples (higher O content) had lower rates of degradation, which was similar to the CHS samples.

The rate of DOC degradation in the MUN samples was found to be dependent on the H:C ratio of the TDS and the chloride concentration of the water (Eq. 5.5). The relationship between the rate and the chloride concentration was positive, suggesting that the higher the chloride concentration, the higher the rate of degradation. This relationship did not hold for the other two sites, as CHS had the highest rate of degradation but the lowest chloride concentration, and DBS had an intermediate chloride concentration and the lowest rate of degradation. The negative relationship between the MUN rate of DOC degradation and the H:C ratio showed that the higher the hydrogen and the lower carbon content of the TDS, the lower the rate of degradation. This was similar to the DBS and hydrogen relationship. The CHS TDS samples had the lowest H:C ratios of the TDS samples, and had the highest rates of degradation, and so this relationship could be applied to the other sites.

The n value for equation 5.3 was limited by the availability of ^{13}C NMR data, and so another regression was carried out using only variables available for the majority of the samples.

CHS, using only variables available for all samples:

$$Rate_0 = 0.80TGA - 3.54\Delta H - 55.77C_{ox} + 0.68\left(\frac{[C]}{[N]}\right) + 31.39$$

$$\qquad (0.41) \qquad (1.44) \qquad (27.42) \qquad (0.50) \qquad (23.55)$$

n=20, $r^2 = 0.39$ *(Eq. 5.6)*

where *[N]* is the molar concentrations of nitrogen, C_{ox} is the carbon oxidation state, TGA is the final weight of the sample after heating to 1000 °C (%), ΔH is the gross heat of the sample (MJ/kg) and all other terms are as described above. This equation shows that as the C_{ox} and gross heat decrease, and the C:N ratio and final weight increases, the rate of DOC degradation will increase.

The final weight was a proxy measurement related to the total organic content of a sample, and so samples with a high final weight percent contained more material that would not burn, and were therefore composed of more recalcitrant matter. However, within the CHS samples, the range of final weights was strongly negatively correlated (r^2=0.65) with the percentage weight loss in the EXO 2 range (400-500 °C), which was attributed to the recalcitrant pool, with aromatic-rich C and lignin components (Lopez-Capel et al., 2008). This correlation suggested that samples with a low EXO 2 had a high final weight, and therefore a high initial rate of DOC loss.

The relationship between initial rate and gross heat was negative, showing that as the gross heat of a sample increased, the initial rate decreased. Samples with higher gross heats have more complex structures that require greater energy to break down, and so these samples were less available to bio- and photo-degradation and had lower initial rates of degradation as a result.

The C_{ox} had a strong negative correlation with the hydrogen content, indicating that samples with high hydrogen contents had lower C_{ox} values and were more reduced than oxidised. In equation 5.3, there was a positive relationship between the initial rate and the hydrogen content, relating to the reduction of the TDS; a similar relationship existed in equation 5.6, as the relationship between C_{ox} and initial rate was negative, but the correlation between hydrogen and C_{ox} was also negative, implying a positive relationship between hydrogen content and initial rate. Also, in equation 5.3, there was a strong positive relationship between initial rate and the OR of the samples. C_{ox} and OR are strongly negatively correlated, so if initial rate should have a positive relationship with OR, it would have a negative relationship with C_{ox}.

The positive relationship between initial rate and C:N (Eq. 5.6) showed that when the nitrogen content of the sample was low, and the carbon content was high, there was a higher initial rate of DOC degradation. This was similar to the result of equation 5.5, which found a low carbon content resulted in a lower rate of degradation.

As in equation 5.3, these relationships generally point to the faster rates of DOC degradation being attributed to TDS samples that were more reduced, and lower rates could be attributed to samples that were more oxidised, with higher C_{ox} and gross heats. The high reaction speeds of the breakdown of TDS by light exposure and microbes may explain the differences between the source materials

and the headwater TDS, as they may have already started working on it and changed the composition very quickly.

The regression analyses (Eq. 5.3-5.6) showed which compositional covariates were significant in determining the rate of the DOC bio- and photo-degradation. This and the results of the PCA in sections 5.3.2 and 5.3.3 showed the importance of using multiple methods of analysis, as the significant variables came from the Elemental Analysis, ^{13}C NMR, TGA, Bomb Calorimetry and water analysis.

5.3.5. Discussion

The main finding of sections 5.3.2, 5.3.3 and 5.3.4 was the potential oxidation pathway of the organic material as it travels downstream, from the least oxidised matter in the peat, vegetation and litter, to the intermediate oxygen content of the TDS from CHS, and the most oxidised material, the TDS from DBS and MUN. Even though there were no measurements of the elemental composition of the DOC in previous chapters, this finding links with trends shown in the variables that can be used as proxies for DOC composition, the absorbance at 400 nm, the specific absorbance and the E4:E6 ratio.

The results of section 5.3.4 in particular highlight the role that redox plays in the degradation of DOC, with the more oxidised samples (as found at DBS and MUN) having lower degradation rates. This relationship holds for the samples from CHS as well, with the individual samples with higher oxygen contents having lower rates of DOC loss.

Numerous papers have used many of the same analytical methods as in this chapter in various combinations, e.g. Abdulla et al. (2010b) used Elemental Analysis, $\delta^{13}C$, FTIR, ^{13}C NMR and DOC concentration; and Reiche et al. (2010) used Elemental Analysis, Pyrolysis GC/MS and TGA. Poirier et al., (2005), point out the advantages of using several methods, and themselves use Elemental Analysis, Pyrolysis GC/MS, FTIR and ^{13}C NMR to compare composition of soil organic matter fractions. It is certainly not a new idea to use several techniques in one paper; Levesque and Dinel (1978) used Elemental Analysis and Bomb Calorimetry to look at the composition of peats and vegetation. Chadwick et al. (2004) and Masiello et al (2008) presented a method for using Bomb Calorimetry data to calculate oxidative ratios, and Davis et al. (1999) showed correlations between peak heights from FTIR and the O:C and E4:E6 ratios of organic compounds.

The PCAs in this chapter used variables from the different analysis methods, but there was little specific comparison of the different analytical techniques. In order to compare the techniques, correlations between all the data, regardless of type of organic matter, were identified, and some examples are displayed in Figure 5.22. All the correlations shown were significant ($p<0.0001$), and had r^2 of at least 0.66. The purpose of these correlations was to identify simply the factors that show similar patterns across all the sites in this chapter, such as the pH and molar concentrations of oxygen and carbon, which show opposite trends: samples with high oxygen content have higher pHs, and samples with higher carbon concentrations have lower pHs. The correlation between oxygen concentration and TGA final weight shows that the samples with a higher oxygen concentration had higher final weights, so that not as much of the matter was lost in heating. The correlation between carbon concentration and the TGA final weight again shows the opposite; samples with higher carbon content had lower TGA final weights, suggesting more mass was lost. The gross heat values correlate negatively with C_{ox} and positively with the carbon content, showing that the gross heat of a sample increased with a high carbon content and decreased with a high C_{ox} value. The correlation between absorbance at 400 nm and both EXO 1 and carbon content was positive, with both increasing as absorbance increased. The sulphate and nitrate concentrations both correlate positively with the O:C ratio, showing as the anions increase, so does the O:C ratio. These correlations nearly all include one or more of the variables from the elemental analysis (carbon, oxygen, C_{ox}, O:C), suggesting that this method of analysing samples was the most useful.

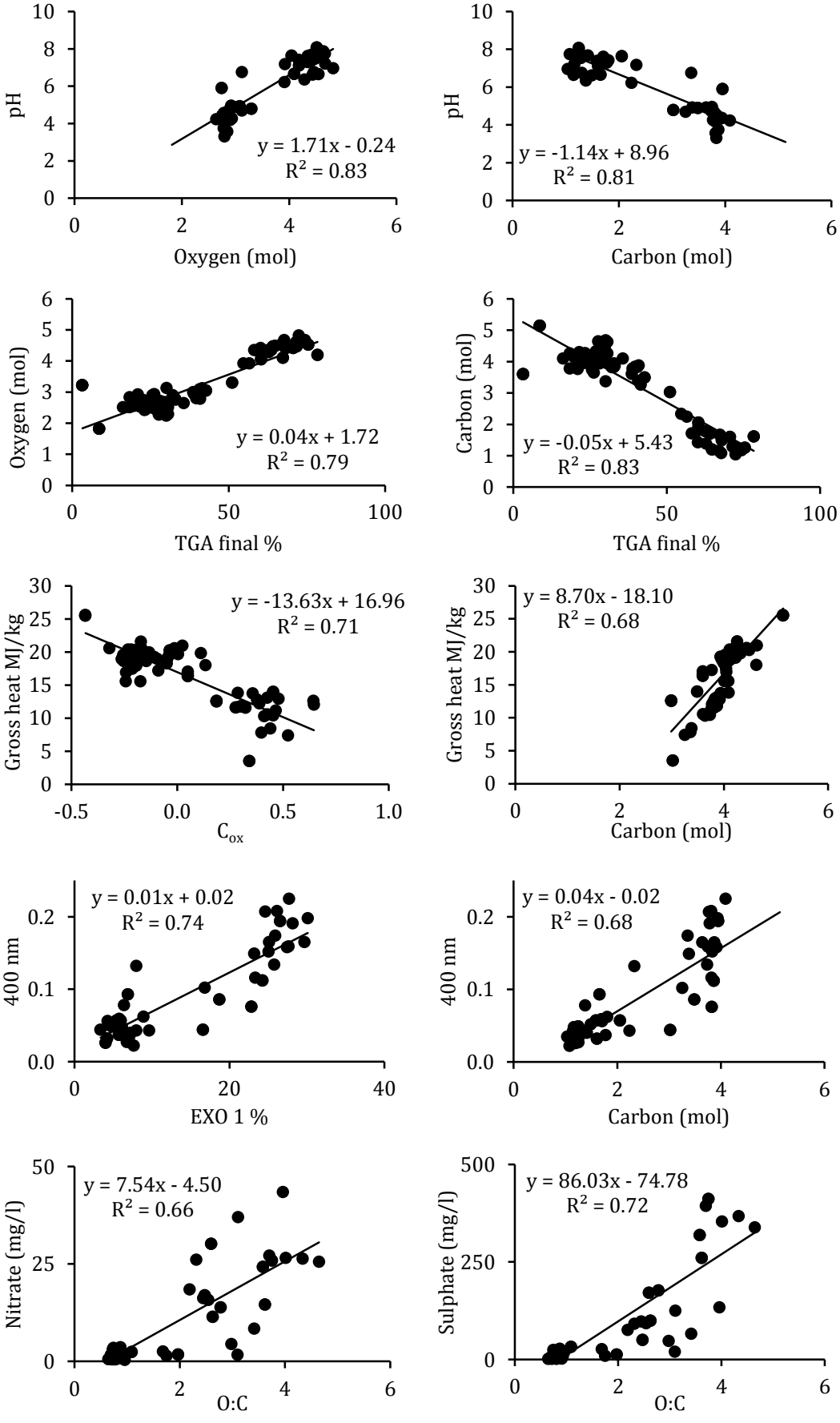
The changes in composition between the CHS and DBS TDS show that as the DOC travels downstream it loses carbon and gains oxygen and nitrogen. It becomes more resistant to thermal degradation, as the DBS samples lose smaller proportions of their weight during the EXO 1 and EXO 2 temperature ranges, and have higher final weights from the TGA than the CHS samples. This chapter has attributed these compositional changes to the in-stream processing of organic carbon that occurs in the river. However, it is possible that none of the DOC released at CHS reaches DBS, and the DOC measured there is actually the result of release from POC, in-stream production and anthropogenic sources of DOC. As outlined in Chapter 1, release of DOC from POC can be caused by photo- and bio-degradation (Billett et al., 2010; Pawson et al., 2006). In-stream production of DOC in headwater streams is generally low (Cole and Caraco, 2001; Eatherall et al., 2000), but production of DOC

further from the source could contribute to the total C pool in the water. Dawson et al. (2001b) state that as stream order increases, the sources of organic carbon change from being dominated by terrestrial inputs to being the products of in-stream primary production and organic carbon transported from upstream.

Anthropogenic sources of DOC, such as waste water treatment (Kempe, 1984), can contribute to the DOC in the water between the two sites on the River Tees, and so the differences in composition could be due to the DOC being from a different source rather than degradation in the water. Raymond and Bauer (2001b) suggest that differences in the composition in terrestrially derived organic matter could be due to the extensive modification and remineralisation that takes place in rivers, rather than being derived from a different source. It would be interesting to analyse the composition of the TSS from DBS and MUN sites as this may help to explain the changes in the TDS, however the organic content of this is likely to be so low that several of the analytical techniques used in this chapter may not yield any useable data for these samples.

One draw-back of the DOC degradation rates analysis is that some of the rates were derived from Chapter 2, which looked at the daily, rather than hourly, rates of DOC change. These daily rates were divided by 24 in order to get an hourly rate; however this assumes a constant rate of degradation over the whole 24 hours, which Chapters 3 and 4 have shown is not likely. This means that the rates derived from this method are probably underestimates, as there would have been approximately 12 hours of darkness in that first day of Chapter 2 experiments when the change in DOC concentration would have been negligible and therefore the daily rates will represent the rates for the 12 hours of daylight only.

Figure 5.22. Examples of correlations between data from different analytical methods.



5.4. Conclusion

Comparing the source and downriver TDS showed that the composition changed significantly over the course of the river, which ties in to the results of the previous chapters (2, 3 and 4) that suggest that the DOC becomes less degradable and more recalcitrant over time. The changes in composition could explain this reduction in degradability, as the material has been 'processed' by light exposure and the microbes in the river.

Comparing the headwater TDS to source materials showed that the composition of the TDS was significantly different to all source materials, and it was consistently more oxidised than the other materials. This means either that is derives from another, unmeasured, source, or that it is a complex mixture of material from all the sources. The relationship with oxidation could also be a part of the mechanism for DOC release, and therefore not the result of the difference between the source materials and river C, but the overall cause of the differences.

Comparing the initial rates of degradation in the light to the composition found that different factors were relevant at different sites, so one model could not be found to explain the variation at all sites. The ANCOVA and regression analyses were dominated by the elemental composition data, suggesting that the molar compositional changes in the TDS as it moves downstream are primarily responsible for the differences in the DOC degradation rates. The molar compositional changes reflect an oxidation pathway, with the more oxidised samples (as found at DBS and MUN) having lower degradation rates.

Chapter 6:
Impact of nutrient-addition on DOC degradation

6.1. Introduction and Aims

The aim of this chapter was to investigate whether the biological degradation of DOC is limited by the nutrient concentration of the river water, as suggested in Chapter 4, and by Bengtsson and Torneman (2004), Evans et al. (2005), Kling et al. (1991), and Marschner and Kalbitz (2003). The photodegradation of DOC is unlikely to be affected by the nutrient concentrations.

As noted in Chapters 2 and 4, after 10 days there is still DOC present in the samples, even with rapid changes within the first 2 days. One possible reason for the decline in DOC degradation rates and the lingering presence of DOC is that there are insufficient nutrients for the biota to utilise to degrade the DOC faster. If the DOC biodegradation was limited by nutrient availability, adding nutrients to the water should increase the rate of degradation, as well as decreasing the final DOC concentration. In addition to a nutrient solution being added at the beginning of the experiment for comparison to a no-nutrient control experiment, a batch of water had nutrients added after 24 hours, to find whether this caused an increase in the rate of degradation after the initial fast rate of DOC loss had slowed (as found in previous chapters). The initial rate of DOC loss may have slowed due to the biota running out of available nutrients, and so adding more would allow an increase in the rate of biodegradation of DOC.

If this method was successful at decreasing the final DOC concentrations to almost zero, it could be deployed as a safer method of DOC removal from drinking water, as exposing the water to light and adding a nutrient solution could be safer and more effective at removing DOC than the methods currently used by water treatment companies.

6.2. Approach and Methodology

This chapter adapts the method of Chapters 2, 3 and 4, to conduct in-situ degradation measurements of DOC from the headwater and former tidal limit of the River Tees in North-East England over periods of up to 70 hours, with a nutrient solution added at the beginning and after 24 hours of each experiment.

6.2.1. Field sites

This chapter used the two River Tees sites used in Chapter 4: Cottage Hill Sike and Broken Scar in Darlington. These are the source water within Moor House NNR and the former tidal limit, respectively (CHS and DBS; Figure 2.1, Table 2.1). If nutrient limitation is the cause of the decline in degradation rates then adding nutrients may have a relatively large impact on fresh samples from the headwater, which can be considered highly degradable, in comparison to those from the outlet of the Tees which may be considered highly degraded already.

6.2.2. Degradation measurements

The degradation measurements were made as in Chapters 2 and 4, outside of the laboratory in ambient light and temperature conditions (rather than indoors under artificially controlled conditions).

Water samples were taken on a bi-monthly (every other month) basis from the two sites on the River Tees. Each degradation experiment spanned approximately 70 hours with sacrificial sampling taking place at hours 0, 1, 2, 8, and then at dawn and dusk on days 2, 3 and 4, with three nutrient treatments:

1. B = No nutrients added, control
2. N_0 = Nutrient added at t_0
3. N_{24} = Nutrient added at t_{24}

The nutrient solution contained 0.1 M of potassium nitrate (KNO_3) and tri-calcium phosphate ($Ca_3O_8P_2$) in deionised water, and 0.1 ml of the solution was added to each water sample. The nutrient solution concentration was calculated to ensure the nutrients were not limiting based upon elemental compositions measured in

Chapter 5, and a small volume of solution was used so as to not to dilute the river water.

All three treatments were kept in the light, so they experienced the full day/night cycle. Replicates were included within each degradation experiment where equipment availability allowed. No hour 0 samples were replicated, but 22% of all other measurements were replicated (88 of 407 samples). As in Chapter 4, a data logger with a PAR (photosynthetically active radiation) meter and thermocouple recorded the radiation levels and air temperature at 15-minute intervals throughout the 70-hour period of each experiment. Radiation and temperature conditions were summarised as the average conditions over the period for each sample and PAR measurements were summed to give the total radiation experienced by any one sample.

The first day of the experiment was conducted at the field sites so the samples were exposed to the same light and temperature conditions as the river. At dusk all tubes were taken to the laboratory and placed outside so they would continue to experience natural light and temperatures with on-going monitoring of these conditions.

6.2.3. Sample analysis

Samples were analysed for DOC, absorbance at 400, 465 and 665 nm, E4:E6, pH, water temperature, conductivity, anion concentration and acidity/alkalinity as in Chapter 4.

Suspended sediment (SS) concentration in each experiment was measured in samples at the beginning, in the middle (at approximately 30 hours) and at the end of each experiment. Samples were filtered through pre-weighed, 0.45 µm, glass fibre filters; dried to 105 °C and the filter paper re-weighed to give the concentration of suspended sediment. The filter papers were then put in a furnace for 4 hours at 550 °C, and then re-weighed. As in previous chapters, the mass lost in the furnace equates to the mass of particulate organic matter (POM), and 47.5% of this was assumed to be particulate organic carbon (POC).

6.2.4. Statistical methodology

The design of the experiment incorporates four factors: Month, Time, Site and Treatment. Each factor has a number of levels: Month has five levels (one for each month sampled); Time has 11 levels (hour 0, 1, 2, 3.75, 9.67, 20.4, 24, 29.5, 42.6, 54.39, 67.67); Site has two levels (CHS and DBS); and Treatment has three levels (B, N_0 and N_{24}). The sample times are the averaged values (each has a standard error) that represent the samples taken on the first day (hour 0, 1, 2, 3.75, 9.67, called t_0, t_1, t_2, t_4 and t_{10}), dawn and dusk on day 2 (hour 20.4 and 29.5), dawn and dusk on day 3 (hour 42.6 and 54.39) and dawn on day 4 (hour 67.67, called t_{70}). Additionally, as nutrient solution was added at hour 24, (called t_{24}) samples were also taken then too. Only four sampling times were exactly the same each month: t_0, t_1, t_2 and t_{24}, and so these four times had no standard error.

Statistical analysis was carried out on the nitrate and phosphate concentrations to show that adding the nutrient solution had caused a change in the nitrate and phosphate concentrations, and to check that the nutrient solution was consistent throughout the experiments.

The concentrations of DOC and POC were checked for normality, and transformed if necessary, and analysed in both absolute and relative terms where the relative value for each sample in an experiment was expressed as the ratio of the measured value to the measurement at t_0 for the same site on that experimental run. The magnitude of the effects and interactions of each significant factor and interaction were calculated, as in previous chapters. For some analyses, the two sites were analysed separately due to the differences between them; often this resulted in better r^2. Main effects plots were used to visualise the data as they show the overall effect of the factor, once all other factors and covariates have been taken into account. The month number was transformed into the sinusoidal function for the regression analysis, as in previous chapters.

As in previous chapters, the initial rates of DOC degradation were calculated, as were the day and night rates for each treatment. Specific to this experiment, the day 2 rates were investigated to see if adding nutrients at t_{24} changed the rate of DOC degradation during that stage of the experiment. Also, the 'nutrient-induced' change in DOC and POC concentrations and 'nutrient-induced' rate of DOC degradation were calculated by subtracting the B treatment values from the N_0 values. Analysis was carried out on the final DOC and POC concentrations to see if

adding nutrients had changed these, relative to the no-nutrient control samples. The rate of DOC degradation in the time following the addition of nutrients to the N_{24} samples was calculated to investigate whether there was a difference from the rate of change before the nutrients were added, and also compared to the same time in the B treatment samples.

6.3. Results and Discussion

In total, there were 373 individual experiments conducted, with complete covariate information and within the context of the factorial design. Summary of the water chemistry at the two sites over the 70 hours of the experiment for treatment B (no nutrients) is given in Table 6.1.

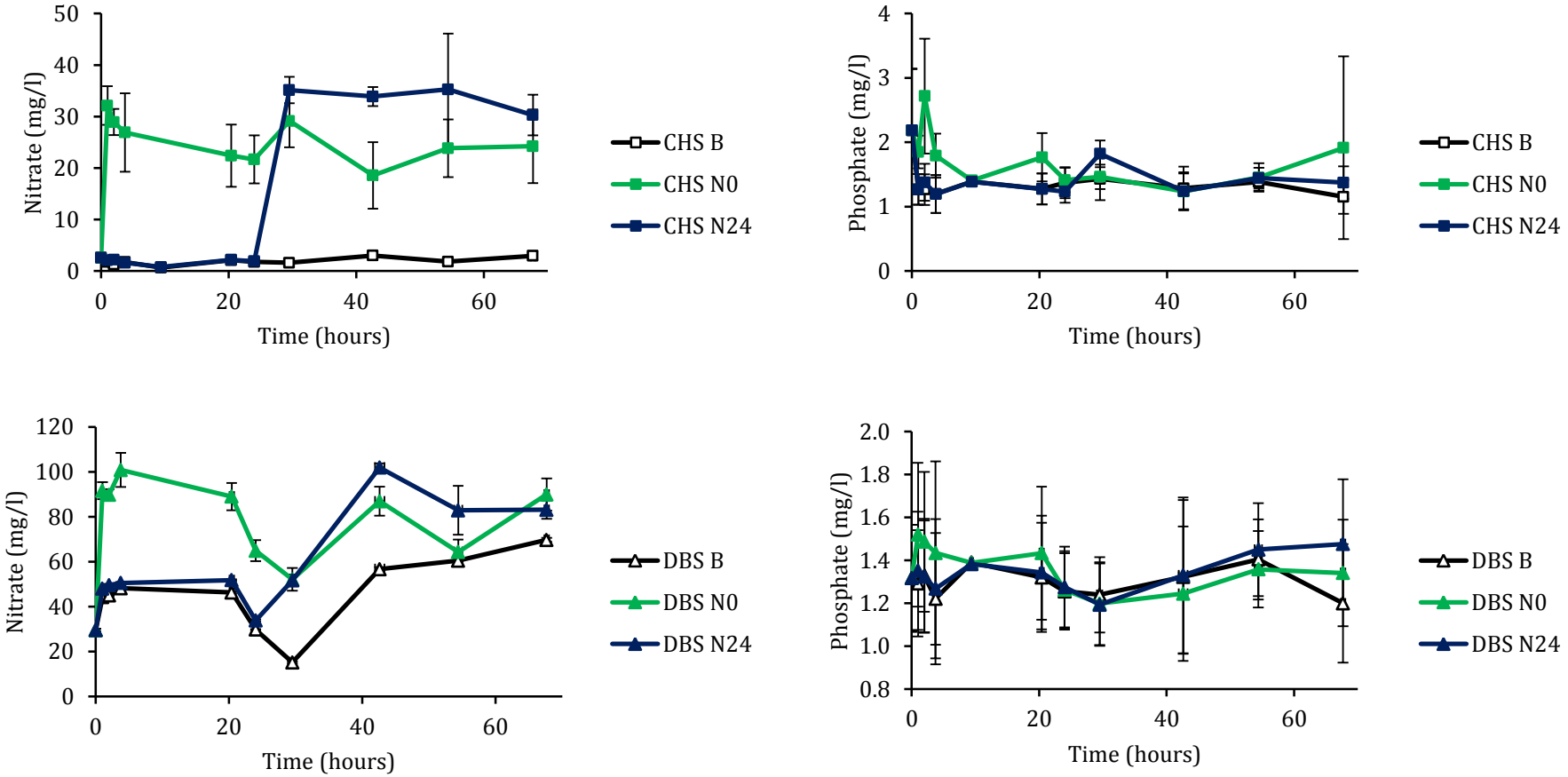
Some of the covariates showed the same pattern at both of the two sites between t_0 and t_{70}, as shown in Table 6.1. The absorbance at 400 nm and bromide concentration decreased, and the conductivity, pH, chloride and fluoride concentrations increased. The E4:E6 ratio and sulphate concentration decreased at CHS and increased at DBS. Of these changes, only two were significantly different between t_0 and t_{70} across both sites: the conductivity ($p=0.0196$) and pH ($p<0.0001$) increases at CHS.

The concentrations of the conservative ions (bromide and chloride) were investigated further to show how much natural variability there was in the dataset. An ANOVA on the whole dataset showed there were significant differences between Treatment, and the interaction of Site*Treatment for the bromide concentrations, and between Site for the chloride concentrations, with DBS having significantly higher chloride concentrations than CHS. Post hoc tests showed that the bromide differences were due to the N_0 treatment being significantly different to both the B and N_{24} treatments. It is unclear why adding a NPK nutrient solution to the water would significantly affect the concentration of a conservative ion such as bromide. Also if the nutrient solution affected the bromide concentration in the N_0 treatment, there is no clear reason why it would not also affect the bromide concentration in the N_{24} treatment once it had been added to that water.

Table 6.1. The average and coefficient of variation (CV - %) of the 12 variables measured from the two sites (CHS and DBS), averaged across all five sampling months. The table shows the initial (t_0) and end (t_{70}) concentrations for each variable from the B (no added nutrients) treatment.

	Cottage Hill Sike (CHS)				Broken Scar (DBS)			
	t_0		t_{70}		t_0		t_{70}	
Determinant	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
POC (mg C/l)	3.68	71	3.59	25	3.14	68	3.18	52
Conductivity (µS/cm)	40.76	25	64.43	32	641.40	58	821.50	21
pH	4.09	16	6.47	5.4	6.94	7.7	7.48	5.5
DOC (mg C/l)	44.44	31	4.82	79	32.50	22	12.97	75
Abs_{400}	0.14	35	0.11	36	0.07	75	0.03	45
E4:E6	5.77	37	4.25	40	2.71	78	2.73	19
Bromide (mg/l)	1.12	56	0.73	86	1.24	57	0.93	78
Chloride (mg/l)	9.64	49	10.18	75	76.29	95	114.64	29
Fluoride (mg/l)	0.24	223	0.50	110	0.27	224	0.58	101
Nitrate (mg/l)	2.58	48	2.93	75	29.48	120	69.68	111
Phosphate (mg/l)	2.18	98	1.15	56	1.32	42	1.20	56
Sulphate (mg/l)	13.84	80	10.71	41	60.93	42	103.25	43

Figure 6.1. The average nitrate and phosphate concentrations at the two sites for the three treatments. The error bars are the standard errors.



