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Sub-daily rates of degradation of fluvial carbon from a peat headwater stream

C.S. Moody[1] & F. Worrall

Department of Earth Sciences, Science Laboratories, South Road, Durham, DH1 3LE, UK

Abstract

In-stream processing of allochthonous dissolved organic carbon (DOC) and particulate organic carbon (POC) in peat-sourced headwaters has been shown to be a significant part of the terrestrial carbon cycle, through photo- and bio-degradation, with both DOC and POC converted to carbon dioxide (CO_2). This study reports a series of 70-hour, in-situ experiments investigating rates of degradation in unfiltered surface water from a headwater stream in the River Tees, North Pennines, UK. Half the samples were exposed to the normal day/night cycle (ambient); half were continuously dark. The study found that the DOC concentration of samples in the ambient treatment declined by 64% over the 70 hours, compared with 6% decline for the samples kept in the dark. For POC, the loss in the ambient treatment was 13%. The average initial rate of loss of DOC in the ambient treatment during the first day of the experiment was 3.36 mg C/l/hour, and the average rate of photo-induced loss over the whole 70 hours was 1.25 mg C/l/hour. Scaling up these losses, the estimate of total organic carbon loss from UK rivers to the atmosphere is 9.4 Tg CO_2/yr which would be 0.94% of the global estimate of CO_2 emissions from streams and rivers from the 2013 IPCC report. Initial rate kinetics in the light were as high as 3rd order, but the study showed that no single rate law could describe the whole diurnal degradation cycle and that separate rate laws were required for night and day processes. The comparison of dark and ambient treatment processes showed no evidence of photo-stimulated bacterial degradation.

Keywords: DOC, POC, in-stream, upland, river, UK

[1] Corresponding author: tel. no. +44 (0)191 334 2295; c.s.moody@durham.ac.uk.

Introduction

Peatlands, as highly organic soils, are an important, if not the most important, source of dissolved (DOC) and particulate (POC) organic carbon to rivers (Aitkenhead et al. 2007; Rothwell et al. 2008; Tipping et al. 2010). Both DOC and POC are important components of the fluvial carbon cycle, facilitate the transport of pollutants (Rothwell et al. 2007); contribute to the nutrient supply and energy sources in the river (Marschner and Kalbitz, 2003; Tipping et al. 2010); and affect the cost of water treatment (Evans et al. 2012). Across the northern hemisphere there have been widespread reports of increasing concentrations of DOC in river water in recent years (Evans et al. 2005; Freeman et al. 2001); and widespread erosion in UK peatlands has led to an increase in POC fluxes into some headwaters (Evans et al. 2006; Pawson et al. 2008).

The fluxes of DOC and POC from World rivers have been measured and modelled (e.g. Harrison et al. 2005), but these studies have calculated flux of organic components at the outlet of the catchments rather than the flux from the terrestrial sources (e.g. peat soils) and thus do not take into account any changes that have occurred along the path of the river, such as in-stream processing of DOC and outgassing of dissolved inorganic carbon (DIC; Worrall et al. 2012) and so are poor estimates of how much carbon is being lost from terrestrial environments and how much carbon is contributed from rivers to the atmosphere. In-stream processes can either decrease or increase the DOC concentration of the stream by sorption to POC and/or the autochthonous production of DOC.

The extent to which the processing of DOC and POC contribute to the release of atmospheric greenhouse gas depends upon the rates of processes that degrade and convert DOC to greenhouse gases. Gennings et al. (2001) state that 40-70% of annual inputs into boreal lakes are evaded to the atmosphere. At a global scale, Cole et al. (2007) estimated that 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. (2009) suggested a lower removal rate of 21%, and Raymond et al. (2013) estimated a value of CO_2 lost from global rivers of 1.8 Pg C/yr and 0.32 Pg C/yr from lakes and reservoirs.