6.3.1. Nutrient concentrations

The nutrient solution added both nitrate and phosphate to the water at t_0 and t_{24}, and the results clearly reflect this. The concentrations of both increase immediately in the N_0 treatment, and both show an increase after t_{24} in the N_{24} treatment, while the B treatment with no nutrients shows no changes throughout the experiment time (Figure 6.1). However, the phosphate concentration quickly returns to the same level as the B treatment at both sites, suggesting that it is utilised very quickly by in-stream biota.

An ANOVA showed that the added nutrients significantly increased the nitrate ($p<0.0001$) and phosphate ($p=0.0069$) concentrations at both sites. There were also significant differences in the nitrate concentration between the treatments with nutrients added at the beginning (N_0) and those with nutrient added at 24 hours (N_{24}). However, the first samples after the nutrient solution was added were not significantly different from each other; i.e. the t_1 samples from the N_0 treatment were not significantly different from the t_{30} samples from the N_{24} treatment showing there were no significant differences in the actual nutrient solutions between addition times and months. The interaction of Treatment*Site was not significant, suggesting that the nutrient concentrations changed in the same way at both sites.

The effect of adding nutrients on the conductivity and pH were investigated further. At CHS, there was a significant effect of treatment and sample time on the conductivity (with the model explained 77% of the total variance, and Treatment, Time and the interaction of Treatment*Time explaining 26, 29 and 17% of the variation respectively). The only significant differences in the pH were between sample times, so not linked to the nutrient additions. At DBS there were no significant differences in the conductivity or the pH of the nutrient additions at either time.

6.3.2. DOC concentrations

The average DOC concentration for all sites and all treatments decreased during the 70 hours of each experiment (Figure 6.2). The decline followed a roughly diurnal cycle, with a large decrease in DOC concentration during the first day, between t_0 and t_{10}, followed by a lower rate of decrease over the rest of the experiment. For

CHS B samples, the DOC concentration fell to just 35% of the starting concentration by t_{10}, and decreased by another 18% over the last 60 hours, to a final concentration of 17%. As in Chapter 4, the decline in DOC concentration was not as dramatic for DBS samples as it was for CHS, but still the DOC declined to 40% of the initial concentration (DBS B samples). The average decline in DOC concentration across all five months for all sites for all B samples was from 37.63 to 8.89 mg C/l after 70 hours: when concentrations were judged relative to the t_0 concentration at each site, the average decline over 70 hours was 76%. For N_0 the decline was 74%, for N_{24} it was 72%. The graphs in Figure 6.2 show that the DOC concentration did not decrease as rapidly in the N_0 treatment as compared to the others, for either site, and there appears to be no difference in the concentrations between the treatments after 40 hours.

The ANOVA on the relative DOC concentrations showed that all four factors were significant, along with four interactions: Site*Time, Site*Month, Treatment*Month and Time*Month. The factor that explained the largest proportion of the variance was the Time of the experiment, showing that the DOC concentrations change significantly with time. Adding covariates found that fewer factors and interactions were significant (Table 6.2), but three covariates were significant. The DOC_0 (the initial DOC concentration), POC_0 (the initial POC concentration) and phosphate concentrations explained approximately 2% of the variation each. Site, Treatment and Month were no longer significant; this is likely due to the inclusion of covariates that differed between the sites, treatments and months. Collectively, the covariates explained 6% of the variation, a larger proportion than the Site and Treatment factors explained in the ANOVA.

Figure 6.2. The average relative DOC concentration for the three treatments at the two sites. The error bars are the standard errors.

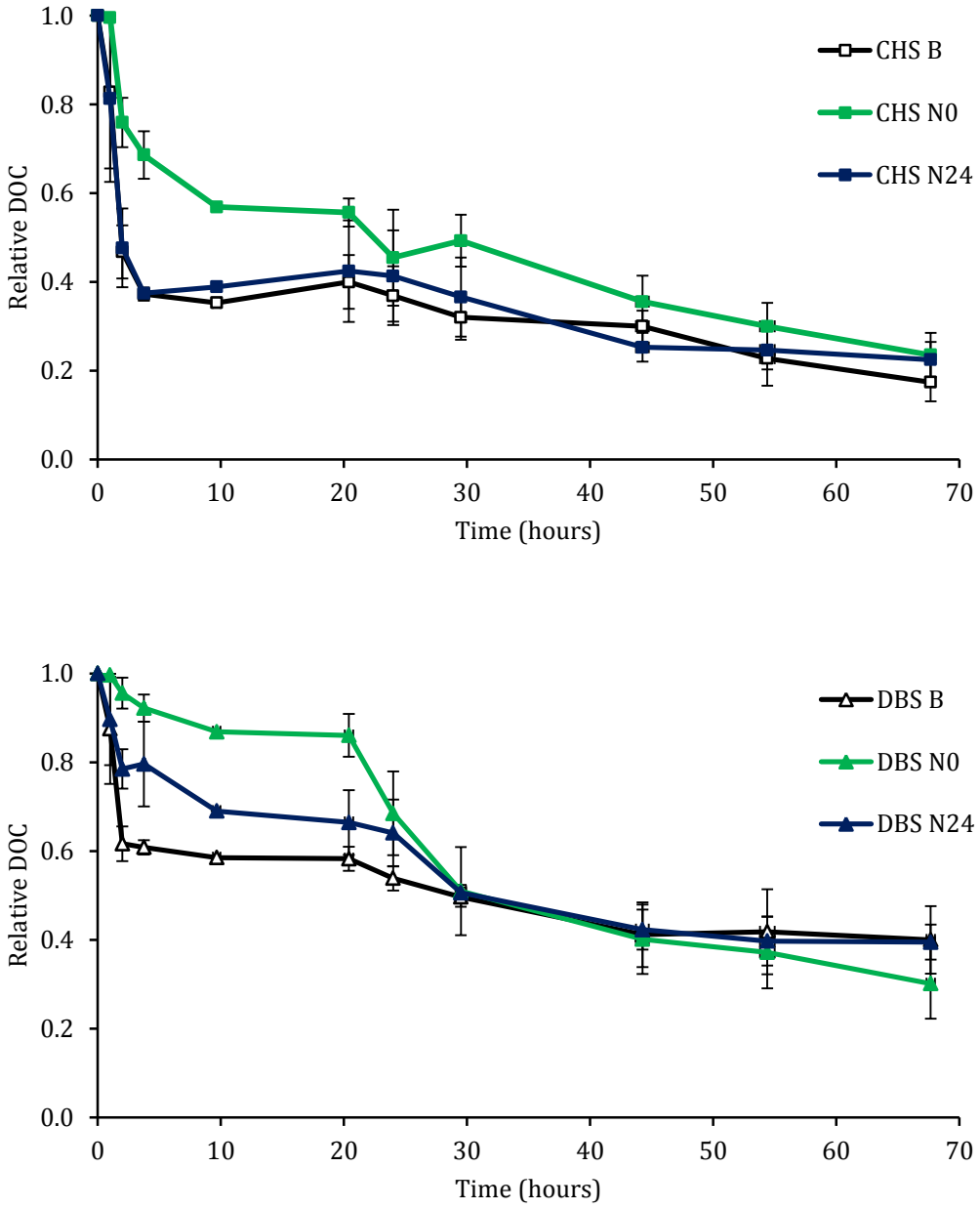


Table 6.2. Results of ANOVA for relative DOC concentrations for all experiments across all treatments.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	<0.0001	2.05
POC_0	na	-	<0.0001	2.23
Phosphate	na	-	<0.0001	1.63
Site	<0.0001	2.26	ns	-
Treatment	0.0210	0.47	ns	-
Time	<0.0001	39.94	<0.0001	40.20
Month	<0.0001	5.85	ns	-
Site*Time	0.0038	1.36	0.0014	1.66
Site*Month	<0.0001	2.98	ns	-
Treatment*Month	<0.0001	2.88	<0.0001	2.53
Time*Month	<0.0001	9.25	<0.0001	10.88
Error		10.64		15.81

Guided by the ANOVA and ANCOVA, the following significant regression equation was calculated:

$$ln\Delta DOC = -0.41\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + 0.28lnt + 0.05DOC_0 - 0.30POC_0 + 0.96$$
$$(0.08) \qquad (0.02) \quad (0.004) \quad (0.04) \quad (0.12)$$

n=342, r^2=0.55 *(Eq. 6.1)*

where ΔDOC is the change in DOC concentration, DOC_0 is the initial concentration of DOC, POC_0 is the initial concentration of POC (all in mg C/l), t is the time of the experiment in hours and m is the month number. The numbers in brackets beneath each parameter is the standard error of that parameter.

As in previous chapters, the regression analysis was also carried out for the two sites separately. For CHS, the r^2 increased to 0.61, but the DOC_0 was no longer

significant. The r^2 did not increase for the DBS data (0.47), but all the same parameters, plus phosphate concentration, were significant:

CHS:

$$ln\Delta DOC = \underset{(0.12)}{1.62}\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + \underset{(0.03)}{0.27lnt} + \underset{(0.06)}{0.73POC_0} - \underset{(0.22)}{0.26}$$

n=163, r^2=0.61 *(Eq. 6.2)*

DBS:

$$ln\Delta DOC = \underset{(0.4)}{1.26}\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + \underset{(0.03)}{0.28lnt} - \underset{(0.05)}{0.17DOC_0} + \underset{(0.32)}{1.08POC_0} - \underset{(0.22)}{1.00PO_4^{3-}} + \underset{(0.97)}{5.25}$$

n=163, r^2=0.47 *(Eq. 6.3)*

where PO_4^{3-} is the phosphate concentration (mg/l) and all other terms are as described above.

These results show that, as in Chapters 2 and 4, the change in DOC concentration can be modelled using the month, time since the start of the experiment and the DOC_0 concentration. Interestingly, this chapter found the POC_0 concentration to be significant in both the ANCOVA and regression analysis, which has not been significant in the earlier chapters. The DBS equation in Chapter 4 (Eq. 4.3) found the chloride and nitrate ion concentrations to be significant, whereas this DBS equation found the phosphate concentration to be significant, perhaps due to the added nutrient solution in ⅔ of the samples.

6.3.3. POC concentrations

The average POC_0 concentration at CHS was 3.68 mg C/l, and at DBS was 3.14 mg C/l; the average POC_{70} concentration at CHS was 4.64 mg C/l, and at DBS was 3.53 mg C/l. These represent increases of 26 and 12% at CHS and DBS respectively. For both sites, the large increases were observed in January and March, whereas the POC decreased in the other three months (May, July and September; Figure 6.3).

An ANOVA on the relative POC concentrations found that Month explained the majority of the variation (42%). Time was also significant, as were the interactions of Time*Month, and Site*Month. Adding covariates found that the POC_0 and chloride concentrations were significant, however Time was no longer significant (Table 6.3). The significance of the initial POC concentration is not surprising, and shows that the change in the POC concentration is dependent on the starting amount of POC. The chloride concentration significance is harder to explain, as there is unlikely to be a direct mechanistic interaction between the POC and chloride. It may be that the chloride reflects the pH or conductivity differences between the two sites.

As there were no significant differences between the treatments in the ANOVA or ANCOVA, it was concluded that there was no 'nutrient-induced' POC degradation, and so no further analysis of POC was carried out.

Figure 6.3. The main effects plot for the log of the relative POC concentrations, where negative numbers represent a decrease in POC concentrations over the time of the experiment and positive numbers represent an increase. The error bars are the standard errors.

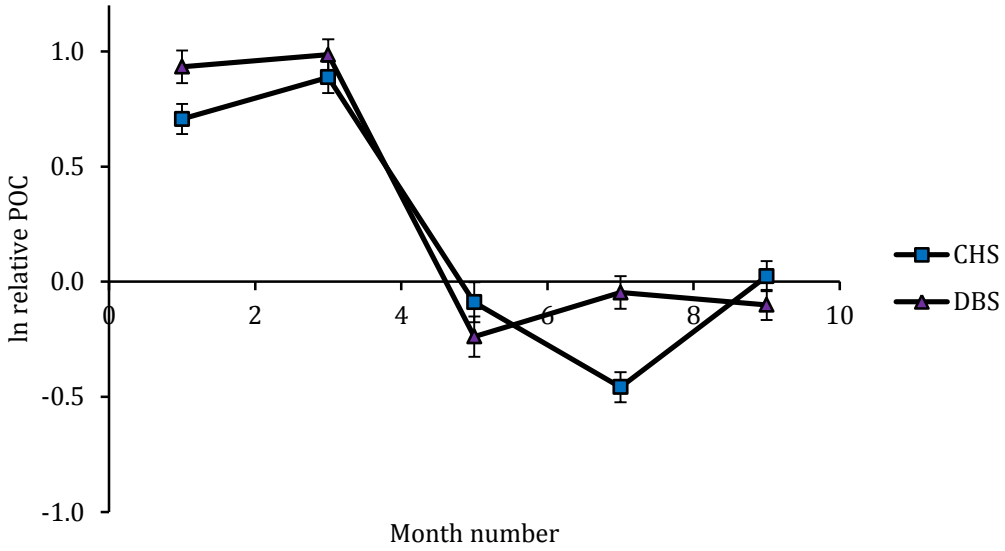


Table 6.3. Results of ANOVA for relative POC concentrations for all experiments across all treatments.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
POC_0	na	-	<0.0001	2.81
Chloride	na	-	<0.0001	0.65
Time	<0.0001	3.70	ns	-
Month	<0.0001	41.68	<0.0001	26.80
Site*Month	<0.0001	2.03	-	-
Time*Month	<0.0001	17.25	-	-
Error		26.51		15.10

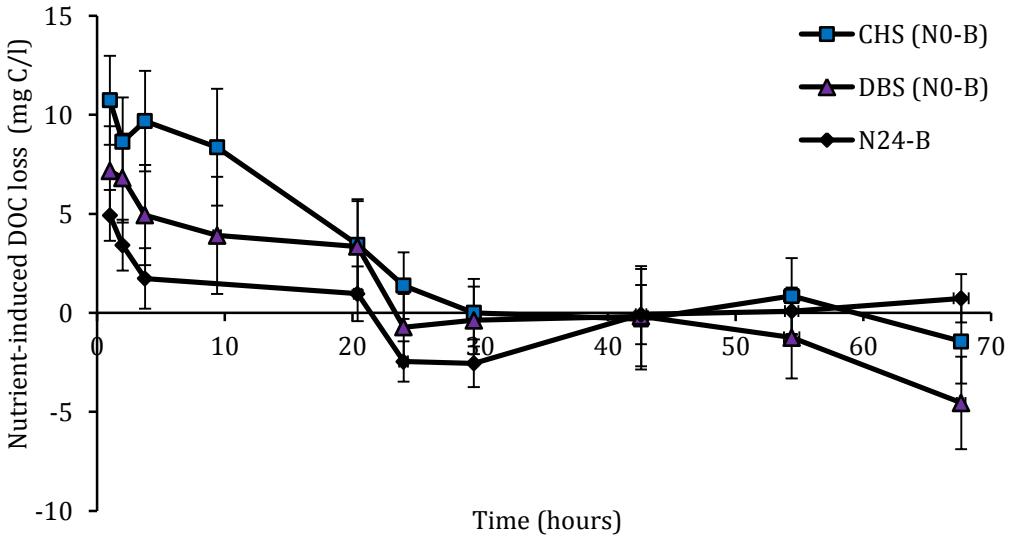
6.3.4. Nutrient-induced DOC degradation

The nutrient-induced changes in DOC concentration were calculated by subtracting the B treatment from the N_0 treatment data. This looked at the difference that adding nutrients had on the DOC concentration; when the DOC concentration was

higher in the nutrient treatment, this did not imply that the added nutrients had added DOC to the water, rather that the DOC decline was not as great as in the control treatment without nutrients.

The change due to the nutrients in the N_0 treatment ranged from -18.67 mg C/l to 25.73 mg C/l, and the average change at CHS was 3.38 mg C/l, and at DBS was 1.41 mg C/l, showing that the nutrients had a greater effect at CHS. The average values show that the nutrient addition did not increase the loss of DOC; in fact the DOC concentrations in the N_0 treatment were on average higher than the DOC concentrations in the B treatment. The magnitude of the difference between the two treatments decreased with time, to approximately zero at t_{30}, then fell to negative numbers (where the B concentration was higher than the N_0 concentration) during the last ten hours of the experiment (Figure 6.4).

Figure 6.4. The main effects plot of the difference between N_0 and B for the two sites, and the difference between N_{24} and B treatments. The error bars are the standard errors.



An ANOVA included three factors: Time, Site and Month; there was no Treatment factor. The results showed that the three factors were all significant, as was the interaction of Site*Month (Table 6.4). Month explained the largest proportion of the variation (35%). Once covariates were added to the model, three

were found to be significant, but the r^2 did not increase; in fact it stayed the same as without covariates. The significant covariates were the phosphate concentration, which was also significant in the previous section ANCOVA, and the absorbance at 400 nm and the E4:E6 ratio, both of which can be related to the DOC concentration and composition.

Table 6.4. The results of the ANOVA on the difference between the N_0 and B treatments.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
E4:E6	na	-	0.0031	2.69
Abs_{400}	na	-	<0.0001	7.83
Phosphate	na	-	0.0024	2.84
Site	0.0312	1.04	<0.0001	11.13
Time	<0.0001	16.64	<0.0001	16.73
Month	<0.0001	35.06	<0.0001	28.20
Site*Month	<0.0001	15.68	ns	-
Error		5.89		5.94

Guided by the results of the ANOVA and ANCOVA, the following regression equation was calculated:

$$\Delta DOC_{nutrients0} = \underset{(0.54)}{-2.60} lnt \underset{(10.85)}{- 39.38} Abs_{400} \underset{(0.697)}{+ 1.49} PO_4^{3-} \underset{(2.26)}{+ 11.0}$$

n=106, r^2=0.28 *(Eq. 6.4)*

where $\Delta DOC_{nutrients0}$ is the change in DOC concentration due to nutrient addition at time 0, Abs_{400} is the absorbance at 400 nm and all other terms are as described above. As in the previous sections and chapters, the regressions were also calculated for the two sites separately:

CHS:

$$\Delta DOC_{nutrients0} = -3.13lnt - 84.09Abs_{400} + 24.71$$
$$(0.80) \quad (20.59) \quad (4.12)$$

n=57, r^2=0.39 *(Eq. 6.5)*

DBS:

$$\Delta DOC_{nutrients0} = -2.52lnt - 68.98Abs_{400} + 12.30$$
$$(0.57) \quad (14.38) \quad (1.94)$$

n=55, r^2=0.44 *(Eq. 6.6)*

where all terms are as described above. The more negative time parameter for the CHS equation (Eq. 6.5) shows that the DOC changes more with time than in the DBS samples. The lack of a significant phosphate term in equations 6.5 and 6.6 suggest that the phosphate was significant in equation 6.4 as it explained the differences between the two sites. The significance of the absorbance at 400 nm suggests that there is an effect of the composition of the DOC on the DOC degradation.

The negative relationship between the parameter estimates for the time factor for both CHS and DBS, when analysed together and separately, suggests that the nutrients had the largest effect on the DOC concentration when t was small, when the nutrients were not limiting, rather than when t was larger, towards the end of the experiment, when it was expected that the nutrients would be limiting the loss of DOC. The higher DOC concentration in the nutrient treatment suggests that the added nutrients did not cause the DOC concentration to decrease as much as it did in the control treatment.

A similar analytical method was applied to the difference between the DOC concentration in the B treatment and N_{24} treatment. The change due to the nutrients in the N_{24} treatment ranged from -12.02 mg C/l to 19.61 mg C/l; the average change at CHS was 1.19 mg C/l, and at DBS was 1.16 mg C/l. The average values show that the nutrient addition at t_{24} did not increase the loss of DOC, and as in the N_0 treatment, the DOC concentrations in the N_{24} treatment were on average higher than the DOC concentrations in the B treatment. As with the N_0 and B

differences, the magnitude of the difference between the two treatments decreased with time (Figure 6.4).

The ANOVA showed that there were significant differences between Time and Month, and between the interactions of Site*Month and Time*Month (Table 6.5). The largest proportion of the variation was explained by the Site*Month interaction (28%). Further analysis of the difference between the times showed that there were significant differences in the DOC concentrations at t_1 and t_2. It is interesting to note that there were also significant differences in the DOC concentrations between the N_{24} and B treatments at t_{24} and t_{30}, showing that adding the nutrients did significantly affect the DOC concentration. However, as with the N_0 and B analysis, these were not the differences that were expected, as adding the nutrients increased the DOC concentration relative to the control B treatment DOC concentrations. The effect of the nutrients on the DOC concentration change at t_{24} is investigated further in section 6.3.5.6.

Table 6.5. The results of the ANOVA on the difference between the DOC concentrations in the N_{24} and B treatments.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	<0.0001	12.57
Time	0.0021	6.71	0.0009	13.19
Month	<0.0001	9.58	ns	-
Site*Month	<0.0001	27.87	ns	-
Time*Month	0.0097	18.71	0.0027	18.85
Error		26.36		28.30

Guided by the results of the ANOVA and ANCOVA on the difference between the N_{24} and B treatments, the following regression equation was calculated:

$$\Delta DOC_{nutrients24} = -1.48lnt - 0.22DOC_0 + 13.58$$
$$(0.37) \quad (0.04) \quad (1.99)$$

$n=111$, $r^2=0.28$ *(Eq. 6.7)*

where $\Delta DOC_{nutrients24}$ is the change in DOC concentration due to nutrient addition at t_{24} and all other terms are as described above. The regression analysis was carried out on the two sites separately, as even though there was no significant Site factor in the ANOVA or ANCOVA, the interaction of Site*Month was significant.

CHS:

$$\Delta DOC_{nutrients24} = \underset{(0.57)}{-0.95}lnt - \underset{(0.06)}{0.40}DOC_0 - \underset{(0.71)}{1.66}\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + \underset{(3.24)}{21.67}$$

n=56, r^2=0.48 *(Eq. 6.8)*

DBS:

$$\Delta DOC_{nutrients24} = \underset{(0.30)}{-2.02}lnt - \underset{(0.37)}{0.98}\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + \underset{(0.90)}{6.72}$$

n=55, r^2=0.50 *(Eq. 6.9)*

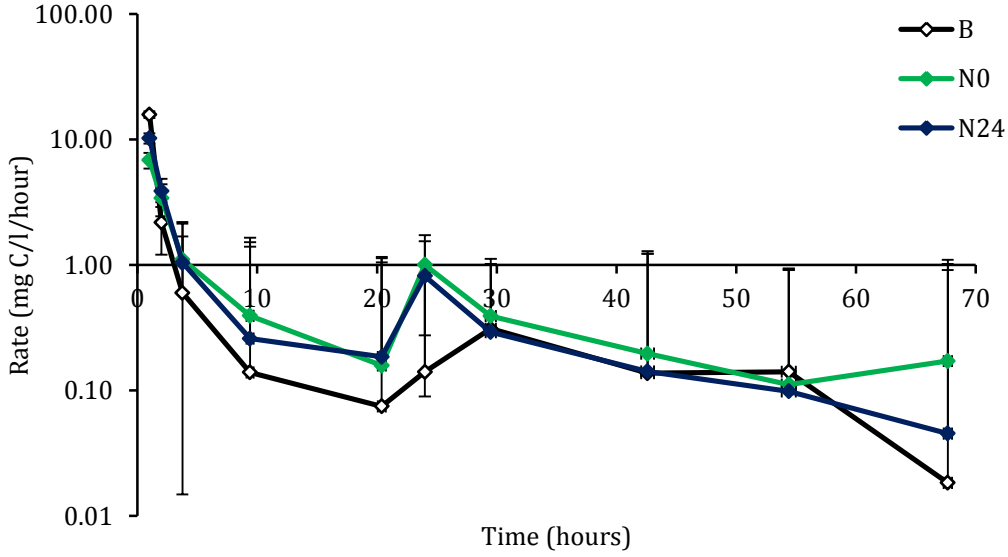
where all terms are as described above. As with the N_0 and B differences, the negative relationship between the parameter estimates for the time factor for both CHS and DBS, when analysed together and separately, suggests that the nutrients had the largest effect on the DOC concentration when t was small, when the nutrients were not limiting, rather than when t was larger, towards the end of the experiment, when it was expected that the nutrients would be limiting the loss of DOC.

6.3.5. Rate of DOC degradation

The rate of DOC degradation varied from -6.13 mg C/l/hour to 31.87 mg C/l/hour, with the average rate of change being 1.52 mg C/l/hour. The average values for CHS and DBS were 1.92 and 1.10 mg C/l/hour respectively. Negative rates, where the DOC concentration increased, were observed in 54 of 343 cases; only four rates

were less than -1 mg C/l/hour. The majority of the t_1, t_2, t_4 and t_{10} rates were higher than the rates after t_{10}, showing that the DOC concentration initially fell, and the remainder of the rates are close to zero, showing that the DOC changed little after t_{10} (Figure 6.5).

Figure 6.5. The main effects plot for the rates of DOC change in all three treatments, displayed on a log scale to show better the differences between the smaller rates. The error bars are the standard errors.



An ANOVA on these data showed that the Site, Time and Month factors were significant, as were the interactions of Site*Time, Treatment*Time, and Time*Month (Table 6.6). As Treatment was significant in an interaction, further analysis of the rates was carried out for the three treatments separately, to investigate further which covariates and factors were significant for each. From Figure 6.5 it appears that the significant differences between the treatments may be solely due to the differences in the DOC concentrations on the first day of the experiment, as there are few differences between the rates after t_{10}. The increase in rate in the two nutrient treatments at t_{24} is odd, as this is before the nutrients were added to the N_{24} treatment, and 24 hours after the nutrients were added to the N_0 treatment. The increase was not significant, and there were no significant differences between t_{20}, t_{24} and t_{30}.

This section also includes the differences in the DOC degradation rates between the B and N_0, and the B and N_{24} treatments.

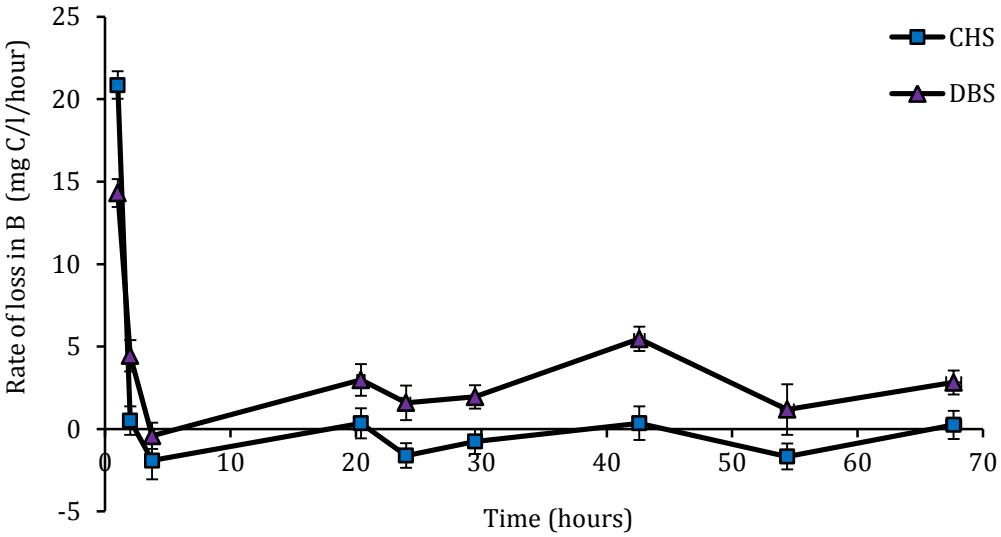
Table 6.6. The results of the ANOVA on the rate of DOC degradation.

	Without covariates	
Factor	p	ω^2
Site	0.0029	0.97
Time	<0.0001	46.57
Month	0.0449	0.71
Site*Time	<0.0001	3.94
Treatment*Time	0.0002	4.14
Time*Month	0.0232	2.26
Error		8.73

6.3.5.1. Rate of DOC degradation with no added nutrients (B)

The rate of degradation in the B treatment samples varied from -2.39 mg C/l/hour to 31.41 mg C/l/hour, with the average rate of change being 1.68 mg C/l/hour. The average values for CHS and DBS were 2.10 and 1.26 mg C/l/hour respectively. Negative rates, where the DOC concentration increased, were observed in 21 of 119 cases; only three of those were from CHS, and only one rate was less than -1 mg C/l/hour. The majority of the rates were positive, showing that the DOC concentration fell over time. The 18 highest rates (showing the greatest loss of DOC) all fell within the first few hours of the experiment, and the majority of these rates were for CHS samples, showing that large proportions of the DOC from CHS was lost at the very beginning of the experiment (Figure 6.6).

Figure 6.6. The main effects plot for the rates of DOC change in the B treatment for the two sites. The error bars are the standard errors.



An ANOVA on the rate of degradation in the B treatment showed that the largest majority of the variation was explained by the Time factor (76%), with the Site and Month factors explaining small amounts. Two interactions were also significant: Site*Time and Time*Month, both of which explained small amounts of the variation. The error term explained another small amount, but was actually the second largest source of variation (Table 6.7).

The ANCOVA found that Site and Month were no longer significant as individual factors; however both were still significant interactions with Time. Four covariates were significant, DOC_0, POC_0, chloride and nitrate, but collectively they only explained 3.33% of the variance. Again, Time explained the largest proportion of the variation (76%). Adding covariates increased the r^2 and decreased the proportion of the variation explained by the error term.

Table 6.7. The results of the ANOVA on the rate of DOC change in the B treatment.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	0.0171	0.51
POC_0	na	-	0.0085	0.65
Chloride	na	-	0.0002	1.45
Nitrate	na	-	0.0062	0.72
Site	0.0061	0.83	ns	-
Time	<0.0001	76.09	<0.0001	76.44
Month	0.0176	1.04	ns	-
Site*Time	<0.0001	3.92	<0.0001	3.85
Time*Month	0.0025	4.50	<0.0001	6.70
Error		6.00		3.89

Guided by the results of the ANOVA and ANCOVA, the following regression equation was calculated:

$$Rate_B = \underset{(0.27)}{-2.39}\,lnt + \underset{(0.034)}{0.07}\,DOC_0 + \underset{(1.42)}{6.40}$$

n=118, r^2=0.43 *(Eq. 6.10)*

where $Rate_B$ is the rate of change of DOC concentration in the B treatment (mg C/l/hour), and all other terms are as described above. The rate equation shows that the rate of DOC change in the B treatment increased with increasing DOC_0 concentration. Calculating the regressions for the two sites separately found that the r^2 increased slightly:

CHS:

$$Rate_B = \underset{(0.45)}{-2.91}\,lnt + \underset{(0.048)}{0.046}\,DOC_0 + \underset{(2.56)}{8.28}$$

n=60, r^2=0.43 *(Eq. 6.11)*

DBS:

$$Rate_B = -1.90lnt + 0.003Cl^- + 6.46$$
$$(0.27) \quad (0.004) \quad (1.02)$$

n=44, r^2=0.45 *(Eq. 6.12)*

where all terms are as described above. These equations show that the rate of DOC degradation in the B treatment (equivalent of the 'light' treatment in Chapters 2, 3 and 4) is dependent on the initial DOC concentration and the time since the start of the experiment (and the chloride concentration for the DBS samples). As stated earlier, the chloride concentration significance is hard to explain, as there is unlikely to be a direct mechanistic interaction between the DOC and chloride. In Chapter 4, the rate of DOC degradation in the light was found to be dependent on the time, temperature and PAR, showing that environmental factors were important. Neither temperature nor PAR was found to be significant in any regression equations in this chapter. In Chapter 2, the rate of degradation in the light was dependent on the DOC_0 concentration and time since the start of the experiment, similar to this chapter, but also on the temperature, similar to Chapter 4.

6.3.5.2. Rate of DOC degradation with added nutrients (N_0)

The rate of degradation in the N_0 treatment samples varied from -6.13 mg C/l/hour to 19.16 mg C/l/hour, with the average rate of change being 1.29 mg C/l/hour. The average values for CHS and DBS were 1.60 and 0.97 mg C/l/hour respectively. Negative rates, where the DOC concentration increased, were observed in 18 of 113 cases, and only two rates were less than -1 mg C/l/hour. The majority of the rates were positive, showing that the DOC concentration fell over time. The 11 highest rates (indicating the largest loss of DOC) all fell within the first two hours of the experiment, showing that large proportions of the DOC were lost at the very beginning of the experiment (Figure 6.7).

Figure 6.7. The main effects plot for the rates of DOC change in the N_0 treatment for the two sites. The error bars are the standard errors.

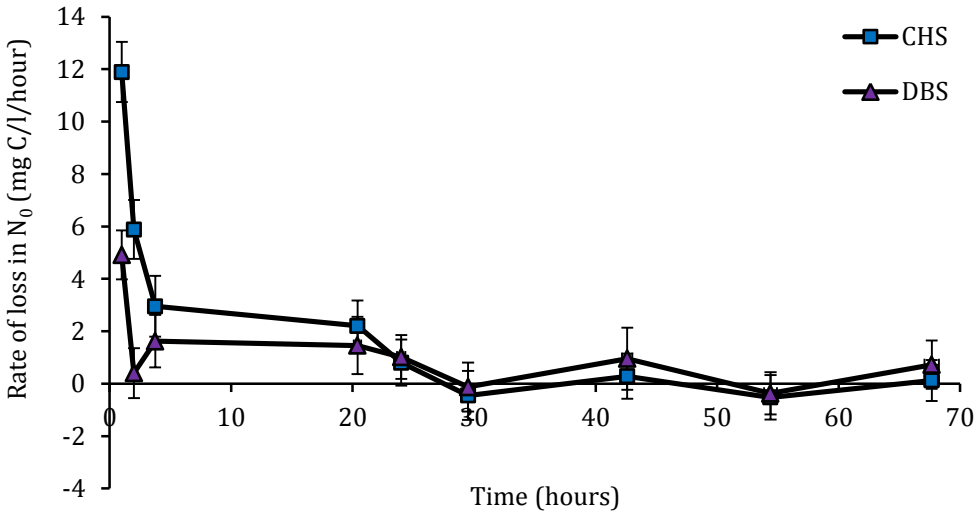


Table 6.8. The results of the ANOVA on the rate of DOC change in the N_0 treatment.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
pH	na	-	0.0273	0.68
DOC_0	na	-	0.0044	1.29
Abs_{400}	na	-	0.0001	2.69
Chloride	na	-	<0.0001	3.10
Nitrate	na	-	0.0033	1.39
Sulphate	na	-	0.0009	1.88
Site	0.0126	1.22	ns	-
Time	<0.0001	37.20	<0.0001	26.65
Site*Time	0.0249	2.61	0.0002	5.39
Time*Month	<0.0001	34.16	<0.0001	38.53
Error		11.78		10.02

An ANOVA on the rate of degradation in the N_0 treatment showed that Site, Time and the interactions of Site*Time and Time*Month were significant (Table 6.8). The largest proportion of the variation was once again explained by the Time (37%), followed closely by the interaction of Time*Month (34%). The ANCOVA

found that six covariates were significant, explaining 11% of the variation between them. Site was no longer significant, but the rest of the factors and interactions significant in the ANOVA were also significant in the ANCOVA. Adding the covariates increased the r^2 and decreased the error term slightly.

Guided by the results of the ANOVA and ANCOVA, the following regression equation was calculated:

$$Rate_{N0} = -1.32lnt + 0.02DOC_0 - 0.01NO_3^- + 4.73$$
$$\quad (0.23) \quad (0.025) \quad (0.006) \quad (1.36)$$

n=83, r^2=0.32 *(Eq. 6.13)*

where $Rate_{N0}$ is the rate of DOC change in the N_0 treatment, NO_3^- is the nitrate concentrations (mg/l) and all other terms are as described above. The rate equation shows that the rate of DOC change in the N_0 treatment increased with increasing DOC_0 concentration, and was significantly affected by the time and nitrate concentration. The significance of the nitrate concentration suggests that the biodegradation is affected by the nutrient concentration. Calculating the regressions for the two sites separately found that the r^2 increased for CHS but decreased for DBS:

CHS:

$$Rate_{N0} = -2.39lnt + 0.59\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + 1.64pH + 0.042DOC_0 + 2.98$$
$$\quad (0.42) \quad (0.34) \qquad\qquad (0.81) \quad (0.03) \quad (4.24)$$

n=57, r^2=0.50 *(Eq. 6.14)*

DBS:

$$Rate_{N0} = -0.64lnt - 0.007NO_3^- + 3.23$$
$$\quad (0.29) \quad (0.007) \quad (1.15)$$

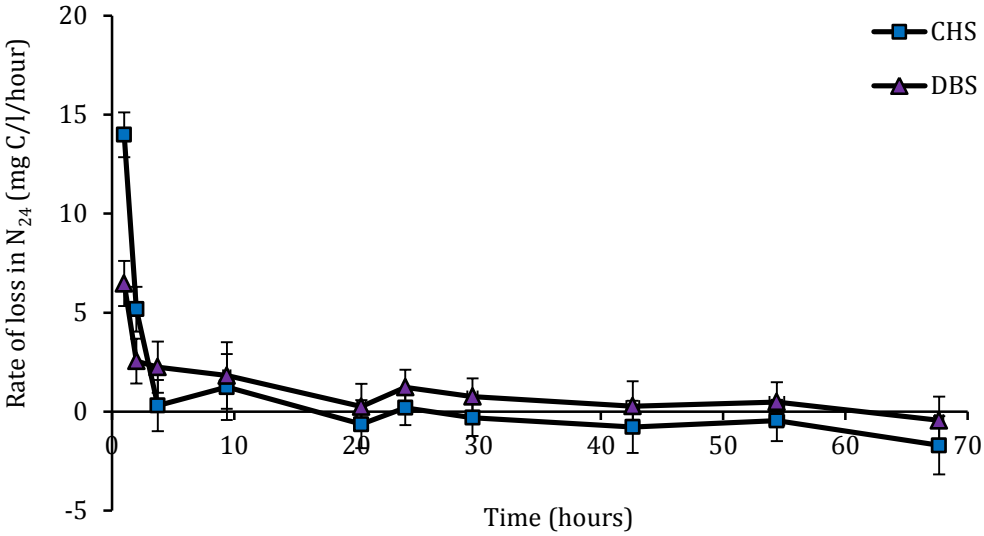
n=41, r^2=0.12 *(Eq. 6.15)*

where all terms are as described above. The significance of the nitrate concentration in these equations reflects the increased concentration in these samples compared with the B treatment.

6.3.5.3. Rate of DOC degradation with added nutrients at t_{24} (N_{24})

The rate of degradation in the N_{24} treatment samples varied from -3.00 mg C/l/hour to 31.87 mg C/l/hour, with the average rate of change being 1.57 mg C/l/hour. The average values for CHS and DBS were 2.07 and 1.08 mg C/l/hour respectively. Negative rates, where the DOC concentration increased, were observed in 15 of 112 cases, and only one rate was less than -1 mg C/l/hour. The majority of the rates were positive, showing that the DOC concentration fell over time. The 11 highest rates (the largest loss of DOC) all fell within the first few hours of the experiment, showing that a large proportion of the DOC was lost at the very beginning of the experiment (Figure 6.8).

Figure 6.8. The main effects plot for the rates of DOC change in the N_{24} treatment for the two sites. The error bars are the standard errors.



An ANOVA on the rate of degradation in the N_{24} treatment showed that the three factors were significant, along with two interactions: Site*Time and Time*Month. As in the N_0 treatment ANOVA, the Time, and Time*Month interaction explain the largest proportions of the variation, 33% each (Table 6.9).

The ANCOVA found that the only significant covariate was the DOC_0 concentration, which explained the smallest proportion of the variation (0.86%). The Site and Month factors were no longer significant.

Table 6.9. The results of the ANOVA on the rate of DOC change in the N_{24} treatment.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	0.0340	0.86
Site	0.0324	0.92	ns	-
Time	<0.0001	33.33	<0.0001	34.02
Month	0.0384	1.65	ns	-
Site*Time	0.0029	4.88	0.0035	4.76
Time*Month	<0.0001	32.72	<0.0001	32.71
Error		12.23		14.47

Guided by the results of the ANOVA and ANCOVA, the following regression equation was calculated:

$$Rate_{N24} = -1.81lnt + 0.07DOC_0 + 3.97$$
$$\qquad\quad (0.30) \qquad (0.034) \qquad (1.61)$$

n=112, r^2=0.27 *(Eq. 6.16)*

where $Rate_{N24}$ is the rate of DOC change in the N_{24} treatment and all other terms are as described above. The rate equation shows that the rate of DOC change in the N_{24} treatment increased with increasing DOC_0 concentration, and was significantly affected by the time of the experiment. Calculating the regressions for the two sites separately found that the r^2 increased for CHS and DBS, but the DOC_0 was not significant for DBS:

CHS:

$$Rate_{N24} = -2.56lnt + 0.08DOC_0 + 5.73$$
$$(0.55) \quad (0.058) \quad (3.10)$$

n=56, r^2=0.31 *(Eq. 6.17)*

DBS:

$$Rate_{N24} = -1.06lnt + 3.98$$
$$(0.21) \quad (0.65)$$

n=56, r^2=0.32 *(Eq. 6.18)*

where all terms are as described above. As in the $Rate_B$ equations (Eq. 6.10 and 6.11), these equations are dependent only on the time since the start of the experiment and the initial DOC concentration. As the nitrate concentration was significant in the previous section rate equations ($Rate_{N0}$; Eq. 6.13 and 6.15), it is perhaps surprising that there was no significant nutrient factor in these equations.

6.3.5.4. Comparing rate results across the three treatments

The range of the rates and the number of negative rates (indicating an increase in the DOC concentration) were similar in all treatments. In all treatments the average rate was higher for CHS than for DBS, showing that the rate of DOC degradation was on average higher in water from the headwater site than from the tidal limit site.

The ANOVAs on the three treatments separately (Tables 6.7, 6.8 and 6.9) showed that the Time factor explained the majority of the variation, followed by the interaction of Time*Month. The smallest proportion of the variation was explained by Site.

The ANCOVA results show that the DOC_0 was a consistent significant covariate, but only explained a small proportion of the variation. Other covariates were found to be significant in the rate of DOC change in the B (POC_0, chloride, nitrate) and the N_0 treatment (pH, absorbance at 400 nm, chloride, nitrate, sulphate), but none other than the DOC_0 were significant in the N_{24} treatment.

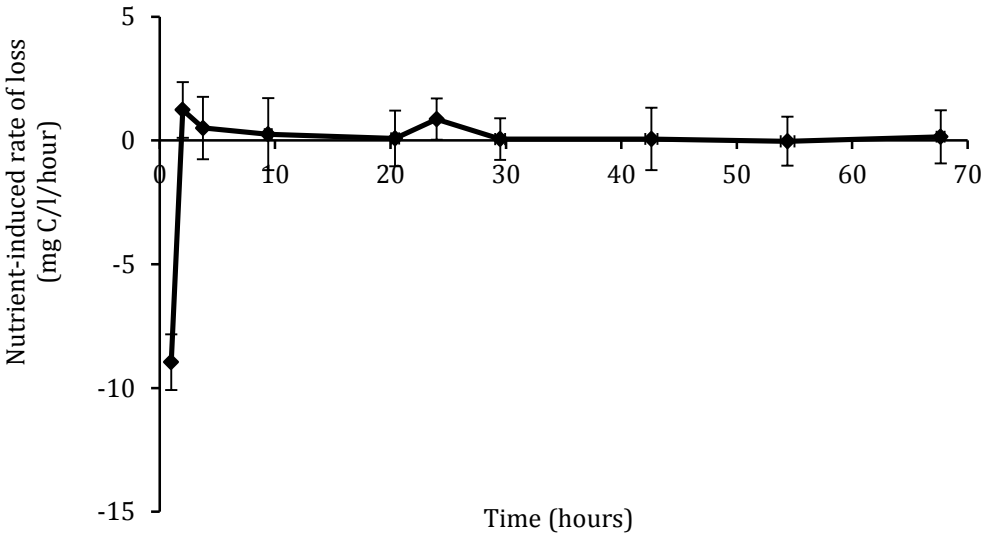
The regression equations (Eq. 6.10-6.18) all had the Time factor as a significant parameter, and the relationship between rate and time was always negative, indicating that there were larger rates of degradation when the t was small, as would be expected in the control treatment. This relationship holds for the nutrient treatments where it would be expected that the addition of nutrients would increase the rate of degradation towards the end of the experiment, when t would be large, as the 'native' nutrient concentrations would be low, and the effect of the added nutrients would be greatest.

The initial DOC concentration was a significant positive parameter in all the equations where the two sites were considered together (Eq. 6.10, 6.13 and 6.16), and in all equations for the CHS samples (Eq. 6.11, 6.14 and 6.17), but never in the DBS sample equations (Eq. 6.12, 6.15 and 6.18). This shows that the initial concentration of DOC influences the degradation at CHS, but not at DBS, where other factors, such as the chloride and nitrate concentrations were more influential.

6.3.5.5. Rate of nutrient-induced DOC degradation (B and N_0)

The rate of degradation due to nutrient addition was calculated by subtracting the B treatment rates from the N_0 treatment rates. The rates varied from -25.73 mg C/l/hour to 13.44 mg C/l/hour, with the average rate of nutrient-induced change being -0.47 mg C/l/hour. The median value was 0.07 mg C/l/hour, indicating that in the majority of cases there was little difference between the B and N_0 rates. The average values for CHS and DBS were -0.60 and -0.34 mg C/l/hour respectively (Figure 6.9).

Figure 6.9. The average rates of nutrient-induced DOC change, averaged across both sites. Error bars are the standard errors.



An ANOVA on the rate of nutrient-induced degradation found that there was no significant effect of the Site factor. Time, Month and the Time*Month interaction were significant, and explained 37, 1 and 40% of the variation respectively (Table 6.10). The ANCOVA found that no covariates were significant; no regression equations could be calculated. The lack of significant effects shows that there were no other factors that could account for the differences in rates between the B and N_0 treatments. Figure 6.9 suggests that the largest difference in DOC rates occurred in the first hour of the experiment, and so further analysis was carried out on the initial rates of DOC change.

Table 6.10. The results of the ANOVA on the rate of nutrient-induced DOC change.

	Without covariates	
Factor	p	ω^2
Time	<0.0001	36.71
Month	0.0355	1.39
Time*Month	<0.0001	40.18
Error		7.86

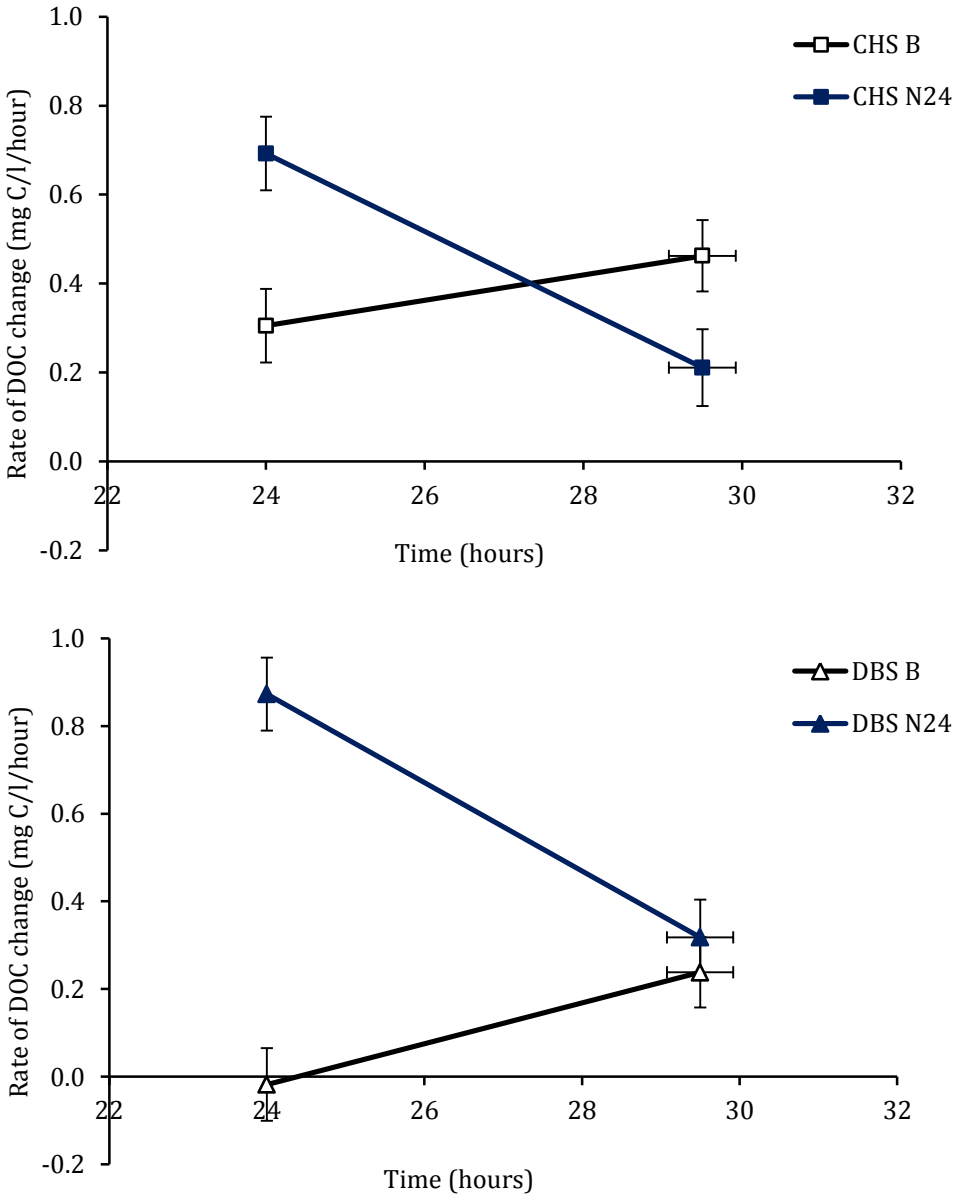
6.3.5.6. Rate of nutrient-induced DOC degradation (B and N_{24})

To investigate the differences between the rates of degradation and the nutrient addition treatment N_{24}, the rates of degradation in the N_{24} treatment at t_{24} (before nutrients were added) and t_{30} (after nutrients were added) were compared to see what affect the nutrients had on the DOC concentrations. They were also compared to the rates at the same times in the B treatment. Previous sections (e.g. sections 6.3.5.2, 6.3.5.3) have shown that the nutrient addition had a greater effect on the CHS samples, but the change in the DBS samples would also show interesting results, and so this analysis was applied to both sites separately.

For the CHS samples, in the N_{24} treatment, the average rate of loss decreased from 0.75 to 0.25 mg C/l/hour, whereas in the same period, the rate in the B treatment increased from 0.28 to 0.40 mg C/l/hour (Figure 6.10). An ANOVA on these CHS data found that there were significant differences ($p=0.0007$, explaining 10% of the variation) in the interaction between Time (t_{24} and t_{30}) and Treatment (N_{24} and B). Further analysis shows that all combinations are significantly different from each other.

For the DBS samples, in the N_{24} treatment, the average rate of loss decreased from 0.89 to 0.34 mg C/l/hour, whereas in the same period, the rate in the B treatment increased from -0.001 to 0.23 mg C/l/hour (Figure 6.10). An ANOVA on these DBS data found that there were significant differences in the interaction of Time*Treatment ($p=0.0409$, explaining 6% of the variation).

Figure 6.10. The main effects plot showing the rate of DOC change at t_{24} and t_{30} for the N_{24} and B treatments for the CHS samples, and for the DBS samples. Error bars are the standard errors.



These were not the expected results, as it was hypothesized that adding nutrients after 24 hours, when the rate of DOC loss was low and all nutrients had been used, would increase the rate of DOC loss in the water, especially in the CHS water where the initial nutrient concentrations are much lower than at DBS. It is interesting that the rate at t_{30} (dusk) was higher than the rate at t_{20} (dawn) for both

sites B treatment, and perhaps this increase reflects the increased rate of DOC degradation after a day of exposure to light. In the DBS B water, the t_{20} dawn rate of DOC change was practically zero, showing that there was hardly any change in the DOC concentration during the preceding night.

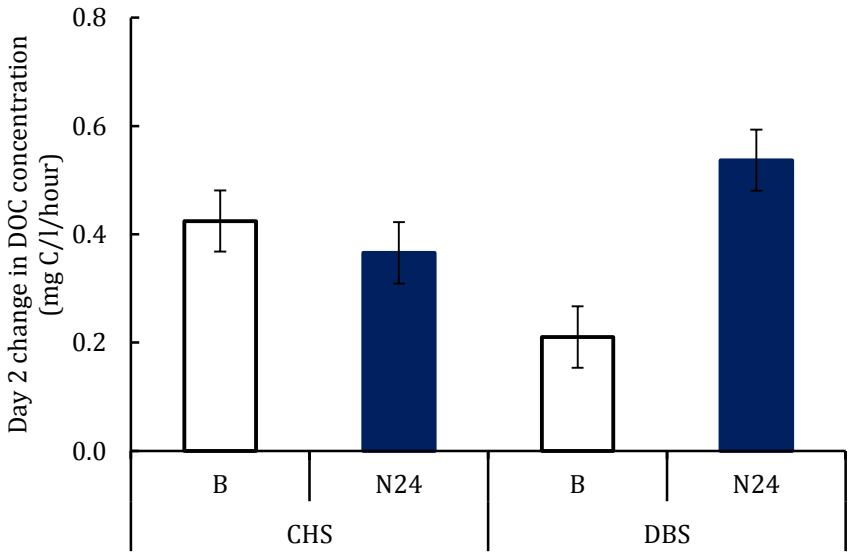
The N_{24} nutrients were added at t_{24}, approximately half way through day 2 of the experiment. In order to see the magnitude of the effect of nutrients on the DOC concentrations, the day 2 rates were calculated, for both sites, for the B and N_{24} treatments, by subtracting the DOC_{30} concentrations (dusk on day 2) from the DOC_{20} concentrations (dawn on day 2), then dividing that by the number of hours between the t_{20} and t_{30} sampling times. This effectively showed the magnitude of the change between dawn and dusk, the gradient of the lines in Figure 6.10.

Analysis of the day 2 changes in DOC concentrations showed that there were significant differences between Treatment, Month, and the interactions of Month*Site, Month*Treatment and Site*Treatment (Table 6.11). The Month factor explained the largest proportion of the variation (37%), followed by the interaction of Month*Site (16%) and Treatment, which explained the smallest proportion (3%). However, the interaction of Site*Treatment explained 7%, and is shown in Figure 6.11. Within CHS, the highest rate of DOC change between t_{20} and t_{30} was in the B treatment. These were the samples that were exposed to light, with no nutrient addition, and perhaps this change in DOC concentration is due to the increased photodegradation of DOC. In the CHS N_{24} water there was a lower magnitude of change in the DOC concentration. In the DBS treatment, the largest change in DOC concentration was in the N_{24} treatment, showing that adding the nutrients had a greater effect on the N_{24} treatment DOC in the DBS water.

Table 6.11. The results of the ANOVA on the difference between the dawn and dusk DOC concentrations on day 2 in the B and N_{24} treatments.

	Without covariates	
Factor	p	ω^2
Month	<0.0001	36.73
Treatment	0.0472	2.78
Month*Site	0.0016	15.64
Month*Treatment	0.0029	13.52
Site*Treatment	0.0065	6.61
Error		6.97

Figure 6.11. The main effects plot showing the magnitude of the DOC change on day 2 of the experiment. Error bars are the standard errors. A high magnitude of change does not necessarily imply a large DOC loss, merely a larger difference between the dawn and dusk DOC concentrations.



Post hoc tests show that there were significant differences between the B and N_{24} treatments at both sites. Adding nutrients did not increase the rate of DOC degradation, in fact it significantly decreased the rate at both sites. The lower rate of loss of DOC in the CHS samples with the nutrient addition is not the expected result, as it was hypothesized that adding nutrients at t_{24} would increase the rate of DOC

loss. The larger change in DOC concentration in the nutrient treatment for the DBS water indicates that adding nutrients decreased the rate of degradation quite significantly compared to the B treatment (Figure 6.10).