Lakes and reservoirs have residence times of weeks to years, which are far longer than the residence times of rivers and especially for rivers in the UK – in-stream residence time in the UK at median flow is only 26.7 hours (Worrall et al. 2014a). Also, due to the long residence times of lakes and reservoirs, the DOC will be “old”, having been in the fluvial network for a longer time. “Young” DOC is readily biodegradable (Marschner and Kalbitz, 2003), and “old” DOC is more refractory (Southwell et al. 2011). Preferential degradation of “young” DOC means that large rivers, reservoirs, lakes and the sea will have larger proportions of “old”, less degradable DOC, and so the rates of degradation of DOC would be lower than in smaller rivers and their headwaters (Raymond and

Bauer, 2001). Cory et al. (2014) found that of the 6.11 Gg C/year (0.4g C/m^2/yr) DOC converted to CO^2 in a river basin, up to 92% could be attributed to light processes in headwater streams (photo-mineralized, photo-oxidised or photo-stimulated bacterial respiration). Worrall et al. (2014b) found that the total fluvial flux of carbon from the terrestrial source was 5.0 Tg C/yr (22.2 g C/m^2/yr) with 3.2 Tg C/yr lost to the atmosphere – equivalent to 13.9 g C/m^2/yr or a total loss rate of 63% and including a 20% net loss of POC across watersheds. Moody et al. (2013) performed experimental observations of the fate of DOC and POC in "young", fresh, peat stream water from the River Tees, northern England, and found an average 73% loss of the DOC over 10 days, with the majority of the loss occurring in the first two days, and between 38 and 87% removal of peat-derived POC. If the majority of degradation and loss of DOC and POC is occurring over a period of 2 days and the residence time of UK rivers is of the order of 1 day then degradation processes need to be considered on the order of hours and not days. As photodegradation, by definition, requires light, the DOC concentration in a stream is likely to exhibit a diurnal cycle of degradation which would not be readily observed if daily or longer timescales were considered (Worrall et al. 2013). Therefore, the aim of this study is to consider fluvial carbon dynamics over periods of hours and not days, with the specific aims of quantifying the changes in DOC and POC concentrations that occur in the normal, day/night cycle compared with changes that occur in total darkness, and attributing a proportion of the change to the effect of the waters exposure to sunlight. The rates of DOC concentration changes in the two treatments, and during each stage of the experiment and during the first hour of the experiments were also quantified with the aim of approximating the order of the reactions, and to investigate the potential for photo-stimulated bacterial degradation of DOC (Cory et al. 2014). This study considered the net changes in DOC concentration in water from the headwater of the River Tees in North-East England over periods of up to 70 hours.

Materials and Methods

Study Site

This study used Cottage Hill Sike (Figure 1; UK national grid ref: NY 744 327) within the Moor House National Nature Reserve (NNR), the most extensively studied of all UK peatlands (Billett et al. 2010), and has a catchment area of 0.2 km^2, with 100% peat cover. The Moor House NNR is part of the Environmental Change Network (ECN) monitoring programme which means that DOC concentration has been monitored in the stream water weekly since 1993 (www.ecn.ac.uk – Sykes and Lane, 1996; Worrall et al. 2009).

Degradation measurements

The degradation experiments were carried out in natural, ambient light and temperature conditions (rather than indoors under artificially controlled conditions). The study considered two treatments, one in which the water was always exposed to ambient light (thus experiencing both night and daylight conditions); and one in which all water samples were exposed to ambient temperature but were covered and therefore always in darkness. These treatments, henceforward referred to as 'ambient' and 'dark' were employed so as to distinguish between components of degradation (i.e. the difference between ambient and dark DOC concentration changes is the photo-induced DOC change). Experiments were conducted each month over the course of a year (except January when samples were not obtained as poor weather conditions prevented access to Moor House NNR) so that samples were taken across a range of both meteorological conditions and DOC concentrations and compositions. The water was not pre-filtered, so this study could consider the net fate of DOC and could include production from POC or adsorption by it, as well as in-situ production of both DOC and POC. The measurements made were net changes in the DOC concentrations, and it was assumed that both production and degradation of DOC and POC were happening in the water.