6.3.6. Initial rate of DOC degradation

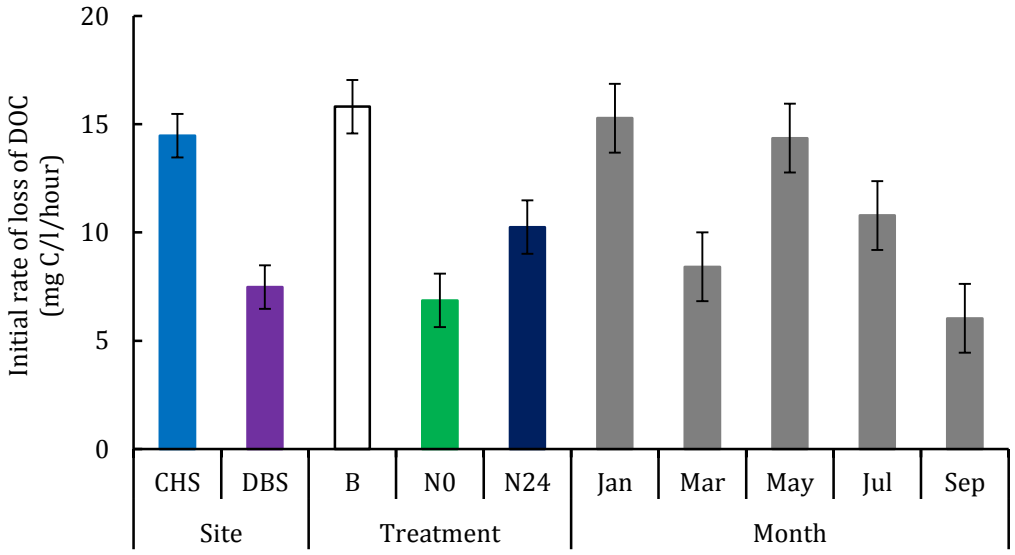
The initial rate of degradation in the first hour of the experiment, for the three treatments and two sites, varied from -0.23 mg C/l/hour to 31.87 mg C/l/hour, with the average rate of change being 10.97 mg C/l/hour. The average values for the sites and treatments are shown in Table 6.12. The rate of change for the N_{24} treatment is the same as for the other two treatments, in the initial hour of the experiment, rather than the first hour after nutrient addition.

Table 6.12. The average initial rates of degradation in the three treatments and two sites, in mg C/l/hour.

Treatment	CHS	DBS	All sites
B	19.82	11.81	15.81
N_0	9.08	4.63	6.86
N_{24}	14.50	6.00	10.25
All treatments	14.47	7.48	10.97

Negative rates, where the DOC concentration increased, were observed in only one of 30 cases. The other 29 rates were positive, showing that the DOC concentration fell during the first hour of the experiment.

Figure 6.12. The main effects plot for the rates of DOC change in first hour of the experiment for the two sites. The error bars are the standard errors.



An ANOVA on the rate of degradation in the first hour of the experiments included the Month, Treatment and Site factors; no Time factor was included, as the ANOVA was only applied to the rate in the first hour. The results showed that all three factors were significant, and explained approximately the same amount of the variation each. Site*Month and Treatment*Month were significant interactions, and explained 14 and 28% of the variation respectively (Table 6.13).

The ANCOVA found that four covariates were significant: conductivity, DOC_0, absorbance at 400 nm and phosphate concentration, collectively explaining 31.54% of the variation in the data. The Treatment and Month factors and Treatment*Month interaction were also significant. The r^2 of the model was high, but the error term represented a large proportion of this (Table 6.13). The ANCOVA showed that even when the covariates were taken into account, there were still significant differences between the initial rates of DOC degradation in the different treatments. Figure 6.12 showed that the rate was significantly lower in the treatments with added nutrients compared with the B treatment with no nutrients, indicating that adding nutrients decreased the rate of DOC degradation, which is the opposite of what was expected.

Table 6.13. The results of the ANOVA on the initial rate of DOC change.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
Conductivity	na	-	<0.0001	16.50
DOC_0	na	-	0.0002	6.38
Abs_{400}	na	-	0.0003	5.63
Phosphate	na	-	0.0028	3.03
Site	0.0006	13.72	ns	-
Treatment	0.0015	14.81	0.0006	6.59
Month	0.0098	11.94	0.0207	3.14
Site*Month	0.0065	13.84	ns	-
Treatment*Month	0.0032	27.87	0.0009	13.32
Error		11.84		43.07

Guided by the results of the ANOVA and ANCOVA, regression equations were calculated (Table 6.14). As Treatment was a significant factor in both the ANOVA and ANCOVA, the regression analysis was carried out separately by Site and by Treatment, and using both the rate and the log of the rate. The equation with the best r^2 is shown below; no significant equation could be calculated for DBS. The relationship between $Rate_0$ and DOC_0 is positive in all cases, showing that the higher the DOC_0, the higher the initial rate, i.e. more DOC is lost in the first hour when the initial DOC is high. The initial rate is also related to the conductivity, absorbance at 400 nm and phosphate concentrations in the majority of the equations.

The DOC_0 was significant in all equations, showing that the initial rate of DOC degradation is dependent on the initial DOC concentration, and this relationship was positive, so as the initial concentration of DOC increases, so does the initial rate of degradation (Table 6.14). The conductivity was a significant negative parameter in the three treatments, and the 'all data' equations, but not in the CHS equation, indicating that this factor may explain the differences between the two sites. The negative relationship means that when the conductivity is high, the initial rate of DOC degradation is low, as has shown to be the case at DBS, whereas where the conductivity is low, at CHS, the rate is higher. The absorbance at 400 nm and the phosphate concentration were also significant negative parameters in three of the equations. The significance of the absorbance at 400 nm suggests that there is an effect of the composition of the DOC on the initial rate of degradation. The

phosphate concentration may reflect the importance of nutrients in the biodegradation of DOC.

The Month factor was significant in only one of the equations (B rate), suggesting that there wasn't a seasonal cycle of degradation for the other treatments. The relationship between the sinusoidal factor and the initial rate in the B treatment may also reflect the dependence of the rate on the initial DOC concentration, as the DOC_0 concentration changes significantly with month and between the sites.

In order to compare the initial rate of degradation in this chapter with the rate in Chapter 4, the regression equation was also calculated for CHS B, as those samples are the equivalent of the light treatment in Chapter 4.

CHS B:

$$lnRate_0 = 1.46 lnDOC_0 - 2.61$$
$$(0.44) \qquad (1.65)$$

n=5, r^2=0.79 *(Eq. 6.19)*

where all terms are as described above. The CHS B equation (Eq. 6.19) shows that this rate is a first order reaction (1.46 ± 0.44), in contrast to Chapter 4, which found it to be a second or third order reaction.

Table 6.14. The regression equations on the initial rate data, showing the significant parameter estimates and the standard errors. The month factor was transformed using the sinusoidal function (Eq. 2.1).

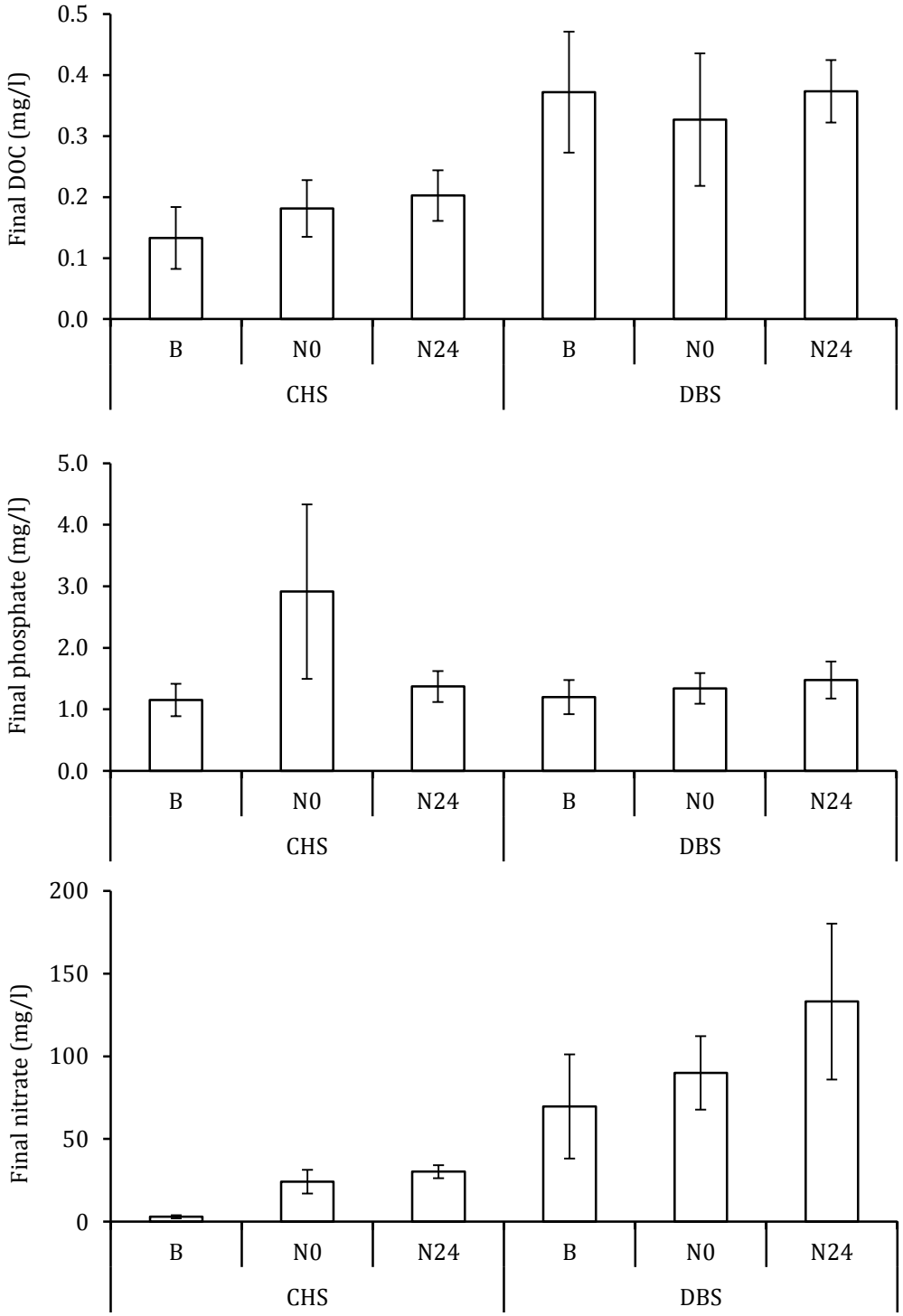
Data	n	r^2	$Rate_0$ or	Significant parameter estimates (SE)					Intercept
			$lnRate_0$	cond	DOC_0	Abs_{400}	PO_4^{3-}	Sinusoidal	
all data	24	0.60	$Rate_0$	-0.02	0.40	-88.26	-6.83	ns	21.90
				(0.005)	(0.19)	(46.84)	(2.32)		(8.41)
CHS	12	0.63	$Rate_0$	ns	0.48	-92.38	-10.25	ns	22.24
					(0.30)	(65.93)	(3.83)		(12.23)
B	10	0.90	$lnRate_0$	-0.0003	0.03	ns	ns	0.13	1.56
				(0.002)	(0.006)			(0.06)	(0.25)
N_0	7	0.84	$lnRate_0$	-0.001	0.006	ns	-2.46	ns	6.91
				(0.001)	(0.034)		(0.74)		(1.84)
N_{24}	10	0.77	$Rate_0$	-0.02	0.85	-184.95	ns	ns	4.64
				(0.008)	(0.22)	(54.28)			(9.66)

6.3.7. Final concentrations (t_{70})

The aim of this chapter was to discover if nutrients were limiting the degradation of DOC over 70 hours, and so the final concentrations was analysed (Figure 6.13). The average final DOC concentration was lowest for CHS B, at just 13% of the starting concentration. Both nutrient treatments had higher final DOC concentrations than the nutrient-free B treatment (18 and 20% for N_0 and N_{24}). For DBS, the lowest final concentration was 33% of the initial concentration in the N_0 nutrient treatment. Both the B and N_{24} treatments had higher final concentrations of 37%. As found previously (section 6.3.3), there were no significant differences in POC concentrations between the treatments.

An ANOVA on the final DOC and POC concentrations found no significant differences between the Treatments, showing that the treatment had no significant effect on the final concentrations of either. An ANOVA on the nitrate and phosphate final concentrations found no significant differences between the Treatments for the phosphate, but significant differences for the nitrate concentrations, with all Sites and Treatments being significantly different from each other. The lowest concentrations were found in the CHS B combination, where there were no additions of nutrients. The second lowest concentration of nitrate was found in the CHS N_0 samples, which had nutrients added at t_0, and the concentration was still significantly higher than the CHS B treatment even after 70 hours. The highest CHS nitrate concentration was in the CHS N_{24} treatment, where the nutrients were added at t_{24}. These results point to the nitrate not being used very quickly at all in the CHS water, as the final concentrations were significantly higher for both nutrient treatments than for the control, and significantly higher again in the nutrient treatment that received the nutrients last (N_{24}). The DBS treatments followed the same pattern, showing that the nitrate was not limiting at either site. This reflects the theory that the phosphate is utilised more rapidly in the water, whereas the nitrate is less limiting and is not consumed as quickly, so the concentrations stay higher for longer.

Figure 6.13. The average final relative DOC and phosphate concentrations, which were not significantly different between sites and treatments; and the nitrate concentrations, which were significantly different. Error bars are the standard errors.



6.3.9. Discussion

Adding a nutrient solution at the beginning of the experiment significantly reduced the initial rate of DOC degradation compared to the rate without nutrient solution. Also, adding nutrient solution at t_{24} significantly reduced that rate of DOC loss compared with the preceding rate, and compared to the same time in the B treatment. There were no significant differences in the final DOC or POC concentrations between the treatments. These results show that the nutrient solution did not increase the rate of DOC loss, or the total amount of DOC or POC lost from the water.

There were significant differences in the initial rate between the treatments, but no significant differences in the final concentrations, suggesting that the rate of DOC degradation in the nutrient treatments must have 'caught up' with the rate on the control samples at some point over the experiment. The nutrient-induced changes in DOC concentration show that there were positive differences at the beginning of the experiment, and negative differences towards the end of the experiment, showing that the 'catch up' in the rate probably happened during the middle of the experiment (Figure 6.4). The reason for the initial rate being slower in the nutrient treatments than in the control samples may be that there is another process that is also limited by nutrients that takes precedent over the degradation of DOC, and therefore uses all the available nutrients, making the rate of DOC loss less than in the control treatment.

The findings of this chapter are different to the findings reported by Bano et al. (1998), who added nutrient solutions to acidic freshwater wetland samples and found that bacterial production rates were enhanced in irradiated samples by 34% (no nutrients), 63% (nitrates) and 74% (nitrates and phosphates) relative to the samples kept in the dark. They also found that when DOM was exposed to sunlight, it was phosphate that limited the rate of bacterial productivity, with the other nutrients resulting in smaller increases than the phosphate treatment. However, their study had dark controls for rate comparisons, whereas this chapter did not, and so it is possible that the nutrient-enhanced rate of DOC degradation would have been greater than the rate of DOC degradation in the dark. Still, the finding of Bano et al. (1998), that the rate was enhanced by nutrients compared to a no-nutrient control is the opposite of the findings of this chapter. The third nutrient added by this experiment was potassium, and it is possible that this negated the effects of the

added nitrates and phosphates in some way, and therefore caused the differences seen. The effect of the potassium on the rates of DOC degradation could not be assessed in this chapter, and so there is no way to know whether it enhanced or worsened the effects of nutrient addition.

Moran et al. (2000) added a nutrient solution to their biodegradation experiments to avoid any nitrate and phosphate limitation; however the effects of this are not discussed in detail. Søndergaard and Middleboe (1995) suggested that bacterial nutrient-limitation would only exist in nutrient-poor and low-productivity systems, which would explain why the CHS water samples reacted more to the addition of nutrients, as the initial concentrations of nitrate were much lower than at DBS. Stutter et al. (2013) found that with the addition of an N and P nutrient solution, 90% of the DOC was respired after 41 days, and the nutrient solution caused an increased in the total amount of DOC respired in 9 out of 12 cases. These results are the opposite of the results found by this chapter.

The rate of DOC degradation decreased at both sites when the nutrient solution was added, whether at the beginning of the experiment, or after 24 hours, which was unexpected, as it was hypothesized that the nutrient addition would increase the rate of DOC degradation, due to an increase in the rate of biodegradation in previously nutrient-limited water. However, it is possible that there was an increase in the biodegradation, that cause bacterial and algal growth in the water, which in turn caused the lower light penetration in the water, and so there was a lower rate of photodegradation in the water. Light-attenuation has been shown to decrease the rate of photodegradation (Osburn et al., 2009). It is possible that the nutrient addition did cause an increase rate of loss of DOC, but also caused an increase in the rate of bio-production of DOC. As only the net changes in DOC were measured, and it was not possible to measure in-situ bio-production of DOC, it is likely the losses measured in previous chapters are where the degradation of DOC far out-strips the production (which is possibly limited by nutrient availability), and this chapter merely shows the magnitude of the bio-production that goes on simultaneously with the biodegradation. It is also possible that there is another factor limiting the degradation of DOC, or perhaps the remaining DOC is so recalcitrant that not even prolonged exposure to light and microbes can break it down any further (as suggested in Moran and Zepp (1997)).

The significance of various covariates in the different ANCOVA and regression models suggests that the degradation of DOC is dependent on/controlled

by a number of other processes in the water. The change in DOC concentration (Table 6.2, Eq. 6.1, 6.2, 6.3) was found to be influenced by the initial organic carbon contents, with both DOC and POC found to be significant. The significance of these factors was quite logical; the loss of DOC depends on the starting concentration of the organic carbon in both forms. The significance of the phosphate was harder to explain; it is likely due to the added nutrient solution and differences in the concentration of the 'natural' phosphate in the water. Other anions were also significant in other analyses:

- Chloride: POC concentrations (Table 6.3), rate of degradation in B (Table 6.7, Eq. 6.12), rate of degradation in N_0 (Table 6.8)
- Nitrate: rate of degradation in B (Table 6.7, 6.8, Eq. 6.13, 6.15)
- Phosphate: nutrient-induced DOC change (Table 6.4, Eq. 6.4), initial rate of DOC degradation (Table 6.13, 6.14)
- Sulphate: rate of degradation in N_0 (Table 6.8)

The change in the POC concentrations (Table 6.3) was also influenced by the initial concentraiton of the POC, another logical covariate. The chloride concentration was also significant in the ANCOVA; however it explained a very small proportion of the variance (0.65%), and the relationship between DOC and chloride is harder to explain.

The E4:E6 ratio and absorbance at 400 nm were significant in various analyses (Table 6.4, 6.8, 6.13, Eq. 6.4, 6.5, 6.6) showing that the composition of the DOC affects the nutrient-induced change in DOC and the rate of DOC degradation, as also shown in Chapter 5.

6.4. Conclusion

The main findings of this chapter are shown in Table 6.15. The rate of DOC degradation does not appear to be limited by nutrients; in fact adding nutrients decreased the rate of DOC loss. There were significantly lower initial rates of degradation in the nutrient treatments, and significantly lower t_{30} rates of degradation after adding nutrients at t_{24}, compared with the control treatment. There were no significant differences in the final DOC concentrations between the sites or treatments. However, this chapter still found similar total loss of DOC in the

light (in the B treatment) to the losses in Chapters 2 and 4, showing that these results are consistent.

As in Chapters 2, 3 and 4, there were significant differences in the loss of DOC between the sites, and the initial DOC was often a significant covariate in the analyses, showing that the site differences in DOC type and concentration play a large role in determining the DOC degradation rates. The rate of DOC degradation declined over time, suggesting a decrease in the 'degradability', or an increase in the recalcitrance, of DOC.

Table 6.15. The main findings of Chapter 6.

Sites studied	Teesdale: CHS, DBS
Range of catchment areas (km^2)	0.2 – 818.4
Range of percentage peat cover (%)	33.9 – 100
Duration of experiments	5 months, for 70 hours
Sample size	373
Net DOC loss in the control (B) treatment (%)	76
Net DOC loss in the N_0 treatment (%)	74
Net DOC loss in the N_{24} treatment (%)	72
Initial rate of DOC loss in the B treatment (mg C/l)	15.8
Initial rate of DOC loss in the N_0 treatment (mg C/l)	6.9
Initial rate of DOC loss in the N_{24} treatment (mg C/l)	10.3
Range of rate of DOC loss in the B treatment (mg C/l/hour)	31.4 – -2.4
Range of rate of DOC loss in the N_0 treatment (mg C/l/hour)	19.2 – -6.1
Range of rate of DOC loss in the N_{24} treatment (mg C/l/hour)	31.9 – -3.0
Significant covariates:	
Change in DOC concentrations	Initial DOC, initial POC, phosphate
B rate	Initial DOC, initial POC, chloride, nitrate
N_0 rate	Initial DOC, pH, absorbance at 400 nm, chloride, nitrate, sulphate
N_{24} rate	Initial DOC
What to investigate next?	The effect of nutrient concentrations on the bio-degradation of DOC in a high nutrient river system, due to the interesting result that the added nutrients did not increase the total amount of DOC lost from the water.

Chapter 7:
Lowland confluence and DOC degradation

7.1. Introduction and Aims

The aim of this chapter was to investigate the rates of DOC degradation in a lowland river, and how these are influenced by the confluence with an upland river. Chapters 2, 3, 4 and 6 have looked at the DOC dynamics of different sites on upland, peat-sourced rivers and found significant differences between the DOC concentrations and rates of loss at the different sites. Chapter 5 has shown that there were significant differences in the composition of the DOC from the upland and lowland rivers and sites. This chapter intends to measure the DOC concentrations across a lowland river, with sites above and below a town and water treatment works, and above and below the confluence of the lowland and upland rivers, to investigate the effects that all these features have on the DOC dynamics.

Lowland rivers tend to have higher nutrient loads due to a greater density of urbanisation and more intensive agriculture compared with the low-intensity sheep grazing and low human populations of the catchments of upland rivers (Jarvie et al., 2008). The DOC concentrations in rivers from catchments that contain no peat should be low relative to DOC concentrations in upland rivers (Neal et al., 1998), as there is a direct relationship between percentage peat cover and DOC flux in the river and peats are pre-dominantly a feature of upland areas in the UK (Austnes et al., 2010). Kay et al. (2009) state that 'only catchments dominated by organic soils will generate DOC levels significant to the water industry'; however, anthropogenic inputs of DOC into the water (e.g. effluent discharge) can cause the DOC concentrations to be higher than expected (Eatherall et al., 2000). The relationship between discharge sites and the concentrations of nutrients and metals in the water was studied by Rothwell et al. (2010); they found significant correlations between proximity and density of sewage and trade discharge sites and the pH, base cations, nutrients and metals. The correlations with the proximity were mostly negative, indicating higher pH, base cations, nutrients and metals closer to the point-source input. The correlations with the density were mostly positive, indicating that the higher the number of sewage and trade discharges, the higher the base cations and

nutrients. They concluded that human activities, specifically point source nutrient inputs, have a significant effect on the water chemistry in rivers in North West England.

It is possible to hypothesize that lowland rivers, high in nutrients, with point-source anthropogenic inputs of labile DOC, such as waste water treatment outlets, may lead to renewed degradation of DOC from other sources, in the case of this chapter, the DOC coming from peat soils in the uplands, making the DOC dynamics below the confluence different to those in either contributing river. The water chemistry of the contributing rivers may provide information about the mixing ratio of the two waters below the confluence.

This chapter analysed the DOC concentrations of two sites on a lowland river, including one site that is downriver of a waste water treatment works, and compared the rates of degradation of DOC from these lowland sites with the rates from an upland river and from one site below the confluence of the two rivers. It was hypothesized that there would be differences between the upland and lowland sites, differences between the chemistry above and below the waste water treatment works, and the water chemistry of the two rivers would affect the water chemistry below the confluence.

7.2. Approach and Methodology

This chapter adapts the method of Chapters 2, 3 and 4, to conduct degradation measurements of DOC from four sites on the rivers Skerne and Tees in North-East England over periods of up to 70 hours.

7.2.1. Field sites

This chapter uses one of the sites used in Chapter 4 (Broken Scar, DBS, Figure 2.1, Table 2.1), and three new sites (Figure 7.1, Table 7.1). Two of the sites (MUN and OXY) were on the river Skerne, a lowland river that runs 50 km from its source near Trimdon in County Durham, to its confluence with the river Tees, at Croft-on-Tees, just south of Darlington. These two Skerne sites were close to two NRFA sites (25020 and 25004, www.ceh.ac.uk/data/nrfa/index.html). The NRFA site closest to MUN (4 km north, at Preston le Skerne; NZ 292 237) has a catchment area of 147

km^2, with an altitude range between 67.5 and 219.2 m AOD. It contains 5.2% woodland, 52% arable and horticulture, 29.1% grassland and 4.9% urban land use. There is no mountain, heath or bog land in the catchment. The majority of the underlying geology is high permeability Magnesian Limestone, with mixed permeability superficial deposits, mainly boulder clay. The average rainfall data for the period of 1961 to 1990 was 654 mm/yr for the catchment. Between MUN and OXY is the town of Darlington, and a waste water treatment plant, and the second NRFA Skerne site (4 km north, Skerne at South Park; NZ 283 129). This site has a catchment area of 250.1 km^2, with an altitude range of 34.1 and 219.2 m AOD and is 5.1% woodland, 47.2% arable and horticulture, 29.3% grassland and 9.1% urban land use; again, there is no mountain, heath or bog within the catchment. The average rainfall data for the period of 1961 to 1990 was 644 mm/yr for the catchment. The underlying geology of the catchment is the same as the Preston-le-Skerne NRFA catchment. Using the catchment area of the NRFA sites, and the distance of river between the NRFA site and experiment site, the catchment area at MUN and OXY were calculated.

The fourth site (COT) was the furthest down the Tees catchment, approximately 100 m below the Skerne/Tees confluence, and 200 m from OXY. The catchment area and percentage peat cover of COT were calculated using the data from NRFA, OXY and DBS sites.

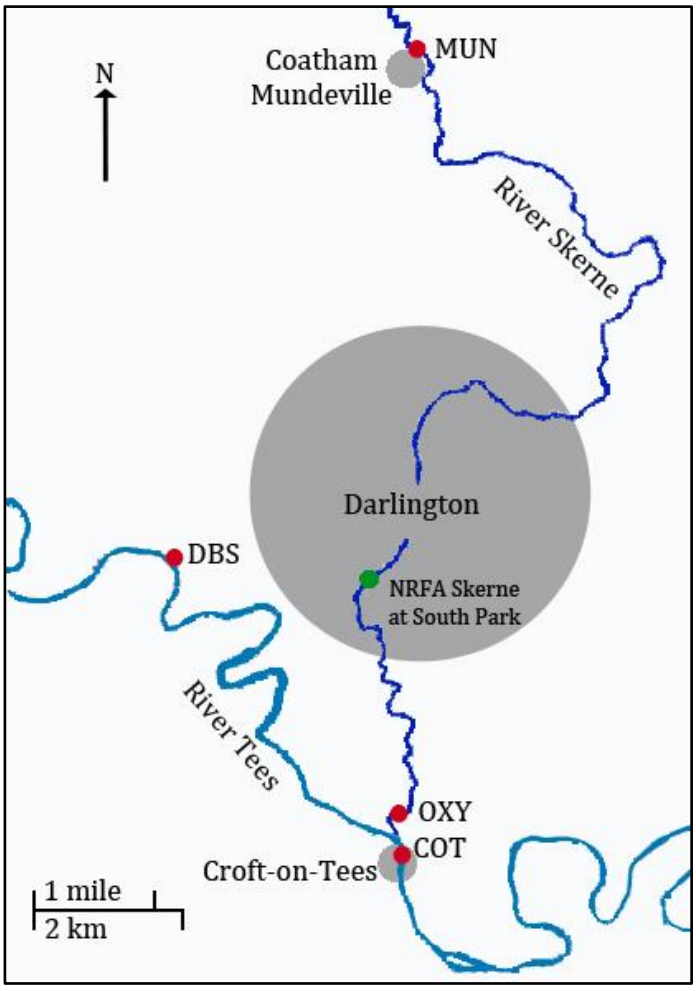
The Broken Scar gauging station is on the Tees, 8.1 km North West of the confluence with the Skerne (DBS, NRFA site 25001). The catchment area differs from the Skerne sites, as it contains much lower urban land use (0.4%) and much higher mountain, heath or bog land (33.9%). The maximum altitude in the catchment is much higher than the Skerne catchment, at 884.8 m AOD, and the average rainfall data for the period of 1961 to 1990 was almost twice as much as that for the Skerne sites, 1141 mm/yr.