Each degradation experiment spanned approximately 70 hours with sacrificial sampling taking place at approximately hour 0, 1, 2, 8, and then at dawn and dusk on day 2, 3 and 4, with ambient and dark treatments on each month. Fixed numbers of hours since the start of the experiment were not used in the experiment because changes in initial river sampling time and changes in day length would mean that samples in daylight one month could be in darkness in a subsequent month, and thus samples were taken relative to dawn and dusk for each period of experimentation each month. Replicate samples (where more than one water sample per treatment and time was sampled) were included within each degradation experiment and over the course of the year each combination of factors was replicated, resulting in more than 11 samples for each sampling time and treatment combination. No hour 0 samples were replicated, but 47% of all other measurements were replicated (187 of 398 samples), approximately 12 replicates per month across all treatments and sampling times. Replication was limited by practical constraints of the amount of equipment available and the time taken to process DOC analysis to ensure the short timescales at the beginning of the experiment.

The sampled stream water was poured into acid-washed, quartz glass tubes, so they were approximately half full, with an air headspace, 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 put outside in trays, with all tubes lying at a slight angle (approximately 15°) to prevent rainfall entering and the sample evaporating or pouring out. The angling of the tubes also stopped the ambient 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, which could have shaded the water only at the peak of the growing season. 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. 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. UV radiation was not measured.

Sample analysis

Samples for DOC analysis were filtered to 0.45 µm, and then "fixed" with concentrated sulphuric acid. There was no visible flocculation after the addition of 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 measurement of DOC concentration was calibrated using standards of oxalic acid of 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 with deionised water so as to be within this range; the need for dilution was judged based on colour of the water. At each sampling time a duplicate sample was filtered to 0.45 µm, and used for further analysis. Absorbance at 400 nm was measured to provide a basic (visible) colour reading and the specific absorbance was taken as the absorbance at 400 nm divided by the DOC concentration of the sample. All optical measurements were performed using a UV–Vis spectrophotometer, with a 1 cm cuvette. Blanks of deionised water were used.

Suspended sediment (SS) concentration in each monthly experiment was measured in 50 ml samples at the beginning, middle and end of each experiment. Samples were filtered through pre-weighed, 0.45 µm, Advantec glass fibre filters; dried to 105 °C and the filter paper re-weighed to give the concentration of suspended sediment. In some months, 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). The suspended sediment concentrations were measured in each of the 11 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 62 POM measurements. Extrapolating

from the six months of data, the percentage of POM, and therefore POC, was calculated and applied to the whole suspended sediment data set, resulting in a year of calculated POC concentrations.

Conductivity, pH and water temperature of water samples as it left each quartz glass vial were measured by electrode methods to provide covariate information in ANCOVAs (analysis of covariance statistics).

Statistical methodology

The design of the experiment incorporated three factors: month, sample time and treatment. The month factor had 11 levels (one for each calendar month sampled except for January when weather prevented sampling); sample time had 10 levels (with average times (hh:mm) since start of experiment as: 0:00, 1:00, 2:00, 4:22, 9:00, 21:58, 30:58, 45:05, 54:29, and 68:52); and treatment had two levels (ambient and dark). The sample times are the averaged values (each has a standard error) that represent the samples taken on the first day (average hours 0:00, 1:00, 2:00, 4:22, 9:00), dawn and dusk on day 2 (average hours 21:58, 30:), dawn and dusk on day 3 (hours 45:05, 54:29) and dawn on day 4 (average hour 68:52, henceforward referred to as t_{70}).