These sites were chosen due to their proximity to NRFA monitoring sites and ease of river access. They provided the ideal sites to study the confluence and the effects of a large town on the water chemistry. The DBS site was used as it the DOC concentrations and degradations have been analysed in Chapters 2, 4 and 6, so comparisons with these other chapters could be made.

Table 7.1. The site codes, catchment areas, and percentage peat of the four sites used in this chapter.

Site and River	Site code	National Grid reference	Catchment area (km^2)	Peat cover (%)
Broken Scar, Tees	DBS	NZ 259 137	818.4	33.9
Coatham Mundeville, Skerne	MUN	NZ 291 207	157.5	0
Oxneyfield bridge, Skerne	OXY	NZ 289 102	253.5	0
Croft-on-Tees, Tees	COT	NZ 288 098	1085.95	25.55

Figure 7.1. A map showing the location of the Skerne and Tees sites, and the NRFA gauging station at South Park, in relation to Darlington, UK.



7.2.2. Degradation measurements

The degradation measurements were made as in Chapter 4, outside of the laboratory in ambient light and temperature conditions (rather than indoors under artificially controlled conditions).

Water samples were taken on a bi-monthly (every other month) basis from the four sites. Each degradation experiment spanned approximately 70 hours with sacrificial sampling taking place at hour 0, 1, 2, 8, and then at dawn and dusk on day 2, 3 and 4, with two treatments, light and dark. Replicates were included within each degradation experiment where equipment availability allowed. No hour 0 (t_0) samples were replicated, but 6% of all other measurements were replicated (23 of 407 samples). As in Chapter 4, a data logger with a PAR (photosynthetically active radiation) meter and thermocouple recorded the radiation levels and air temperature at 15-minute intervals throughout the 70-hour period of each experiment. Radiation and temperature conditions were summarised as the average conditions over the period for each sample, and PAR measurements were summed to give the total radiation experienced by any one sample. The radiation measurements were treated in this way because a sample after 70 hours may have experienced the same average radiation as a sample after 1 day but will have received a larger total radiation dose.

The first day of the experiment was conducted at the field sites so the samples were exposed to the same light and temperature conditions as the river. At dusk all tubes were taken to the laboratory and placed outside so they would continue to experience natural light and temperatures with on-going monitoring of these conditions.

7.2.3. Sample analysis

Samples were analysed for DOC, absorbance at 400, 465 and 665 nm, E4:E6, pH, water temperature and conductivity as in Chapter 4. The anion concentrations were measured in samples at t_0, t_{70} and in the middle of the experiment.

Suspended sediment (SS) concentration in each experiment was measured in samples at t_0, t_{70} and in the middle of each experiment. Samples were filtered through pre-weighed, 0.45 µm, glass fibre filters; dried to 105 °C and the filter paper re-weighed to give the concentration of suspended sediment. The filter papers were then put in a furnace for 4 hours at 550 °C, and then re-weighed. As in previous

chapters, the mass lost in the furnace equates to the mass of particulate organic matter (POM), and 47.5% of this was assumed to be particulate organic carbon (POC).

7.2.4. Statistical methodology

The design of the experiment incorporates four factors: Month, Time, Site and Treatment. Each factor has a number of levels: Month has five levels (one for each month sampled); Time has 10 levels (hour 0, 1, 2, 4, 9.17, 20.67, 30, 44.55, 54, 67.33); Site has four levels (COT, DBS, MUN, OXY); and Treatment has two levels (light and dark). The sample times are the averaged values (each has a standard error) that represent the samples taken on the first day (hours 0, 1, 2, 4, 9.17, henceforward called t_0, t_1, t_2, t_4 and t_{10}), dawn and dusk on day 2 (hours 20.67 and 30), dawn and dusk on day 3 (hours 44.55 and 54) and dawn on day 4 (hour 67.33, henceforward called t_{70}).

The concentrations of DOC and POC were analysed in both absolute and relative terms, where the relative value for each sample in an experiment was expressed as the ratio of the measured value to measurement at t_0 for the same site on that experimental run. The data were first tested for normality using the Anderson-Darling test, and then ANOVA, ANCOVA and regression analyses were used. The magnitude of the effects and interactions were calculated using generalized ω^2 as in previous chapters. For some analyses, the sites and/or treatments were analysed separately due to the differences between them, often this resulted in better r^2. Main effects plots were used to visualise the data as they show the overall effect of the factor once all other factors and covariates have been taken in to account. Where the environmental variables (PAR and temperature) were significant, the apparent quantum yield (AQY) and activation energies were calculated, as in previous chapters.

As in the previous chapters, the effect of priming was analysed by comparing the night time DOC degradation rates of the two treatments. Another aspect of priming was analysed, as to whether the addition of high nutrient and anthropogenic DOC water from the Skerne increased the rate of DOC degradation below the confluence of the Skerne and Tees.

One-way ANOVAs were carried out on the t_0 covariates and organic carbon concentrations (bromide, chloride, fluoride, nitrate, phosphate, sulphate, DOC and

POC concentrations, and the conductivity, pH, Abs_{400} and E4:E6) to look for differences between the sites, as outlined in the Introduction and Aims (section 7.1), more specifically to look at the following:

- The differences between upland and lowland river characteristics (comparing DBS and OXY)
- The differences in chemistry between above and below Darlington (comparing MUN and OXY)
- The effect of the confluence on the river chemistry (comparing DBS, OXY and COT)

A mixing analysis was carried out using regression analyses on the t_0 data from the three sites closest to the confluence (DBS, OXY and COT). These were applied to each variable in the data set to look for the regression equations for the confluence site, to see which upstream sites (DBS and OXY) were significant in modelling the water chemistry below the confluence (COT). It was expected that there would be a 1:1 ratio of contributions from the two rivers, if the covariates were behaving conservatively.

A Principal Component Analysis (PCA) was also carried out on the t_0 data, and on the light treatment data for the whole experiment, to look for end-members, to derive a mixing model for the confluence.

7.3. Results and Discussion

In total, results for 375 individual experiments with complete covariate information and within the context of the factorial design are available for analysis. Summary of the water chemistry at the four sites over the 70 hours of the experiment in light conditions are given in Table 7.2.

Some of the covariates showed the same pattern at all four sites between t_0 and t_{70}, as shown in Table 7.2. The E4:E6 and bromide concentration decreased, and the conductivity, pH, chloride and fluoride concentrations increased. The absorbance at 400 nm did not change at COT and OXY, but decreased at DBS and MUN. The nitrate concentration decreased at MUN but increased at the other three sites, whereas the phosphate and sulphate decreased at COT but increased at the three other sites. Of these changes, several were significantly different between t_0

and t_{70} from each site: the pH increase was significant from COT (p=0.0246), DBS (p=0.0191), MUN (p=0.0043) and OXY (p=0.0179), the E4:E6 decrease at DBS (p=0.0392), the fluoride increase at DBS (p=0.0440), MUN (p=0.0005) and OXY (p=0.0053), and the phosphate increase at DBS (p=0.0131) were also significant.

The concentrations of the conservative ions (bromide and chloride) were investigated further to show how much natural variability there was in the dataset. An ANOVA on the whole dataset showed there were no significant differences between Site, Time or Treatment for either anion.

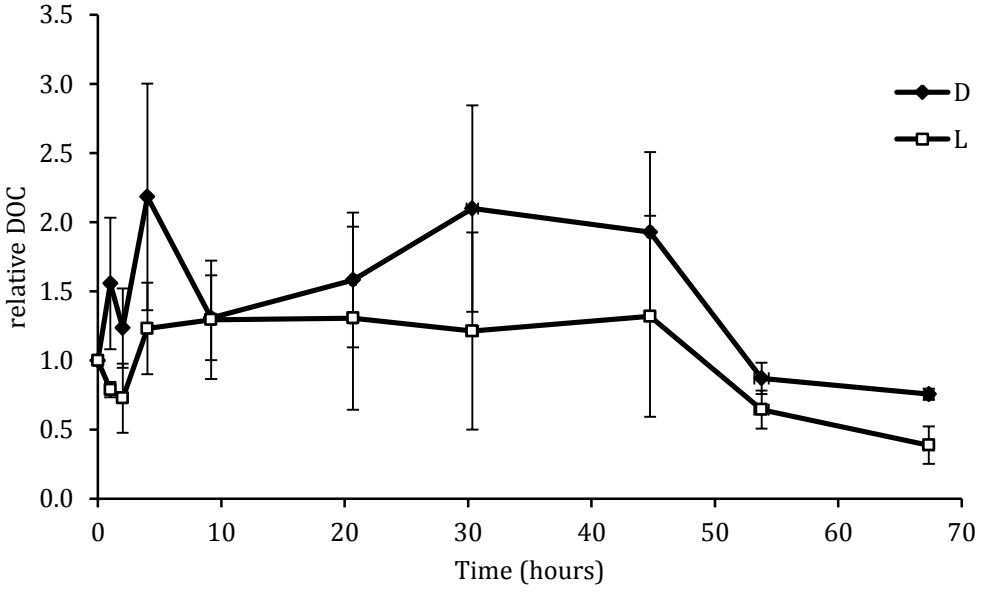
Table 7.2. The average and coefficient of variation (CV - %) of the 12 variables measured from the four sites (COT, DBS, MUN and OXY), averaged across all five sampling months. The table shows the initial (t_0) and end (t_{70}) concentrations for each variable from the light treatment.

	Croft-on-Tees (COT)				Broken Scar (DBS)				Coatham Mundeville (MUN)				Oxneyfield Bridge (OXY)			
	t_0		t_{70}		t_0		t_{70}		t_0		t_{70}		t_0		t_{70}	
Determinant	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
POC (mg C/l)	2.35	90	3.25	30	1.94	73	3.47	13	1.90	71	3.10	4.9	2.18	77	3.82	19
Conductivity (µS/cm)	826.6	24	936.3	17	714.8	35	800.3	25	1005	20	1073	2.1	1034	18	1124	9.6
pH	7.64	4.6	8.19	0.5	6.25	7.6	7.12	1.3	7.49	3	8.02	1.7	7.77	2.3	8.09	1.1
DOC (mg C/l)	26.38	91	9.12	56	28.42	71	2.82	76	28.99	84	17.82	40	46.59	88	7.13	93
Abs_{400}	0.04	18	0.04	60	0.04	52	0.03	60	0.04	34	0.02	47	0.03	51	0.03	32
E4:E6	3.73	48	2.08	42	5.07	52	1.37	19	3.39	59	1.61	22	2.71	60	1.62	35
Bromide (mg/l)	1.51	31	1.03	25	1.39	29	1.09	84	1.53	48	1.07	52	1.47	38	0.97	48
Chloride (mg/l)	79.5	47	140.4	53	63.9	53	77.03	33	87.17	20	103.5	20	98.07	39	138.7	30
Fluoride (mg/l)	0.02	224	0.27	160	0.00	0	0.22	146	0.02	224	0.69	67	0.00	0	0.46	86
Nitrate (mg/l)	20.74	25	25.21	15	16.91	50	26.06	66	23.55	23	19.7	40	21.57	20	23.63	4.2
Phosphate (mg/l)	1.62	7.8	1.58	2.2	1.55	4.4	1.76	11	2.68	91	1.64	7.6	1.57	5.0	1.75	17
Sulphate (mg/l)	337.8	72	256.1	18	92.91	51	104	8.8	331	28	423.2	21	314.7	21	844.9	100

7.3.1. DOC concentrations

The mean average DOC concentration changes in the light and dark were more complicated than in previous chapters, and did not exhibit a clear diurnal cycle (Figure 7.2). The DOC still declined overall, but there were quite large increases in the concentration in both treatments. The dark treatment DOC concentrations increased to approximately twice their starting concentration and stayed high until the last two sampling times when the DOC decreased below the initial concentration. The light treatment DOC concentration initially decreased to approximately 75% of the starting concentration in the first few hours, then increased and stayed higher than the starting concentration until decreasing in the last two sampling times, as in the dark DOC concentrations. Generally the dark DOC concentration was higher than the light DOC concentration; however they were very close at t_{10} and towards the end of the experiment.

Figure 7.2. The main effects plot of relative DOC concentration change for light and dark over the course of the experiment. D = dark, L = light. Error bars are standard errors.



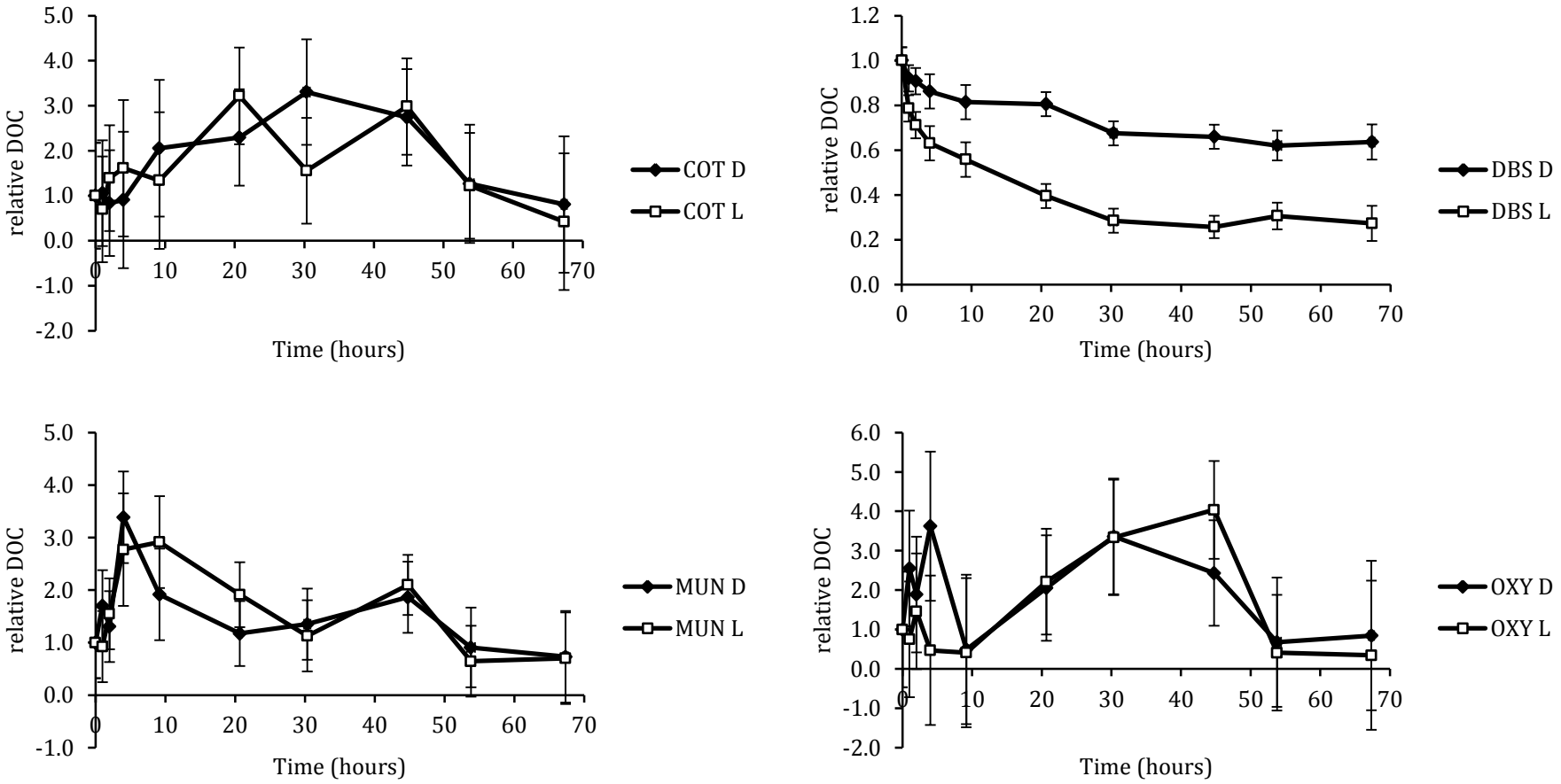
The average decline in DOC concentration across all months for all sites for samples in daylight was from 36.52 to 9.40 mg C/l after 70 hours: when

concentrations were judged relative to the DOC_0 concentration (initial DOC concentration at t_0) at each site the average decline over 70 hours was 74%. For experiments only in the dark the average decline over a 70-hour period was 29%. The average difference across all sites and all times between samples in light and dark was 6.95 mg C/l; DOC_{70} concentrations (DOC concentration at t_{70}) of samples kept in the light were on average 45% lower than those kept in the dark when judged relative to the DOC_0.

When the average DOC concentrations for each site were viewed separately (Figure 7.3), the COT water DOC concentrations increased in both treatments towards the middle of the experiment, then decreased towards the end, with both the light and dark DOC_{70} concentrations lower than DOC_0. The average DOC concentrations in the MUN water initially increased and peaked at approximately t_{10}, then decreased towards the end of the experiment. Again, both the light and dark DOC_{70} concentrations lower than DOC_0. The OXY water DOC concentrations were highly variable, with increases and decreases observed throughout the experiment. The average DOC concentrations in both treatments showed an increase between t_{10} and t_{30}, where the dark treatment decreased to the end of the experiment and the light treatment increased for one more sampling time, then decreased as well. Again, both the light and dark DOC_{70} concentrations lower than DOC_0. These three sites have very large error bars on the DOC concentrations at all times and in both treatments, showing that the concentrations were large highly variable. There are no obvious differences between the two treatments, and they do not exhibit the diurnal cycle of DOC observed in the previous chapters.

The DBS water followed a similar pattern as in previous chapters (Chapters 2, 4 and 6), displaying clear differences between the two treatments and a steady decline in DOC concentrations (Figure 7.3), rather than the less clear response observed when all four sites were analysed together (Figure 7.2). The error bars are much smaller on the DOC concentrations are much smaller than for the three other sites, showing that the DOC concentrations were much less variable throughout the experiment. The average decline in DOC concentration across all months for DBS for samples in daylight was from 28.42 to 4.16 mg C/l after 70 hours: when concentrations were judged relative to the DOC_0 concentration the average decline over 70 hours was 82%. For DBS samples in the dark the average decline over a 70-hour period was 36%. The average difference between samples in light and dark

Figure 7.3. The main effects plot of relative DOC concentration change by site, for light and dark over the course of the experiment. D = dark, L = light. Error bars are standard errors.



was 8.37 mg C/l; DOC_{70} concentrations of DBS samples kept in the light were on average 53% lower than those kept in the dark when judged relative to the DOC_0.

The large error bars on the DOC concentrations from COT, MUN and OXY show the data was highly variable. An ANOVA on the standard errors of the DOC concentrations from all four sites showed that the errors, and therefore the variability of the data, were significantly smaller from DBS than the other three sites (p=0.0007). The same ANOVA was applied to the conductivity, pH and anion concentrations to find whether this variability was specific to the DOC concentrations or was found in other variables too. The ANOVA on the conductivity standard errors showed that there were significant differences between the sites (p0.0014), with the DBS site having significantly higher standard errors than the other three sites. The ANOVA on the pH data showed the same as the conductivity (p=0.0016). The ANOVA on the anion concentrations showed that there were no significant differences in the standard errors between the four sites for any of the anions measured (bromide p=0.5357, chloride p=0.4817, fluoride p=0.3617, nitrate p=0.6877, phosphate p=0.8151, sulphate p=0.2635). This showed that the large variability in the DOC concentrations from COT, MUN and OXY was not accompanied by large variation in the other variables, and the low variability in the DOC from DBS was not accompanied by small variation in the other variables.

Of all the experiments run, there were 131 samples, almost a third of the total of 375 samples, where an increase in DOC concentration was observed relative to the initial DOC concentration. In two of the cases there was a higher DOC_{70} concentration than t_0. As in previous chapters, no raw water samples were filtered prior to inclusion in the experiment it was possible that particles or the microbial population within the sample generated DOC over the course of the experiments. Samples where there was an increase in DOC over the course of the experiment were not removed from the analysis, as the experiment was interested in the conversion of POC to DOC.

7.3.1.1. ANOVA on DOC concentrations

The Anderson–Darling test showed that neither the distribution of DOC concentration nor relative DOC concentration for the experiments conducted in the light, nor those in the dark, met the condition of normality, therefore all subsequent ANOVA were performed on log-transformed data as that did meet the test

conditions, and the Anderson-Darling test showed that no further transformation was necessary.

When the relative concentration data for both treatments (light and dark) were considered without covariates, all single factors were found to be significant (Table 7.3). The least important single factor was Time (Figure 7.2; explaining only 2.36% of the variance in the original dataset). The most important single factor was Site, explaining 6.77% of the original variance. Four interactions were also significant. Overall the ANOVA of the relative DOC concentration explained 53.25% of the variance in the original data. The error term represented 13.55% of the variance. This error term represents the unexplained variance in the model, which was not only due to sampling or measurement error but also variables, factors or their interactions that were not or could not be included in the ANOVA, such as the river discharge at the start of each experiment.

Table 7.3. Results of ANOVA for relative DOC concentrations for all experiments and all sites across both daylight and dark treatments.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
Sulphate	na	-	<0.0001	3.02
Site	<0.0001	6.77	<0.0001	17.18
Treatment	<0.0001	3.76	<0.0001	8.06
Time	0.0053	2.36	ns	-
Month	<0.0001	6.32	ns	-
Site*Treatment	0.0036	1.69	0.0021	1.85
Site*Time	0.0003	5.63	ns	-
Site*Month	<0.0001	11.73	<0.0001	52.49
Treatment*Time	0.0370	1.44	ns	-
Error		13.55		6.25

Including covariates in the ANOVA showed the most important covariate was the sulphate concentration of the water, which explained 3% of the variation in the data (Table 7.3). Adding the covariates decrease the significance of the Month and Time factors so they became insignificant, as did two interactions. However, the r^2 increased from 0.53 to 0.86, suggesting that the model with fewer factors and

interactions is a better fit of the data. Neither temperature nor PAR were significant covariates.

An ANOVA on the sulphate data itself showed that it different significantly by Site and Month, but not between Treatments or over Time, with Site explaining 23% of the variation in the data. The significance of sulphate as a covariate in the ANCOVA on DOC does not make the Site factor insignificant, which would be expected in the differences between the sites were due to the sulphate concentration.

As the ΣPAR was not significant in the ANCOVA, no apparent quantum yield (AQY) was estimated for changes in DOC concentrations. No activation energies were calculated, as there was no significant effect of temperature either.

Guided by the results of the DOC ANOVA and ANCOVA, a regression analysis was carried out on the light treatment data, and the following regression equation was found:

$$ln\Delta DOC = \underset{(0.09)}{0.68}\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + \underset{(0.06)}{0.22}lnt + \underset{(0.18)}{1.47}$$

n=125, r^2=0.43 *(Eq. 7.1)*

Where ΔDOC is the change in DOC concentration (mg C/l), m is the month number and lnt is the log of the time. The numbers in brackets are the standard errors of each coefficient. The equation (Eq. 7.1) shows that the change in DOC can be calculated from the Month and Time, and is not dependent on any other factors or covariates. In Chapter 4, the change in the DOC concentration (Eq. 4.1) was also shown to be dependent on the month of the year and the time of the experiment, however there were other significant parameters: the pH and the DOC_0. In Chapter 4, the r^2 of the regression equations generally improved when the sites were considered separately, and this was also the case in this chapter. The r^2 increased from 0.43 for all sites to 0.52 for COT alone, 0.66 for DBS alone, 0.49 for MUN alone and 0.47 for OXY alone, with the same two factors (Month and Time) being significant in the equations (Table 7.4).

Table 7.4. The results of the regression analysis on the four sites separately, showing the parameter estimates for month and time, and the standard errors in brackets.

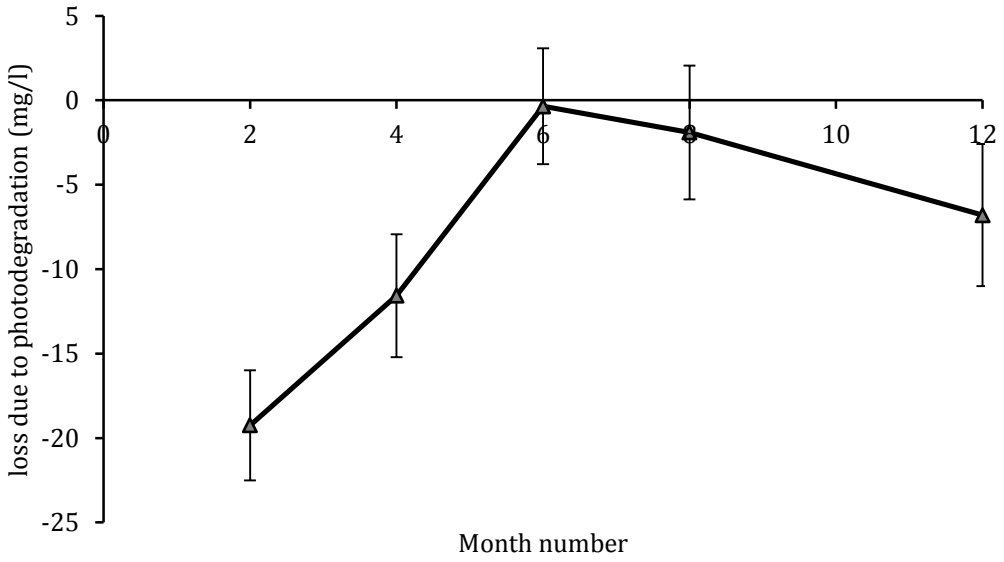
Site	n	r^2	Month (sinusoidal)	ln time	Intercept
COT	27	0.52	0.71 (0.16)	0.15 (0.11)	1.32 (0.31)
DBS	43	0.66	0.45 (0.08)	0.40 (0.06)	1.27 (0.19)
MUN	22	0.49	1.01 (0.24)	0.03 (0.17)	1.42 (0.53)
OXY	33	0.47	0.99 (0.22)	0.16 (0.15)	1.77 (0.43)

The parameter estimates show the relative importance of the Month and Time factors in the change in DOC concentrations between the four sites, e.g. the significantly higher estimate of the Time parameter for DBS showed that the change in DOC varied more through the experiment than at the other sites, which all had significantly lower parameter estimates. DBS also had a significantly lower Month parameter estimate than the other sites, showing that it varied least with the seasonal cycle. The DBS equation had the highest r^2, suggesting that the model of the change in DOC concentrations for that site explained the most variation in that data.

7.3.2. Photo-induced degradation

The difference between the dark and light concentrations in each experiment was taken as the estimate of the impact of photic processes (Figure 7.4). The extent of photo-induced degradation could be estimated in 159 cases and the loss due to photo-induced degradation varied from 92.14 mg C/l to -87.21 mg C/l (i.e. there were 44 occasions where the DOC concentration was observed to increase, implying photo-induced production). Of the 44 occasions where an increase was observed, only 11 were higher than 10 mg C/l, showing the majority of cases have higher dark DOC than light DOC, or a very small difference between the two. The average photo-induced degradation over the 70 hours was -8.60 mg C/l: the dark treatment DOC was on average 8.6 mg C/l higher than the light treatment DOC.

Figure 7.4. The average change in loss due to photo-induced degradation over the course of the experiment. Error bars are the standard errors.



The ANOVA shows that only Month was significant (Table 7.5). The DOC loss due to light was significantly greater in the winter and spring than in the summer (Figure 7.4). The ANCOVA results show that the only significant covariate was the DOC_0 concentration, explaining 8% of the variation, showing that the difference in DOC concentrations between the treatments is influenced by the initial concentration of DOC.

Table 7.5. Results of ANOVA for the difference in DOC concentrations between light and dark treatments

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	<0.0001	8.34
Month	0.0008	9.22	0.0146	4.57
Error		2.35		7.56

Guided by the results of the ANOVA and ANCOVA, the following regression equation was calculated for the loss of DOC due to exposure to light:

$$\Delta DOC_{photo} = -2.07\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) - 0.25DOC_0 - 0.18$$
(1.92) (0.08) (2.94)

n=159, r^2=0.14 *(Eq.* 7.2*)*

where ΔDOC_{photo} is the change in the difference between the dark and light DOC concentrations (mg C/l) and DOC_0 in the initial DOC concentration (mg C/l). Again, the regressions were carried out for each site separately, and the r^2 decreased for COT (0.06) and MUN (0.07), and increased for DBS (0.53) and OXY (0.23) but the parameters were all insignificant except for in the DBS equation. This showed that a large proportion of the photo-induced change in DOC in DBS water was explained by the model, with the month and DOC_0 as significant influences on the DOC concentration (Eq. 7.3). When ΔDOC_{photo} is negative, there is a higher dark DOC than light DOC, and so the negative relationship between ΔDOC_{photo} and DOC_0 shows that when the initial DOC was high, there was higher dark DOC than light DOC, and so a larger amount of photo-induced loss of DOC. For the DBS samples, there were only four out of 41 samples where photo-production of DOC occurred, showing that the majority of DBS samples experienced photo-induced losses of DOC.

DBS:

$$\Delta DOC_{photo} = -3.56\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) - 0.13DOC_0 - 5.01$$
(1.15) (0.07) (2.15)

n=41, r^2=0.53 *(Eq.* 7.3*)*

where all terms are as described as above. The regression analysis only found significant parameters for the DBS site, so no further analysis was carried out on the three other sites.

7.3.3. Rate of degradation in the light

For samples in the light, the degradation rate varied from -43.54 mg C/l/hour to 31.78 mg C/l/hour (Figure 7.5); i.e. increase or no change in DOC concentrations were observed in 44 cases out of 166 (resulting in negative rates). Again, out of the 44 cases that observed negative rates, only two were less than -10 mg C/l/hour, showing that the majority of cases have a positive rate of degradation. The average rate of degradation in the light was 0.97 mg C/l/hour.

The average light rate was negative at t_2, but positive at t_1 and t_4, suggesting that the DOC concentration increased fairly early in the experiment, and then decreased again. This could be due to particulates breaking down and releasing DOC.

The ANOVA of the rate of degradation for samples in the light showed that only Time was significant (Table 7.6). No Treatment factor was included because only experiments in the light were being considered. The variance explained by the Time factor was fairly small, with the error term being quite large. Site was not found to be a significant factor. Adding covariates decreased the error term, and found that the DOC_0 was significant in addition to Time, and explained a larger proportion of the variation than the Time factor.

Table 7.6. The results of ANOVA of the degradation rate of DOC in the light

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	<0.0001	8.94
Time	0.0447	4.30	0.0456	4.35
Error		37.97		4.32

Guided by the results of the ANOVA and ANCOVA, the following regression equation was calculated for the rate of loss of DOC in the light treatment:

$$rate_{light} = -0.63lnt + 0.07DOC_0 + 0.27$$
$$\quad (0.32) \quad (0.02) \quad (1.07)$$

n=166, r^2=0.11 *(Eq. 7.4)*

where $rate_{light}$ is the rate of DOC degradation in the light (mg C/l/hour) and all other terms are as defined above. Again, the regressions were carried out for each site separately, and the r^2 decreased for COT (0.009) and MUN (0.03), and increased for DBS (0.48) and OXY (0.30). The parameters were significant for DBS and OXY but not for COT and MUN.

DBS:

$$rate_{light} = -1.13lnt + 0.06DOC_0 + 2.65$$
$$(0.21) \quad (0.02) \quad (0.75)$$

n=43, r^2=0.48 *(Eq. 7.5)*

OXY:

$$rate_{light} = -1.63lnt + 0.09DOC_0 + 2.62$$
$$(0.67) \quad (0.03) \quad (2.29)$$

n=42, r^2=0.30 *(Eq. 7.6)*

where all terms are as described above.

These regression equations (Eq. 7.4, 7.5 and 7.6) all have a significant, negative relationship between the rate of DOC decline and the time of the experiment (t), and a significant, positive relationship with the initial DOC concentration. This shows that the rate declines as t increases, and a higher rate when the initial DOC concentration is high.

7.3.4. Rate of degradation in the dark

It was possible to calculate the rate of degradation in the dark in 160 experiments, which ranged from -45.58 mg C/l/hour to 51.24 mg C/l/hour, (in 54 cases, an increase or no change in DOC concentration was observed), however, only seven values fell outside the -10 to 10 mg C/l/hour range. The median value for the rates of dark degradation was 0.11 mg C/l/hour, i.e. the majority of the rates were negligible (Figure 7.5).

The average rate of DOC degradation in the dark showed almost the opposite pattern to the light treatment in the first few hours of the experiment, with the t_1 and t_4 rates being negative, but the t_2 rate being positive. From t_{10} onwards there were very few differences between the two treatments (Figure 7.5).

Figure 7.5. Main effects plot of rate of DOC loss in light and dark over time in the experiment. Error bars give the standard error.

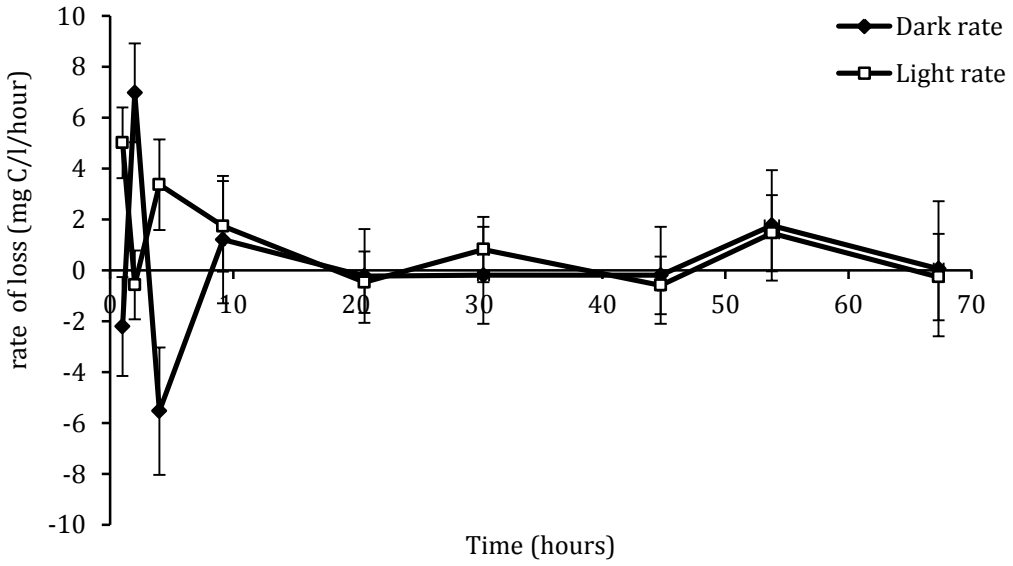


Table 7.7. The results of ANOVA of the degradation rate of DOC in the dark.

	Without covariates	
Factor	p	ω^2
Time	0.0164	5.22
Time*Month	<0.0001	23.43
Error		16.25

For the rate of degradation in the dark, the ANOVA showed that Time and the interaction of Time*Month were significant, explaining 5.22% and 23.43% of the variation respectively (Table 7.7). No covariates, and no regression equation, were found to be significant when the four sites were analysed together. However, a

significant regression equation could be calculated for the rate of degradation in the dark for the DBS water:

DBS:

$$rate_{dark} = \underset{(0.12)}{-0.41}lnt + \underset{(0.01)}{0.02}DOC_0 + \underset{(0.44)}{0.96}$$

n=41, r^2=0.26 *(Eq. 7.7)*

where $rate_{dark}$ is the rate of DOC degradation in the dark (mg C/l/hour) and all other terms are as defined above. The significance of the regression of the DBS samples shows that they could be modelled, whereas the rates for the other three sites were less predictable in the same way. The dark DBS equation (Eq. 7.7) found the same parameters to be significant as the light DBS equation (Eq. 7.5), with similar relationships to the light equation, e.g. a negative relationship with time and a positive relationship with DOC_0. However the DOC_0 and intercept parameter estimates were significantly higher and the t parameter was significantly lower for the light equation than the dark equation. Also the light equation had a much higher r^2, almost double the dark equation r^2, suggesting that the light data was a better fit to the model.

7.3.5. The rate of photo-induced degradation

The rate of the photo-induced degradation could be calculated from 158 experiments and varied from -113.51 mg C/l/hour to 69.65 mg C/l/hour, (in 64 cases an increase or no change was observed). Of all the cases where a negative rate was observed, only five cases were the rates less than -10 mg C/l/hour, showing that the majority of cases showed an increase, or a small decrease (Figure 7.6). The average rate of photo-induced degradation was 0.51 mg C/l/hour, and the median value was 0.10 mg C/l/hour.

Figure 7.6. Main effects plot of rate of DOC loss due to light over time in the experiment. Error bars give the standard error.

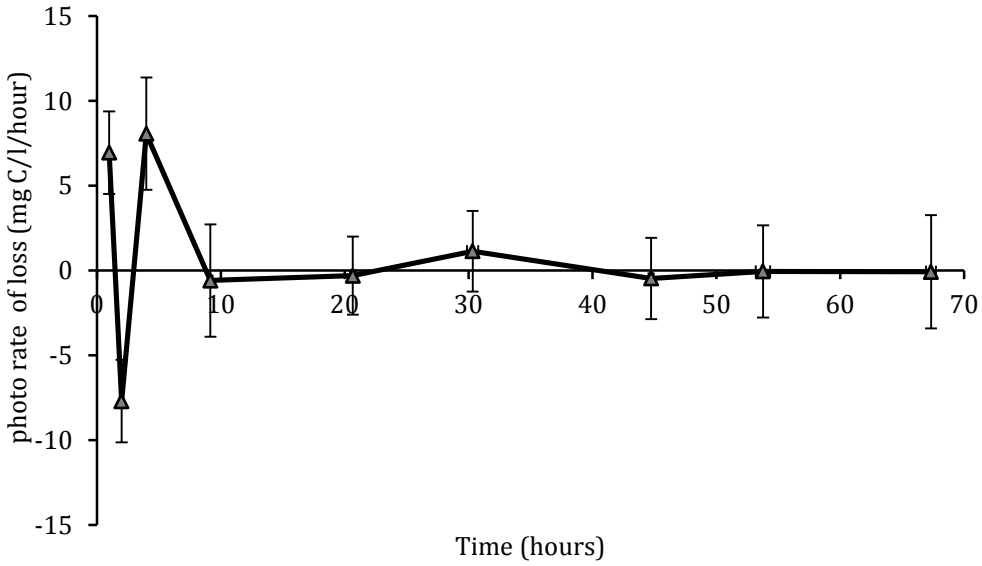


Table 7.8. The results of ANOVA of the photo-induced degradation rate of DOC

	Without covariates	
Factor	p	ω^2
Time	0.0040	7.06
Time*Month	<0.0001	23.44
Error		16.33

Time and the interaction of Time*Month were found to be significant in an ANOVA (Table 7.8). The interaction was the most important component of the ANOVA, explaining 23.44% of the variation. No covariates, and no regression equation, were found to be significant when the whole dataset was analysed. However, as for the dark rate, a significant equation was calculated for the DBS water, which showed that the DBS rate of photo-induced degradation was influenced by the DOC_0, and the time and the month of the experiment (Eq. 7.8).

DBS:

$$rate_{photo} = -0.78lnt + 0.54\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + 0.01DOC_0 + 2.35$$

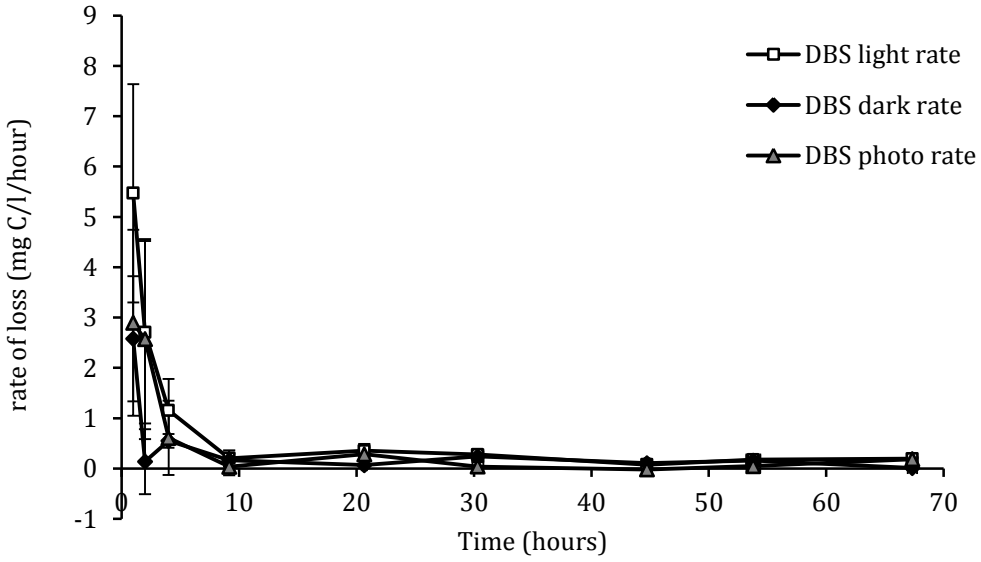
(0.21) (0.39) (0.02) (0.89)

n=41, r^2=0.33 *(Eq. 7.8)*

where all terms are as described above. The relationship between the photo rate and DOC_0 was positive, and negative with the time parameter, indicating that the rate of photo-induced loss increased as the initial DOC concentration increase and as was highest when the *t* was small, i.e. during the first few hours of the experiment.

The light, dark and photo-induced rates have all had significant regression equations for the DBS water (Eq. 7.5, 7.7 and 7.8), but not for the other three sites. The same two parameters were significant in all three equations (time and DOC_0) and the month was also significant in the photo-induced rate. Comparing these equations to those calculated in Chapter 4 for the rates of DOC change in DBS water (Eq. 4.10 and 4.12) show that the r^2 were larger in Chapter 4, and the time of the experiment was also a significant parameter. The graph of the average light, dark and photo-induced DOC rates for DBS (Figure 7.7) shows a difference pattern to the graphs for the averages of all four sites (Figures 7.5 and 7.6), and the graph for DBS is much more similar to the graphs in Chapter 4 (Figures 4.3 and 4.4). It is interesting to note that despite these differences, the ANOVAs showed no significant differences in the rate data between the sites (Tables 7.6, 7.7 and 7.8).

Figure 7.7. The rate of DOC change in the light and dark treatments, and the photo-induced rate for the DBS water. Error bars are the standard errors.



7.3.6. Rate of degradation during each day and night

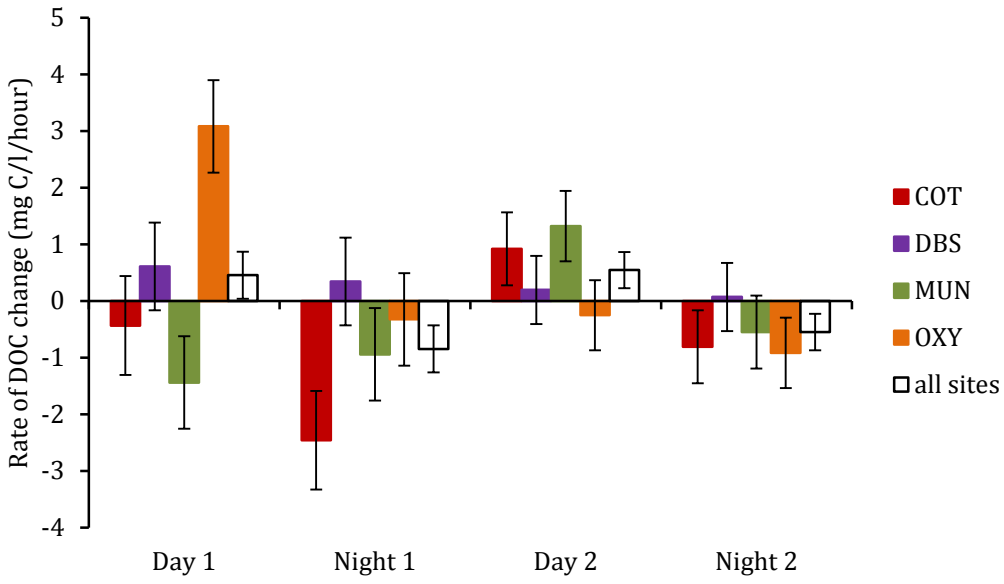
The rates of degradation for each stage of the experiment were calculated as in Chapters 4 and 6. The rates in each stage varied from -9.38 mg C/l/hour to 10.97 mg C/l/hour, with an average value of 0.29 mg C/l/hour. The averages for the light and dark treatments were 0.38 and 0.18 mg C/l/hour respectively.

An ANOVA on the rates of degradation during each stage of the experiment had four factors: Treatment, Site, Stage and Month, with the "Stage" factor having four levels: day 1, night 1, day 2 and night 2. The ANOVA found that Stage was the only significant individual factor (Table 7.9). Two interactions were also significant: Month*Stage and Site*Stage. Month*Stage explained the largest proportion of the variance (10%), followed by Site*Stage (7%). Stage alone only explained 4% of the variation. The post hoc analysis showed that there were significant differences between the following stages: Day 1 and Night 1, Day 1 and Night 2, Day 2 and Night 1, Day 2 and Night 2, i.e. both days were significantly different to both nights, but neither day was significantly different from the other day, and neither night was significantly different from the other night.

Table 7.9. The results of the ANOVA on the rates of degradation in each stage.

	Without covariates	
Factor	p	ω^2
Stage	0.0133	3.58
Month*Stage	0.0012	10.14
Site*Stage	0.0075	7.39
Error		13.62

Figure 7.8. The main effects plot for the rate of DOC change in the first four stages of the experiment for the four sites separately and together. The error bars are the standard errors.



The Stage rates of DOC change are shown in Figure 7.8, and show that the average of all sites has a positive rate during the two days (DOC was lost), and a negative rate during the two nights (DOC was gained). For DBS, the average rate decreased from 0.61 to 0.07 mg C/l/hour from Day 1 to Night 2, and is the only site to have positive average rates for all four stages of the experiment. The other three sites, COT, MUN and OXY had negative average rates for three of the four stages: both COT and MUN gained DOC on Day 1, Night 1 and Night 2, but lost DOC on Day 2, whereas OXY lost DOC on Day 1, and gained DOC on Night 1, Day 2 and Night 2.

However the error bars suggest that the data is spread across both the positive and negative sides of the line, and so DOC was lost and gained in several of these cases (as also shown in Figure 7.3). Post hoc analysis of the Stage*Site interaction showed that COT Night 1 was significantly lower and OXY Day 1 was significantly higher than the other combinations.

7.3.7. Initial rates of degradation

The initial rates of DOC degradation (during the first hour of the experiment) varied from 15.82 to -13.51 mg C/l/hour. The averages for the light and dark treatments were 5.02 and 1.36 mg C/l/hour respectively.

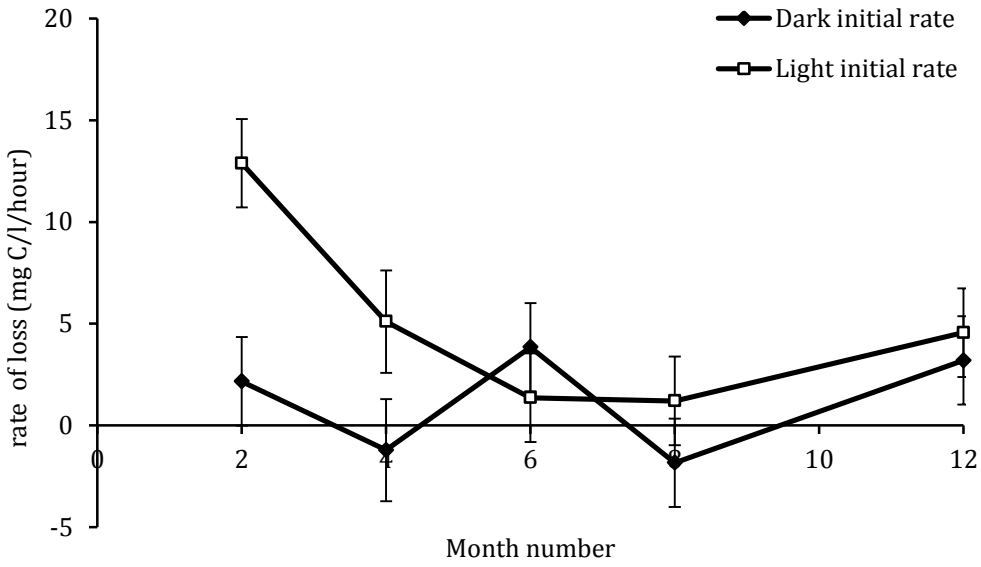
An ANOVA on the rates of degradation during the first hour of the experiment had three factors: Treatment, Site and Month. The ANOVA found Treatment and Month were significant (Table 7.10), but none of the interactions were significant. The Month factor explains the largest proportion of the variance (15.22%). The initial rate in the light shows a seasonal pattern, where the rate of degradation is closer to zero in the middle of the year (Figure 7.9). The rate in the dark has a less clear seasonal pattern, experiencing negative rates (gain of DOC) in two of the five months.

Adding covariates found that Month was no longer significant, and DOC_0 was significant, explaining 11% of the variance. This showed that the rate of degradation in the first hour of the experiments was determined by the initial concentration and whether the water was exposed to light or not.

Table 7.10. The results of the ANOVA on the rates of degradation in the first hour.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	0.0157	11.41
Treatment	0.0242	9.15	0.0272	9.03
Month	0.0367	15.22	ns	-
Error		10.64		4.72

Figure 7.9. The main effects plot for the initial rate of loss in the light and dark treatments for each month. Error bars are the standard errors.



Guided by the results of the ANOVA and ANCOVA, the following regression equation was calculated:

$$lnRate_0 = 0.009DOC_0 + 0.11NO_3^- - 0.001SO_4^{2-} - 1.08$$
$$(0.006) \quad (0.03) \quad (0.001) \quad (0.57)$$

n=34, r^2=0.34 *(Eq. 7.9)*

where $Rate_0$ is the initial rate of DOC loss (mg C/l/hour), and NO_3^- and SO_4^{2-} are the initial concentration of nitrate and sulphate respectively (mg/l). The regression equations for the initial rate of degradation were also calculated for the two treatments separately:

Light:

$$lnRate_0 = 0.68\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + 0.06NO_3^- - 0.001SO_4^{2-} + 0.35$$
$$(0.14) \quad (0.03) \quad (0.001) \quad (0.58)$$

n=17, r^2=0.74 *(Eq. 7.10)*

Dark:

$$lnRate_0 = 0.09NO_3^- - 1.03$$
$$(0.03) \qquad (0.72)$$

n=17, r^2=0.29 *(Eq. 7.11)*

where all terms are as described above. These equations (Eq. 7.10 and 7.11) show that different parameters affect the initial rates of degradation in the light and dark. The dark rate is only influenced by the nitrate concentration of the water, whereas the light rate is affected by the month of the year and the sulphate concentration, in addition to the nitrate concentration of the water. The significance of the nitrate and sulphate concentrations in the regressions is likely to be due to both having significant differences between Site, and so the anion concentration provided a proxy measurement for site.

In Chapter 4, the initial rate of degradation in the light was affected by the DOC_0 and the month of the year (Eq. 4.13), however this was for the upland headwater site (CHS) and so cannot be directly compared to the results above.

7.3.8. Priming

An ANOVA based on the night-time rates, using Treatment, Site and Month as factors, found no significant differences in the rate of degradation over-night between treatments, and so there was no effect of the day of exposure to light on the night-time rates.

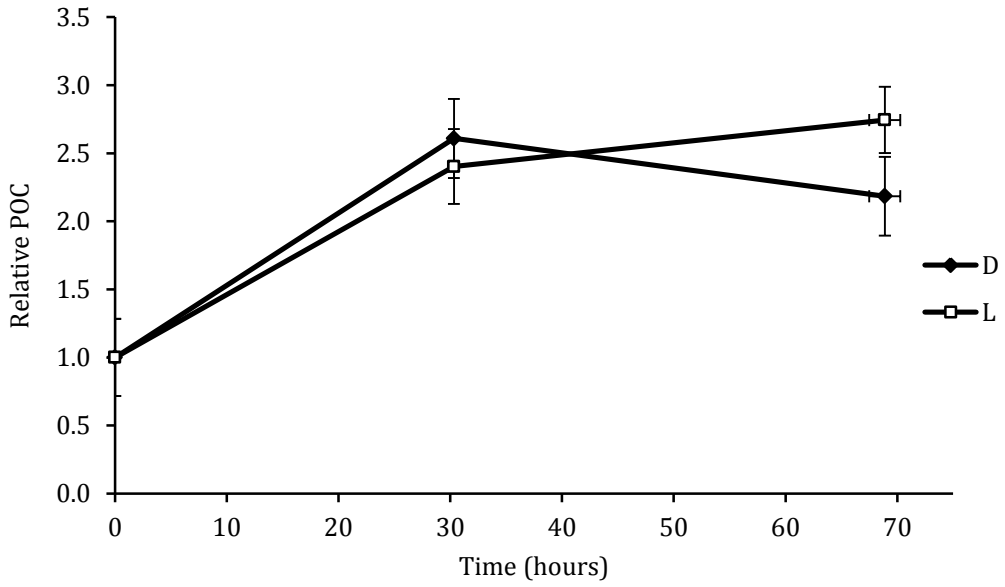
The addition of DOC from point sources on the lowland river also did not have a significant priming effect on the overall rates of DOC degradation in the light or dark, as there were no significant DOC differences between the sites above and below the waste water treatment plant, or below the confluence. However, there were significant differences between the DOC degradation rates during the first four stages of the experiment (Table 7.9, Figure 7.8), where the degradation rates from the site downstream of the waste water treatment plant (OXY) was significantly higher than the rates observed at the other three sites. Comparable differences were not found in the initial rates of DOC degradation, and so the difference must be due to higher rates of DOC degradation between t_2 and t_{10}. This would suggest that

the DOC from OXY was not instantly degraded, but was still labile enough to result in a large loss in the first day.

7.3.9. POC concentrations

The POC concentrations varied from 0.70 mg C/l to 5.65 mg C/l, with the average value of 2.85 mg C/l. The average relative POC in the light treatment was 2.67 and in the dark was 2.40, showing that the POC concentrations increased in both treatments. The relative concentrations in the light treatment increased from t_0 to t_{70}, whereas the POC in the dark treatment increased between t_0 and t_{30}, and then decreased slightly between t_{30} and t_{70} (Figure 7.10).

Figure 7.10. The main effects plot of the change in POC concentration in the two treatments over time. The error bars are the standard errors. D = dark, L = light.

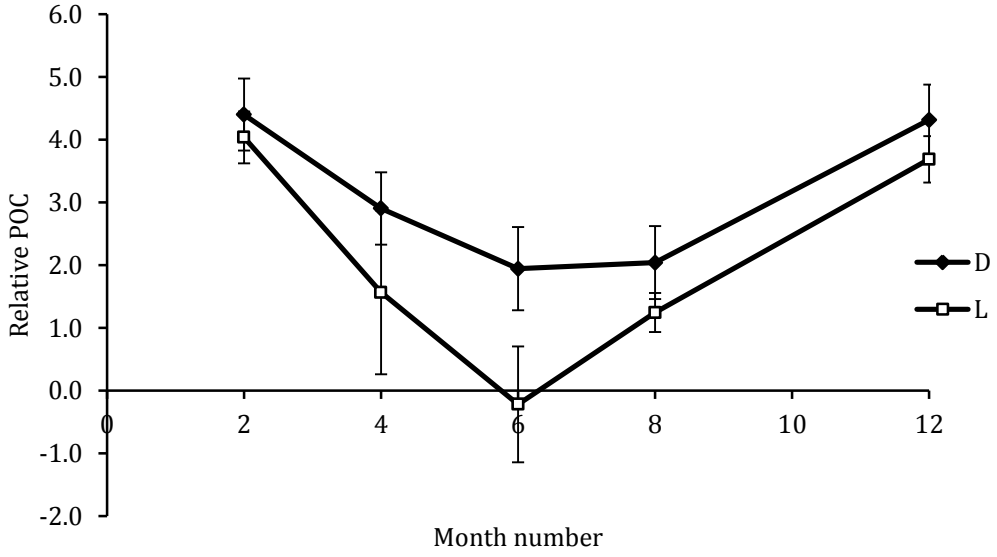


7.3.9.1. ANOVA on POC concentrations

The Anderson-Darling test showed that the 126 POC data points were not normally distributed, and so the data were log-transformed. The Anderson-Darling test on the log-transformed data showed that the data were normally distributed, and so the ANOVA and ANCOVA used this data. The ANOVA on the log of the relative POC concentrations found that there were no significant differences between Sites (Table

7.11). There were significant differences between the other three factors, and the interactions of Treatment*Time, Treatment*Month and Time*Month. The Month factor explained the largest proportion of the variation (37%) and the Treatment factor explained the smallest (0.60%). Applying the same ANOVA to the raw POC values (not relative) found that the Month factor explained a similar proportion of the variation, and so using the relative POC did not eliminate monthly differences.

Figure 7.11. The main effects plot of the change in POC concentration in the two treatments over the months. Error bars are the standard errors. D = dark, L = light.



Adding covariates to the model found that POC_0 and cumulative PAR were significant, explaining 13% and 5% of the variation respectively. The Treatment factor, and Treatment*Time and Time*Month interactions were no longer significant, and the proportion of the variation explained by the whole model decreased. Month still explained the largest proportion of the variation and the interaction of Treatment*Month explained the second largest proportion. The average change in POC was lower in the summer (month 6) than in the other months (Figure 7.11).

As the ΣPAR was significant in the ANCOVA, the apparent quantum yield (AQY) was estimated for the 48 light treatment samples. It was found to vary between 0.04 and 1.34 mmol C/mol photons. The average values for COT, DBS,

MUN and OXY samples were 0.30, 0.28, 0.28 and 0.30 mmol C/mol photons respectively, and the AQY were higher for the middle sample (t_{30}) compared with the end sample (t_{70}). These values are much lower than those calculated for DOC in Chapter 4, where the average value for DBS was 3.87 mmol C/mol photons, indicating that the DOC from DBS is more susceptible to degradation by light than the POC from DBS and the other three sites.

An ANOVA on the POC AQY data found that it was significantly different between Month and Time, and the interaction of Month*Time. There were no significant differences between Site, or between either of the interactions involving Site. The Month factor explained the majority of the variation.

Table 7.11. The results of the ANOVA on the POC concentrations.

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
ΣPAR	na	-	0.0335	4.92
POC_0	na	-	0.0014	13.28
Treatment	0.0015	0.60	ns	-
Time	<0.0001	17.34	0.0201	6.21
Month	<0.0001	37.26	<0.0001	32.32
Treatment*Time	<0.0001	1.48	ns	-
Treatment*Month	<0.0001	2.67	0.0094	15.31
Time*Month	<0.0001	22.52	ns	-
Error		11.57		18.29

Guided by the results of the POC ANOVA and ANCOVA, the following regressing equation was calculated:

$$ln\Delta POC = \underset{(0.14)}{0.71}\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + \underset{(0.1)}{0.31}POC_0 + \underset{(0.003)}{0.03}DOC_0 + \underset{(0.24)}{1.15}$$

n=81, r^2=0.73 *(Eq. 7.12)*

where ΔPOC is the change in the POC concentration (mg C/l), POC_0 is the initial POC concentration (mg C/l) and all other terms are as described above. The ΣPAR was not a significant parameter in this equation. Comparing this equation (Eq. 7.12) to the Chapter 4 change in POC concentrations (Eq. 4.14) found that there were some parameters in common; both equations were significantly influenced by the sinusoidal factor and the initial POC concentration. The magnitude of the sinusoidal parameter is similar in the two equations, 0.71 above and 0.68 in Chapter 4. However, the magnitudes of the effect of the POC_0 concentrations were quite different, 0.31 above and only 0.08 in Chapter 4.

As Treatment was a significant factor in the ANOVA, the regression equations were calculated for these separately, where the ΣPAR was significant in the light equation:

Light:

$$ln\Delta POC = \underset{(0.25)}{0.97}\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + \underset{(0.17)}{0.44}POC_0 + \underset{(0.005)}{0.03}DOC_0 + \underset{(0.0004)}{0.0005}\sum PAR + \underset{(0.50)}{0.50}$$

n=45, r^2=0.72 *(Eq. 7.13)*

Dark:

$$ln\Delta POC = \underset{(0.13)}{0.54}\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + \underset{(0.09)}{0.25}POC_0 + \underset{(0.003)}{0.03}DOC_0 + \underset{(0.23)}{1.50}$$

n=81, r^2=0.85 *(Eq. 7.14)*

where ΣPAR is the cumulative photosynthetically active radiation experienced by a sample (W/m^2) and all other terms are as described above. These two equations show that the POC concentration changes are dependent on the same factors in the light and dark treatments as when analysed together: the month, the POC_0 and the DOC_0. The higher parameter estimates show that the POC in the light is more dependent on the month and the POC_0 than the POC in the dark and the two

treatments together. The parameter estimate for the DOC_0 is the same for all three equations, suggesting that the relationship between the POC change and DOC_0 is the same for both treatments.

7.3.10. Upland vs. Lowland River and Confluence effects

The one-way ANOVA was looking for differences between sites, specifically between the upland (DBS) and lowland (MUN and OXY) sites, and the confluence effect (DBS and OXY above the confluence, and COT below the confluence). The ANOVA found significant differences between the chloride, nitrate, phosphate and sulphate concentrations, the conductivity, pH and E4:E6 ratio (Figure 7.12).

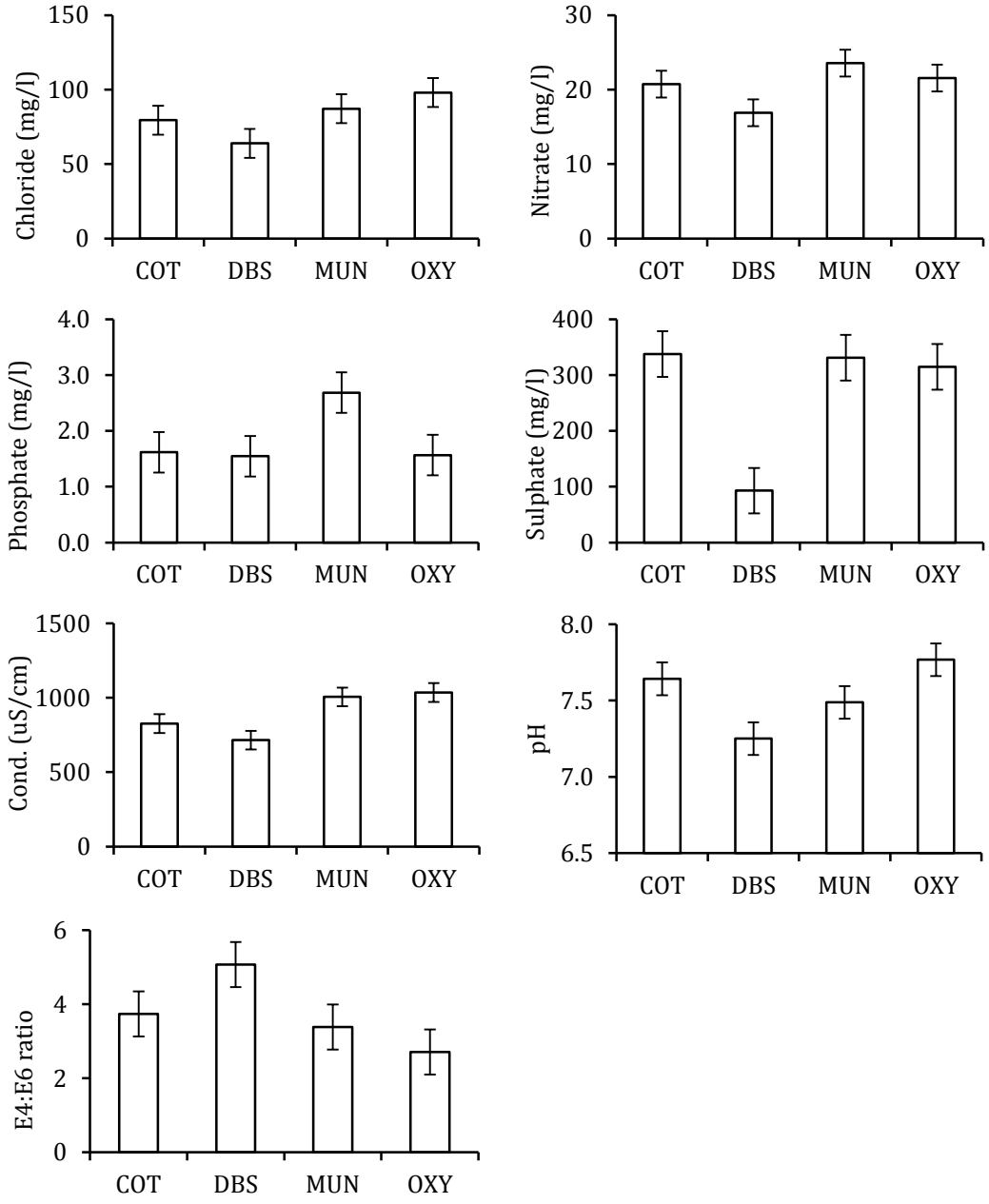
7.3.10.1. Upland vs. Lowland water

The upland site (DBS) had significantly lower chloride, nitrate, phosphate and sulphate concentrations, significantly lower pH and conductivity, and significantly higher E4:E6 ratio than either one or both of the lowland sites (MUN and OXY). Post hoc tests showed that for nitrate and phosphate, DBS was significantly lower than MUN but not OXY; for chloride and pH, DBS was significantly lower, and for E4:E6 DBS was significantly higher, than OXY but not MUN, and for sulphate and conductivity, DBS was significantly lower than both MUN and OXY. These results show that the upland river had lower nutrients, and the upland DOC was significantly more fulvic in composition that the lowland DOC.

7.3.10.2. Above and Below Darlington

The only significant difference between MUN and OXY was the phosphate concentration, with MUN having significantly higher phosphate than OXY. This could be due to the large area of farmland upstream of MUN potentially adding fertilizers to the water. Between MUN and OXY is the town of Darlington and a waste water treatment works, and so it is interesting that the only significant differences in the water chemistry is the phosphate concentration, as it was expected that input from the town and water treatment works would affect the water chemistry of the river.

Figure 7.12. The main effects plots for the initial chloride, nitrate, phosphate and sulphate concentrations, the conductivity, pH and E4:E6 ratio for each site. Error bars are the standard errors.



7.3.10.3. Confluence Effect

The below the confluence site (COT) did not have significantly different chloride, nitrate, phosphate or E4:E6 to the above the confluence sites (DBS and OXY), but did have significantly different sulphate and pH to DBS, but not to OXY, and significantly different conductivity to OXY, but not to DBS. The conductivity was significantly higher at OXY than at COT or DBS, and the sulphate and pH were much lower at DBS than at COT and OXY. These results showed that the conductivity at COT was reduced by the addition of the lower conductivity water from the Tees, and the pH was raised by the addition of the Skerne water.

The regression analyses considered the relative contributions of each river to the water chemistry below the confluence, using data from t_0 for the three sites closest to the confluence (COT, DBS and OXY); for each variable there were five data points (one from each month). No significant equation could be calculated for the fluoride, nitrate, phosphate and sulphate concentrations or the absorbance at 400 nm, but for all other variables, the COT water chemistry could be estimated from the parameter estimates for DBS and OXY values of the same variable (e.g. COT conductivity = DBS conductivity + OXY conductivity) and the results are shown in Table 7.12. The lack of significant equations for the nitrate, phosphate and sulphate concentrations may be due to non-conservative mixing tendencies, and the significant differences between these sites found by the ANOVA in section 7.3.10. However, equations could be calculated for chloride, E4:E6, conductivity and pH, which also had significant differences.

Table 7.12. The results of the regression analysis on the confluence data, showing the parameter estimates for DBS and OXY for each variable. The numbers in brackets are the standard errors of the parameter estimates.

COT	r^2	DBS	OXY	Intercept
POC (mg/l)	0.92	-7.39 (15.19)	7.43 (12.81)	0.51 (1.70)
Conductivity (µS/cm)	0.86	0.59 (0.21)	0.51 (0.29)	-116.86 (325.37)
pH	0.90	0.53 (0.16)	0.51 (0.51)	-0.10 (3.62)
DOC (mg/l)	0.93	1.13 (0.22)	0.05 (0.11)	-8.19 (8.74)
E4:E6	0.91	0.59 (0.15)	0.49 (0.24)	-0.60 (1.08)
Bromide (mg/l)	0.98	0.80 (0.15)	0.30 (0.11)	-0.04 (0.14)
Chloride (mg/l)	0.99	0.55 (0.15)	0.52 (0.13)	-6.51 (7.44)

These equations show the contributions from the Tees (as DBS) and the Skerne (as OXY) to the water chemistry below the confluence (COT). The equations all have very high r^2, suggesting that little else affects the water chemistry beyond the confluence other than the concentrations above the confluence.

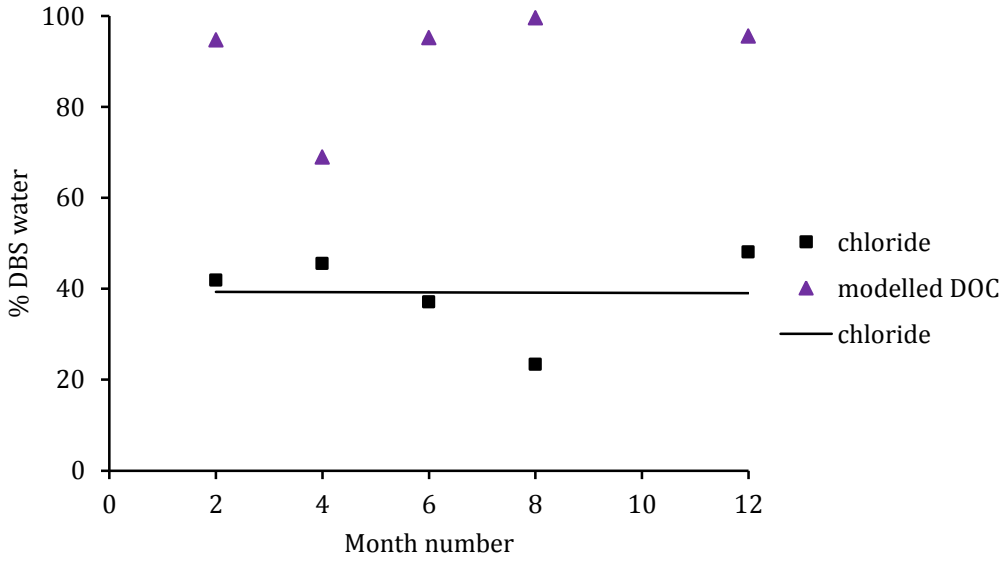
Using these results, the relative contributions to COT could be calculated from the ratio of DBS:OXY for six of the seven variables in Table 7.12. The negative parameter estimate for the POC meant that the relative contribution was negative, and so no ratio was calculated. For the other six variables, the COT water was a mixture of the DBS and OXY waters in the following proportions:

COT conductivity = 54:46
COT pH = 51:49
COT DOC = 96:4
COT E4:E6 = 55:45
COT bromide = 73:27
COT chloride = 51:49

The conductivity, pH, E4:E6 and chloride have very similar ratios, whereas the bromide and DOC have higher upland river contributions than the lowland river; in fact almost all of the DOC at COT comes from DBS (96%, compared with only 4% from OXY), which is an odd result considering the average initial concentrations of DOC are much higher at OXY than at DBS.

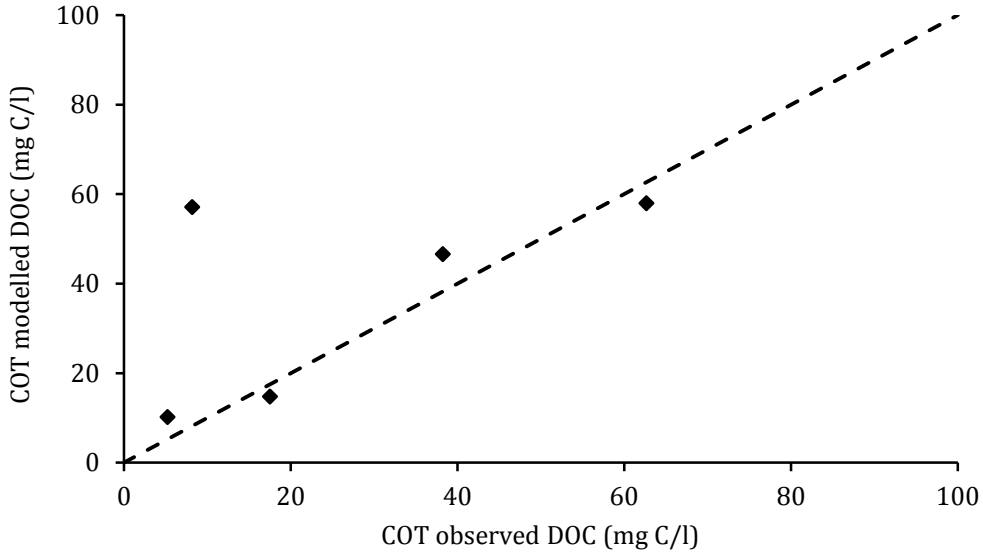
The average of these ratios was 63:37 ± 7, showing that the water chemistry of the upland river (63%) affects the water below the confluence more than the lowland river (37%). However, as chloride is a conservative ion, and the ratio of the relative contributions is very close to 50:50, this would suggest that the mixing ratio of the two rivers maybe closer to 1:1, and they contribute similarly to the water chemistry below the confluence. Figure 7.13 shows the modelled relative contribution from DBS water to the chloride concentration below the confluence each month, compared with the DBS DOC contribution. This showed that each month, the relative contribution of DOC from DBS was higher than the relative contribution of chloride.

Figure 7.13. The modelled percentage contribution of DBS water to the water below the confluence to the DOC and chloride concentrations for each months experiment.



Applying the mixing ratio of chloride (51:49) to the DOC concentrations resulted in the expected DOC concentration at COT, as if the DOC behaved conservatively (Figure 7.14). This showed that three of the months had higher modelled DOC than observed DOC, suggesting that DOC had been consumed at COT, and two months had lower modelled DOC, suggesting production of DOC. However, four of the five data points are very close to the 'observed = expected line', suggesting that in the majority of cases there was no addition or subtraction of DOC at COT relative to the inputs. The one point that has much higher modelled DOC than observed DOC shows that in one month, there was high removal of DOC at the confluence, as was predicted by the initial DOC contribution ratio suggested. This showed that for four out of the five month, the added nutrients and different sources of DOC from the Skerne had no or little effect on the DOC concentrations at COT. An ANOVA on the data, comparing the observed with the expected data, found no significant differences between the two groups ($p=0.4862$). These results showed that the DOC acted more conservatively that the initial DOC contributions ratio suggested.

Figure 7.14. The comparison between observed DOC concentrations at COT, and the modelled DOC, based upon the chloride conservative mixing ratio. The dashed line is the observed = expected line.



A PCA on the t_0 data showed the three confluence sites overlap, and there were no distinct groups. However, there were only 15 data points (three sites over five months) and so the PCA could not consider many variables. Another PCA, carried out on all data for all sites from the light treatment from this chapter, regardless of month or time (n=141), did not show any clear end-members, and so PCA could not be used to derive mixing models.

The ANOVAs on the rate in the light and dark treatments (Tables 7.6 and 7.7), the rate of photo-induced degradation (Table 7.8) and the initial rate of degradation (Table 7.10) showed no differences between the sites, so there was no effect of different nutrient levels or sources of DOC on the confluence on the rates of DOC degradation. This is similar to the results from Chapter 6, where adding nutrients to water did not increase the rate of DOC degradation, and in fact decreased the rate of DOC loss.

7.3.11. Discussion

The main effects plot of the average relative DOC concentrations in the light and dark treatments over the experiment time (Figure 7.2) shows a much more variable

response than those found in Chapters 2, 3, and 4 (Figures 2.2, 3.2 and 4.1), which showed a steady, consistent decline in the DOC concentrations in both treatments. This could mean that the overall percentage loss in this chapter could be merely due to an anomaly in the 'random' DOC concentrations, as the t_{70} concentration happened to be lower than the DOC_0. Looking at the graphs of the four sites individually shows that both treatments in the DBS water followed a similar pattern to those found in previous chapters, whereas the three other sites had less consistent DOC concentrations, with both treatments experiencing large increases and losses of DOC throughout the experiment (Figure 7.3). The reason for this variation is unclear; however the Bartlett and Ross (1988) method used to measure the DOC concentrations was developed for, and tested on, acidic waters, and the three lowland sites were alkali (Table 7.2), and so this may have artificially inflated or decreased the DOC concentrations found in these waters. It was expected that the lowland rivers would have much lower DOC concentrations than the upland river (Austnes et al., 2010; Neal et al., 1998), and so the initial concentrations of DOC in COT, MUN and OXY were higher than expected. COT and MUN had similar initial DOC concentrations to DBS, whereas the OXY concentration was much higher. These higher concentrations could be due to anthropogenic inputs of DOC into the water, or again, it could be due to the measurement method. The lack of colour of the water, and the low absorbance at 400 nm (approximately 0.04 for all four sites, Table 7.2, compared with 0.16 for highly coloured CHS water, Table 4.1) would suggest that the DOC concentrations would be low, and so it is possible that something else in the water was reacting with the DOC-analysis chemicals and resulted in lower than expected absorbance readings and consequently higher DOC concentrations. There were no significant differences in the t_0 specific absorbance of the water (t_0 absorbance at 400 nm / DOC_0), showing that the colour per DOC was not different between the sites. This was not unexpected as there were no significant differences in the DOC concentration or the absorbance at 400 nm between the sites.

The DOC concentrations of COT, MUN and OXY were highly variable, and, as shown in Section 7.3.1, not accompanied by large variance in any other measured variable. This does also point to the method of DOC analysis not being suited to these three sites, probably due to their higher pH. The unknown reliability of highly variable data means that further development of the conclusions and findings of this chapter will not be carried out.

The method for estimating the POC concentrations of POM assumes a consistent C content of all POM from all sites, which was unlikely to be the case as the lowland catchment soils have much lower C contents than the peats of the upland sites, which are the main sources of the particulate matter in the river. Chapter 5 found the C contents of total dissolved solid (TDS) from DBS and MUN to be as low as 8% and 5% respectively, and so it is unlikely that the POM contained almost 50% of C. There were no direct measurements of the elemental composition of suspended sediment for sites used in this chapter in Chapter 5, and so the same percentage C content as used in other chapters were used here for consistency. It may be that in order to compare the DOC and POC of both upland and lowland waters that different methods would be required to clarify the differences.

Lowland rivers tend to have higher nutrient loads than upland rivers, and this was found to be true in this chapter, where DBS had the lowest bromide, chloride, nitrate, phosphate and sulphate concentrations. The nutrient load of nitrate and phosphate in the lowland rivers are of comparable concentrations to the nutrient addition treatments in Chapter 6, with the sites in this chapter nitrate concentrations varying from 16.91 to 23.55 mg/l, and the phosphate concentrations varying from 1.55 to 2.68 mg/l. In Chapter 6, the nitrate concentrations after nutrient addition in the DBS water (either at t_0 or t_{24}) varied between 51.66 and 91.60 mg/l, and the phosphate concentration was between 1.19 and 1.52 mg/l. The concentration of the added nutrient solution in Chapter 6 was calculated so as to avoid any nutrient limitation on the rate of DOC degradation, and so it is unlikely that there was any nutrient limitation in this chapter, especially by phosphates.

The town of Darlington and the water treatment works had a low impact on the water chemistry, with the only significant difference between MUN and OXY sites being the phosphate concentration. There was a larger impact expected, as Rothwell et al. (2010) found significant correlations between proximity to sewage and trade discharge sites and the pH, base cations, nutrients and metals.

7.4. Conclusion

The main findings of Chapter 7 are outlined in Table 7.13. There were significant differences between the four sites in the ANOVA on relative DOC concentrations (Table 7.3), but no significant differences in the photo-induced DOC changes, or the

rates in the light, dark or the photo-induced rate. However, the regression analyses showed that the rate of DOC change in the DBS water could be modelled significantly, whereas the other three sites could not, showing that the upland river follows a similar pattern to those found in the previous chapters.

As in previous chapters, there were significant differences in the loss of DOC between the sites, and the initial DOC was often a significant covariate in the analyses, showing that the site differences in DOC type and concentration play a large role in determining the DOC degradation rates. The rate of DOC degradation declined over time, suggesting a decrease in the 'degradability', or an increase in the recalcitrance, of DOC, even in lowland river systems.

There were no significant differences in rates of DOC degradation below the confluence, suggesting that the mixing of the two rivers did not lead to renewed degradation of upland DOC. Also there were no significant differences in the DOC degradation rates between the upland and lowland rivers, so the higher nutrients in the lowland river did not significantly affect the rate of degradation.

There were significant differences in the water chemistry between the upland and lowland rivers, between above and below the town of Darlington and the waste water treatment plant, and between the sites above and below the confluence, as hypothesized. However, these significant results were not found for the DOC or POC concentrations, only for the chloride, nitrate, phosphate and sulphate concentrations, the conductivity, pH and E4:E6 ratios.

The mixing model results showed that even though there was a larger contribution of DOC from the upland river, this did not significantly alter the expected and observed DOC concentrations at COT, with the observed data from four out of five months having no significant difference from the expected data, modelled based on the conservative mixing ratio of chloride.

Table 7.13. The main findings of Chapter 7.

Sites studied	Teesdale: DBS, COT Skerne: MUN, OXY
Range of catchment areas (km^2)	818.4 – 1085.95
Range of percentage peat cover (%)	0 – 33.9
Duration of experiments	5 months, for 70 hours
Sample size	375
Net DOC loss in the light treatment (%)	74
Net DOC loss in the dark treatment (%)	29
Initial rate of DOC loss in the light treatment (mg C/l)	5.0
Initial rate of DOC loss in the dark treatment (mg C/l)	1.4
Range of rate of DOC loss in the light treatment (mg C/l/hour)	31.8 – -43.5
Range of rate of DOC loss in the dark treatment (mg C/l/hour)	51.2 – -45.6
Significant covariates:	
Change in DOC concentrations	Sulphate
Light rate	Initial DOC
Dark rate	Initial DOC

Chapter 8:
Conclusion

8.1. Overview of Thesis

The aim of this thesis was to address a gap in the IPCC carbon budget, which had no pathway for the loss of carbon from inland waters as CO_2, and to quantify the net losses of carbon along the course of a river. Secondary to this aim was to investigate the mechanisms by which the carbon is lost from rivers; and assess what factors influence the rates and magnitudes of that loss. This was accomplished by measuring the DOC concentrations in over 2500 water samples from upland headwaters, downstream tidal limits and lowland rivers, in light, dark and nutrient treatments over periods of up to 10 days each month; in the context of environmental variables; in comparison to the particulate organic matter; and comparing the rates of DOC loss to the chemical composition of the river water and the molecular composition of the total dissolved solid.

Expanding on the objectives and approach table in Chapter 1 (Table 1.1), Table 8.1 shows the main findings of each chapter.

In Chapters 2, 3, 4, 6 and 7, there were significant differences in the loss of DOC between the sites, and the initial DOC was often a significant covariate in the analyses, showing that the site differences in DOC type and concentration play a large role in determining the DOC degradation rates. The rate of DOC degradation declined over time, suggesting a decrease in the 'degradability', or an increase in the recalcitrance, of DOC. This links with the finding from Chapter 5 that the recalcitrance of the remnant DOC increases, and the DOC increases in oxidation state and decreases in degradability, as it travels downstream.

Table 8.1. The main findings of each chapter.

Chapter	Objective	Approach	Findings
2	Investigate long time scale degradation	Monthly ten-day experiments on the River Tees, with light and dark treatments.	76% loss of DOC in the light treatment, 47% loss of DOC in the dark treatment. The majority of the loss took place in the first two days of the experiment.
3	Investigate shorter time scale degradation	Quarterly, in-situ, 30-hour experiments on the River Ashop, with light and dark treatments, and sub-daily sampling.	86% loss of DOC in the light treatment, 55% loss of DOC in the dark treatment. A clear diurnal cycle, with a low rate of loss of DOC overnight.
4	Investigate medium time scale degradation, combining techniques from Chapters 2 and 3.	Monthly 70-hour experiments on the River Tees, with light and dark treatments, and sub-daily sampling.	35% loss of DOC in the light treatment, 3% loss of DOC in the dark treatment. A clear diurnal cycle, with a low rate of loss of DOC overnight, and much higher rates of loss from the headwater site than the downstream site.
5	Investigate the composition of DOC and relate it to the rates of degradation.	Combined analysis of DOM, POM, vegetation, peat and litter samples with water samples and rates of DOC degradation to produce a model of the factors affecting DOC	The DOC from the upland site was significantly different in composition to the two downstream sites, and significantly different from all potential source materials.

Chapter	Objective	Approach	Findings
		degradation in the river.	The degradation of DOC is an oxidation reaction, with more reduced samples degrading faster.
6	Investigate the effects of nutrients on the rates of DOC degradation, to see if the nutrient concentrations were limiting the biodegradation of DOC.	Bi-monthly 70-hour experiments on the River Tees, with three nutrient treatments.	No significant differences between the three nutrient treatments, suggesting that the biodegradation of DOC is not limited by nutrient availability.
7	Investigate the effects of nutrients in a naturally high nutrient lowland river, and the effect of the confluence of the upland and lowland rivers, and the effect of a town and sewage works on the DOC dynamics.	Bi-monthly 70-hour experiments on the upland River Tees and the lowland River Skerne, and the confluence between the two.	Less reliable DOC measurements, although still clear differences in the DOC concentrations in the upland river. The mixing of the rivers did not lead to renewed degradation of upland DOC.

8.2. Key Objectives and Findings

8.2.1. Photo- and bio-degradation

This thesis quantified net DOC losses due to photo- and bio-degradation by analysing DOC concentrations in light and dark treatments. Table 8.1 shows the average total losses in the light and dark for the headwater site, CHS. All measurements of loss were of the net changes, as there was likely to be production of DOC occurring at the same time as the loss of DOC.

When judged relative to the initial DOC concentrations, the light DOC concentrations across all sites were on average 29%, 30%, 32% and 45% lower than the dark DOC concentrations in Chapters 2, 3, 4 and 7 respectively. The measured losses due to photo-induced degradation ranged from 92 to -111 mg C/l, where positive numbers indicate the light DOC was higher than the dark DOC, i.e. photoproduction, which occurred in only 16% of cases across all chapters. There were significant differences in the rates of DOC decline in the light and dark treatments, showing that exposure to daylight caused a larger decrease in the DOC concentrations compared with samples kept in the dark. The rates of photo-induced degradation from Chapter 2 ranged from 27 to -4 mg C/l/day, and for Chapters 3, 4 and 7 ranged from 70 to -113 mg C/l/day. Negative rates indicate increases of DOC due to photoproduction.

The majority of the degradation occurred in the first two days of the Chapter 2 experiments, with only a small amount of DOC lost between Day 2 and Day 10 of the experiments; on average, only 9 and 4 mg C/l were lost in the dark and light treatments, respectively. Due to the experimental design, there were no samples taken after approximately 70 hours in this experiment, but for comparison with the 70-hour experiments, the Day 2 relative losses (after 48 hours) from the dark and light treatments were 39% and 65%, and the Day 5 relative losses (after 120 hours) were 48% and 72%. These values are comparable to the percentage losses in Chapter 4. The percentage losses in Chapter 3, after 30 hours, were 86% in the light treatment and 55% in the dark treatment, showing that over half of the DOC was lost even in the dark treatment. This showed the importance of biodegradation in the fast breakdown of DOC.

These results showed that in the dark treatment, DOC concentrations were on average higher, with lower rates of degradation, than the DOC concentrations and rates in the light treatment, indicating that exposure to light caused additional loss of DOC at a faster rate, and photoproduction of DOC occurred in only a minority

of cases. The dark rates measured in these experiments are much smaller than the light rates, again showing that the losses of DOC due to photo-degradation are greater than those due to bio-degradation alone. However, the DOC losses in the dark, due to biodegradation alone, still represent significant losses of organic carbon from the water to the atmosphere.

8.2.2. Initial rates and diurnal degradation

An interesting result from this thesis was the incredibly fast rate of initial DOC decline in the light from the headwater site, CHS, and the clear diurnal cycle of DOC degradation. The initial rates were faster than those previously measured in the literature, probably due to the fresh source material from headwater catchments that degrades very quickly.

There were 761 DOC degradation measurements for CHS over the three experiments in Chapters 2, 4 and 6 (Table 8.2). Chapter 2 did not include sub-daily samples, and so the rates of loss data from have been estimated from the data rather than measured, due to the different experimental design, as outlined in the discussion of Chapter 4. There was no dark treatment in Chapter 6, as that chapter had nutrient treatments instead; however, the control treatment in that chapter was in the light, and so could be compared to Chapters 2 and 4 (Table 8.2).

Estimates of the order of the initial rate reaction were 2.3 ± 0.7 (Chapter 4; Eq. 4.13) and 1.5 ± 0.4 (Chapter 6; Eq. 6.19) for CHS DOC water in the light treatment, which showed that the rates were not significantly different from each other, 2nd or 3rd order rates of reaction. However it is most likely to be fractional or mixed order because of the number of processes contributing.

Table 8.2. The average DOC data comparison for CHS. The estimated data from Chapter 2 are shaded. For comparison, the Chapter 3 average rates of DOC degradation during the first hour of the experiment in the light and dark were 23 and 14 mg C/l/hour respectively.

	Chapter		
	2	4	6
Time scale of experiments	10 days	70 hours	70 hours
Total sample size	690	772	373
CHS samples	162	398	201
CHS initial DOC (mg C/l)	111.89	41.75	44.44
CHS light end DOC (mg C/l)	27.03	16.52	4.82
CHS dark end DOC (mg C/l)	38.83	36.42	-
CHS light DOC decline (%)	76	60	89
CHS dark DOC decline (%)	65	13	-
CHS rate of DOC loss in the light in the first day	71.63 mg C/l/24 hours	3.36 mg C/l/hour	2.23 mg C/l/hour
CHS rate of DOC loss in the dark in the first day	48.58 mg C/l/24 hours	0.57 mg C/l/hour	-
CHS rate of DOC loss in the light in the first hour (mg C/l/hour)	2.98	11.57	19.82
CHS rate of DOC loss in the dark in the first hour (mg C/l/hour)	2.02	3.60	-

There were significant differences between the rates of DOC decline in the light treatment between the different stages of the experiment, with Chapters 2, 3, 4 and 6 having significantly higher rates of DOC degradation during the hours of daylight, and significantly lower rates during hours of darkness. This difference was largest on the first day of the experiments, where the highest rates were observed, and less marked throughout the rest of the experiment when the DOC supply had been depleted of the labile carbon. This showed that the kinetics of the reactions would be best summarised by four separate rate equations for the four different stages in the first 48 hours.

These results show the importance of diurnal sampling, and how the estimates of DOC loss have been improved over the course of the thesis, from the coarse estimates of loss generated in Chapter 2 from daily samples over a longer time scale, to the hourly losses in Chapters 3, 4, 6 and 7 that resulted in better modelling and understanding of the behaviour of DOC. The nutrient experiments

added a greater understanding of the limitations on the degradation and the confluence experiment measured the rates of loss on lowland rivers, which are common in the UK.

No experiment found evidence of priming, and so it was concluded that exposure to daylight during the day did not increase the rate of DOC decline over night compared with samples kept in the dark. Also, there was no evidence that the addition of DOC from anthropogenic sources caused a renewed rate of degradation of allochthonous DOC from the uplands.

8.2.3. Limitations on DOC degradation

The DOC concentrations did not reach zero in water from any site, or in any treatment, even after 10 days. The theory that this was due to nutrient concentrations limiting the degradation of DOC was shown to be unlikely by the results of Chapters 6 and 7. The nutrient addition experiment in Chapter 6 found no evidence that the nitrate and phosphate concentrations limited the rate of DOC decline, as there were no significant differences between the nutrient treatments. The initial rates of DOC decline were significantly lower in the treatments after nutrient addition, suggesting that adding nutrients had a deleterious effect on the DOC degradation. Chapter 7 found no effect on the DOC degradation rates of the addition of high nutrient water from the lowland River Skerne to the River Tees, and no differences in the rates of degradation between the sites, and concluded that the high nutrient and anthropogenic sources of DOC in the lowland river did not cause an increase in the rates of DOC loss.

The presence of DOC in bodies of water that have long residence times, such as lakes, reservoirs and the sea, where light exposure and nutrient concentrations are unlikely to be limiting the degradation, suggests that there is a fraction of DOC that is naturally recalcitrant, and so no amount of nutrients, microbes or light exposure will cause it to degrade.

There were often significant differences in the DOC degradation rates between the sites, resulting in better analysis of results when the sites were considered separately, and significant differences in initial concentrations of DOC, suggesting that there was a simple control on the degradation rates, based on the range of initial DOC concentrations that could be expected from each site. This shows that the rates of DOC degradation were also limited by the supply of DOC, and

the results of Chapter 5 suggest that the rates were also affected by the composition, or 'type' of DOC present in the water.

8.2.4. Apparent quantum yields

The apparent quantum yields (AQY) were calculated where there was a significant effect on the DOC of the photosynthetically active radiation (PAR). Values calculated for the change in DOC concentrations in the light treatment in Chapters 2 and 4 showed that the DOC was much more susceptible to degradation by light during the first hour than at the end of the experiment. The AQY of photo-induced degradation showed that samples that gained DOC in the light rather than lost DOC had negative AQYs, however these were less than half the magnitude of the AQYs of the DOC loss. Chapter 7 found PAR to be a significant covariate in the concentrations of POC in the light, with lower AQYs than those found for DOC. These lower values suggest that the POC from the sites in Chapter 7 was less susceptible to degradation by light than the DOC from Chapters 2 and 4.

8.2.5. Activation energies

The activation energies were calculated where there was a significant effect on the DOC of the temperature. The values ranged from 0.19 ± 0.16 kJ/g C to 5.82 ± 1.32 kJ/g C, and all measured values were lower than that reported by Alperin et al. (1994) from marine sediments, suggesting that the DOC from headwaters and upland rivers is more easily degraded than marine sediment. Chapter 4 found that the activation energy was lower for DOC from CHS water than DOC from DBS water, suggesting that the CHS DOC was more easily degraded than the DBS DOC.

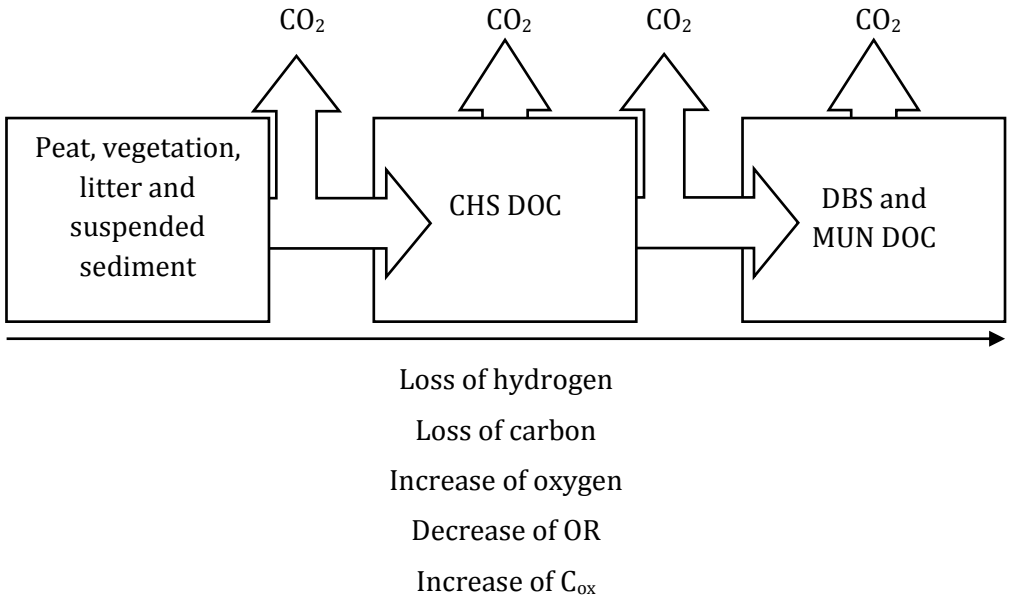
8.2.6. POC dynamics

The measurements of POC concentrations show a large spread in the estimates of POC fluxes, with both gains and losses of POC measured. The losses of POC over the different time scales used in this thesis range from 15% to 87%, and the gains range from 12% to 58%. CHS water in the light treatment consistently lost POC, 87% over 10 days (Chapter 2) and 15% over 70 hours (Chapter 4). Due to the smaller datasets for POC analysis, and the methods of deriving the data, there is little than can be stated conclusively about the dynamics of POC degradation.

8.2.7. Degradation and composition

Chapter 5 found that the initial rate of DOC composition in the light (including both photo- and bio-degradation) could be modelled using the hydrogen content, OR, N-alkyl group abundance and the initial DOC concentration (Eq. 5.3). These relationships pointed to the faster rates of DOC degradation being attributed to DOC that is more reduced, and lower rates attributed to DOC that is more oxidised, with lower hydrogen contents and lower OR. The second equation for CHS (Eq. 5.6) found a similar relationship, albeit using different parameters and a larger data set, and the equation for the DBS and MUN samples (Eq. 5.4 and 5.5) also showed that the more oxidised samples had lower rates of degradation. These results showed that the degradation of DOC is an oxidation process, with the most reduced DOC having higher rates of degradation. The hydrogen content, C_{ox} and OR of the samples reflected their redox state, and the suspended sediment, vegetation, peat, litter, cellulose and lignin samples all had higher hydrogen and OR, and lower C_{ox} than the CHS samples, which in turn had higher hydrogen content than the DBS and MUN samples, supporting the theory that the samples became more oxidised the further they travelled from the source materials, and ultimately became fully oxidised to CO_2 either directly from CHS or DBS, or whilst in-stream between the sites (Figure 8.1).

Figure 8.1. Oxidation pathway of chemical changes in DOC from the source materials to CO_2, adapted from Figure 1.2.



8.2.8. DOC concentrations and climate change

The concentrations of DOC in river waters draining from peatlands in the UK and across Europe and North America has been observed to be increasing, with various reasons presented as the possible cause, including several factors linked to climate change: increasing air temperature (Freeman et al., 2001), increasing atmospheric CO_2 concentrations (Freeman et al., 2004), more variable storm events (Austnes et al., 2010) and increased drought (Worrall et al., 2006). Although the cause of this observed rise is still a matter of debate (Evans et al., 2007; Smith et al., 2007), the trend seems to be ubiquitous. Chapter 2 found an increase in the DOC flux from the headwater site, but no significant trend was observed at the tidal limit site. There was a significant increase in the predicted removal rate, implying that the river is capable of removing most or all of the increase in DOC export from the source, before it reaches the sea. This in turn implies that observed increases in DOC flux from peat soils across the northern hemisphere could translate into large increases in loss of CO_2 to the atmosphere.

The covariate that was significant in the majority of the ANCOVAs and regression analyses was the initial DOC concentration, with higher initial concentrations leading to higher rates of DOC degradation. If the concentrations of DOC in water draining from peatlands continue to increase, as the current trend shows they are, then larger quantities of DOC will be lost in-stream, and the emissions of CO_2 will increase.

The current estimate from this thesis of DOC loss from peat-covered catchment in the UK is 14.7 Tg CO_2/yr, which equates to an additional 2.5% on top of the existing UK total emissions. Globally, the losses of DOC from UK streams represent 0.7% of the total CO_2 emissions from inland waters (Raymond et al., 2013).

8.3. Limitations

As with all experiments, the experiments in these chapters had to make various assumptions about the experimental design and nature of the carbon being studied.

Assumptions of the experimental design included the use of quartz glass as a 'reaction vessel'. Quartz glass was used as it allows all wavelengths of light through,

although this was never tested. It has been used in many experiments on light-induced degradation of DOC, and so it is unlikely to affect the amount of light that the samples are exposed to. It was also assumed that wrapping the vessels in foil excluded all light, again this was not tested. It was also possible that the water temperatures in the vials were raised above the ambient air temperature due to the glass and/or foil wrapping, which could have led to artificially high temperatures and affected the rates of DOC loss. The water depth in the vials was much lower than in the source streams and rivers, which could have led to greater light penetration and therefore photo-degradation, than would have occurred naturally.

There were no t_0 replicates, due to the nature of the experimental design, and it was assumed that the water was homogenous with respect to the DOC concentrations. Once the water was poured in to the quartz glass, all the sampling times involved sacrificial sampling, and it was assumed that the changes in the DOC concentrations were not due to initial variation between the quartz glass tubes. Figure 6.2 shows that there were differences in the DOC concentration between the B and N_{24} nutrient treatments in the DBS water before t_{24}, so there were differences between the treatments before the nutrient solution had been added. This shows that there was some variation in the DOC concentrations between quartz glass tubes that was not due to the treatments. There could have been variation between the other variables in a similar manor.

Concentrated sulphuric acid was added to the filtered water samples for DOC analysis in Chapters 3, 4, 6 and 7, and this was assumed to stop any further changes in the DOC concentrations. However, this was never tested. In Chapter 2 the samples for DOC analysis were frozen and defrosted before analysis, again this was assumed to have no effect on the DOC concentrations in the water.

There could have been a build-up of bacterial biomass as biofilms on the glass or rubber bung, which was not measured, and a visual inspection of the glass and bungs would not have revealed. This would not have contributed towards the increase in POC or suspended sediment, but could have contributed towards the decrease in DOC concentration. However, this would count as a biological use of the DOC, and would have counted towards biodegradation, and so would not distort the measurements of DOC loss.

The carbon content of POM was assumed to not vary between sites, even when the carbon contents of the surrounding soils will have been very different, and so this may have been potential source of error.

Measuring the river flow at the sampling time may have added a further dimension to the analysis that could be carried out, however there were several occasions when this would not have been possible due to safety. The river flow is measured by the ECN at Trout Beck in Moor House, and so there was flow data available for that site.

Chapter 5 made an assumption about the evaporation method; the heat from the oven did not change the composition of the DOC. However, if it did have an effect on the DOC, as the oven was kept at a constant temperature for all evaporations, the effect would hopefully have been the same across all months and sites.

DBS was assumed to be an 'end' point of the analysis, i.e. it was assumed that the DBS DOC was the same DOC as had left CHS several hours earlier and the chemical differences between the two were due to the in-stream processing alone and not due to inputs from other sources along the catchment.

All experiments could benefit from larger data sets or more frequent sampling, and these experiments are no exception. However, most chapters had in excess of 300 data points gathered over 12 months or at least seasonally, and so this is not likely to have restricted the analysis, or to have biased the effect of the seasonal cycle on the DOC. Even so, it is unlikely that every possible combination of flow conditions and weather conditions were experienced throughout the experiments, and so there will be some situations that could not been represented, especially in situations where the weather prevented the sites being visited.

Even though Chapter 3 only had four months of data, and Chapters 6 and 7 only had five months of data, there were still over 300 data points in each experiment, and so it is unlikely they were statistically invalidated due to a smaller sample size. Chapters 6 and 7 were carried out in alternate months, and Chapter 3 was carried out seasonally, and so no experiment has samples from only one season, and all experiments experienced a variety of weather and river flow conditions throughout the year.

8.4. Implications

This thesis has estimated the loss of DOC from rivers to range from 35% to 86%. The lower estimate of 35% is similar to losses reported in other papers: 42% (Cole et al., 2007), 40% (Worrall et al., 2006), and 21% (Battin et al., 2009a). There are reported values for the loss of DOC that are higher than these, e.g. 50% (Jonsson et al., 2007) and 70% (Gennings et al., 2001), but none as high as the upper estimate found in this thesis of 86%. This estimate of DOC loss was from five sites in the Peak District with highly organic soils, high DOC concentrations and small catchment areas. The DOC in these environments is extremely labile and so more degradable, resulting in higher estimates of DOC losses than studies conducted on large rivers, lakes and reservoirs.

Using experiments and sites with residence times closer to those of UK rivers that took the diurnal cycle into account (Chapters 4, 6 and 7), the losses of DOC range from 35% to 76% over 70 hours. To scale up, the UK DOC export estimate for peat-covered catchments of 555-1263 Gg C/yr (Worrall et al., 2012) and the estimate of the POC flux from England and Wales of 120-460 Gg C/yr (Dawson and Smith, 2007) were used, in conjunction with the 15% loss of POC from CHS and the 35-76% range of estimates of DOC loss from this thesis. Applying the lower DOC loss of 35% to the DOC export from peat-covered catchments in the UK would estimate the DOC flux at the source would have been between 854 and 1943 Gg C/yr. Loss of DOC to the atmosphere would be 299 to 680 Gg C/yr, or 1096 to 2493 Gg CO_{2eq}/yr. Applying the higher estimate of 76% loss of DOC to the DOC export from UK peat-covered catchments would estimate the DOC flux at the source would have been between 2313 and 5263 Gg C/yr, and loss of DOC to the atmosphere would be 1758 to 4000 Gg C/yr, or between 6450 to 14678 Gg CO_{2eq}/yr. The 15% loss of POC observed in this thesis would equate to a POC flux at the source of 141-541 Gg C/yr, and loss of POC to the atmosphere would be 21-81 Gg C/yr, or 78-298 Gg CO_{2eq}/yr. These CO_2 emission values assume that 100% of the DOC and POC lost from a catchment is lost as CO_2. The values of DOC and POC loss are the average values from the chapters, and so will have an error figure around them. The lowest and highest estimates of loss from the chapters were used for this analysis so as to cover as much of the range of error as well as the range in the original data as

possible. The DOC and POC concentrations were taken from literature and so represent the range of concentrations found in those studies.

The total CO_2 emissions from the UK in 2012 were 580.5 Tg CO_{2eq} (Department of Energy and Climate Change, 2014). The upper estimate from DOC loss of 14.7 Tg CO_2/yr from peat-covered catchments in the UK is 2.5% of the UK total emissions, and larger than the CO_2 emissions from industrial processes (11 Tg) and the public sector (8 Tg), and close to the waste management (17 Tg) sector, although it is still much lower than the emissions from the energy supply (204 Tg) and transport (122 Tg) sectors (Department of Energy and Climate Change, 2012). The CO_2 from POC losses equates to 0.3 Tg CO_2/yr, and is therefore a smaller flux than from any individual sector; however it increases the total flux of organic carbon from England and Wales to 15 Tg CO_2/yr. Including estimates from Scotland, where there are large expanses of deep peat, would increase this flux even further.

Recent estimates of the global CO_2 emissions from inland waters are 1.8 Pg/yr (1.5-2.1 Pg/yr) from streams and rivers and 0.3 Pg/yr (0.06-0.84 Pg/yr) from lakes and reservoirs (Raymond et al., 2013). The total inland water CO_2 flux from Raymond et al. (2013) is larger than the estimates from the fifth assessment by the IPCC (2013) that has a flux of 1 Pg C/yr degassing from freshwater lakes/reservoirs.

The UK is the 80th largest country in the world, covering 0.16% of the Earth's land area (CIA, 2010). The estimate of total organic carbon loss of 15 Tg CO_2/yr from this thesis for UK peat-covered catchments is 0.7% of the total CO_2 emissions from inland waters from Raymond et al. (2013), or 1.5% of the estimate from the IPCC (2013), meaning that the UK inland water CO_2 emissions account for a larger proportion of the global CO_2 water emissions that the total land area suggests it should. This could be that the total inland water CO_2 flux from the UK is higher than expected due to the disproportionately high contribution of low-order streams to the CO_2 flux found by Raymond et al. (2013). The rivers of the UK are generally small and organic-rich, compared with world rivers, and the majority of DOC and POC losses measured in this thesis were from low-order streams, potentially resulting in over-estimates of loss as CO_2.

The higher than expected contribution from the UK inland waters to the global CO_2 flux than the land area of the UK suggests it should be could also be due to the high percentage of land covered by deep peat in the UK. This is linked to high and increasing DOC fluxes, and therefore high losses of organic carbon as CO_2, especially in low-order streams.

8.5. Further Work

There is further analysis that could be undertaken using existing samples from Chapter 5, for example: further ^{13}C NMR of source materials could help identify the functional groups present and provide further information regarding the sources of DOC and for comparison with the existing CHS and DBS data.

Further work could be carried out on lowland rivers, using different techniques more suited to that environment to measure the DOC and POC concentrations, for comparison with the results gained in this thesis. Investigating the limits of the degradation of DOC and POC could help to explain why there was never 100% loss of the carbon, even after 10 days. Bodies of water with residence times of months and years, e.g. lakes, reservoirs and the sea, also have DOC present, showing that even with longer exposure to light and microbes in natural systems the DOC concentrations do not reach zero, so there must be something limiting further break-down of the DOC compounds. This remaining carbon is probably more in a more recalcitrant form not accessible to microbes or degradable by light. Other potential factors that limit the degradation could be the light intensity and water temperature.

Further experimental work should be undertaken in order to better understand the conversion of POC to DOC, and subsequently to CO_2, as that is one pathway that this thesis did not conclude satisfactorily. The chemical composition of POC, and how this changes downstream as it is subject to in-stream processes, and the effect of POC concentrations on the degradation rates of DOC should be investigated further, with sub-daily samples taken for POC as well as DOC analysis. Total exclusion of particulates from the water before it is exposed to light would clarify that the carbon losses from the water were losses of DOC, rather than POC. Also, the effects of high POC concentrations on the rates of loss of DOC and POC would provide information relevant to inland waters draining highly eroded and eroding sites.

Further work should also investigate the differences in headwater DOC composition before and after exposure to day/night cycle, which would provide a better 'end' point for analysis than using a site further downstream. The initial large volume of water from the headwater would be evaporated, while a duplicate sample is exposed to light for a set period of time, preferably in a quartz glass tank or

similar, allowing exposure to the natural day/night cycle. Water samples would be taken periodically, especially during the first few hours of the experiment, for water chemistry and organic carbon analysis, and then the remaining quantity of water would be evaporated. This would result in two solid DOC samples: a beginning sample and an end sample after exposure to the natural day/night cycle for a set time. The DOC and POC concentration measurements could be backed up by Infra-Red Gas Analyser (IRGA) measurements of the CO_2 concentrations to provide a mass balance for the carbon. Another interesting experiment would be to carry out a DOC degradation and compositional analysis on water taken from an upland reservoir, where the inflow water has a high DOC concentration, and the water in the reservoir has been exposed to light for a considerable length of time, to compare the DOC concentrations, degradation rates and composition of the samples.

Appendices

The appendices are provided on CD, and a brief outline of each is given below:

Appendix 1

DOC and water chemistry data (including pH, conductivity, absorbance, POC and anion data) for Chapters 2, 3, 4, 6 and 7

Appendix 2

DOC and water chemistry data (including pH, conductivity, absorbance, POC and anion data) for Chapter 5

Appendix 3

Chapter 5 composition data, including TGA, STA, $\delta^{13}C$, Elemental Analysis, Bomb Calorimetry, ^{13}C NMR

Appendix 4

Cumulative PAR and temperature data for Chapters 2, 4, 6 and 7

Appendices

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