A similar analysis progression was used to Moody et al. (2013) as the experimental design was similar and this allowed comparisons to be made between the two studies. An analysis of variance (ANOVA) was used to assess the significance of all three factors on the DOC and POC concentrations and where possible the interactions between the factors were also determined. Furthermore, the analysis was repeated including covariates (ANCOVA). The covariates used were: pH, conductivity, specific absorbance; and light and temperature variables. 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 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 hour 0 (t_0) for that experimental run. The magnitude of the effects and interactions of each significant factor and interaction were calculated using the method of Olejnik and Algina (2003). Main effects plots use the least squares means which are marginal means corrected for the influence of all other factors, interactions and covariates, to visualise the data.

Guided by the results of the ANOVA and ANCOVA, stepwise linear regression was used to develop empirical models. 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: $\left(sin\left(\frac{m\pi}{6}\right) + cos\left(\frac{m\pi}{6}\right)\right)$, where m is the month number (January = 1 to

December = 12). This was done to make the month factor a continuous variable, rather than one that changes from a value of 12 to 1 in between December and January. Some of the variables were transformed for the sake of physical-interpretability, e.g. reciprocal of the cumulative absolute temperature.

The change in DOC concentration and rate of degradation of DOC were considered relative to the two treatments; i.e. (i) the rate of degradation in the ambient treatment (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 – this was the estimate of photo-induced DOC change, and used to calculate the apparent quantum yield (see below). The photo-induced DOC concentrations will include the effects of both direct and indirect light exposure, such as the photodegradation of DOC and subsequent biodegradation of photodegradation products.

To perform an initial rate analysis, the rates of DOC degradation were also calculated for the very first hour of each experiment. Worrall et al. (2013) proposed a simple kinetic model for the loss of DOC based upon two zero-order decay processes, one for daylight hours and one for night time. To test this approach the rate of change for the whole days and nights in the first 48 hours of the experiments were calculated. The rates were calculated for day 1 (between t_0 and dusk on day 1), night 1 (between dusk on day 1 and dawn on day 2), day 2 (between dawn and dusk on day 2) and night 2 (between dusk on day 2 and dawn on day 3) of each experiment. These rates then underwent the same ANOVA, ANCOVA and regression process as the DOC concentrations, with the sample time factor being replaced by a "stage" factor with four levels (day 1, night 1, day 2 and night 2).

Photo-stimulated bacterial degradation

Photo-stimulated bacterial degradation could result in an increased rate of organic matter degradation following the addition of labile compounds, for example, the products of photo-degradation could stimulate further biodegradation (Tranvik and Bertilsson 2001). In this study it is hypothesized that photo-stimulated bacterial degradation 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, as stated in Cory et al. (2014). The presence of this effect was tested in two ways. Firstly, if there were an effect then there should be a difference between the night time rates measured in samples that have been exposed to light (ambient) from the night time rate for those samples that have always been in the dark. An ANOVA was performed on the night time rates, using treatment and month as factors with the hypothesis that night time rates would be significantly higher for ambient treatments. Secondly, the ratio of the night time rate in the ambient to that in the dark

231 treatments would be one if there was not photo-stimulated biodegradation; therefore, a single
232 value t-test was used to test whether the ratios of night time rates were different from one.
233
234 **Apparent quantum yields and activation energies**
235 The apparent quantum yields (AQYs – the extent of reaction per unit concentration of incident
236 photons) were estimated using the estimates of photo-induced DOC loss using the cumulative light
237 exposure and the number of hours since the beginning of the experiment. The results are presented
238 as a range, due to some instances of photo-production and therefore negative yields. ANOVA and
239 regression analysis were applied to the AQY values, using month and time as factors.
240 The activation energy was calculated to show the effect of temperature on the rate of
241 degradation in the ambient treatment, using the universal gas constant, 0.692 J/K/g C.
242
243 ## Results
244 In total 398 samples with complete covariate information and within the context of the factorial
245 design were conducted and analysed. Summary of the water chemistry over the 70 hours of the
246 study period in ambient conditions are given in Table 1. The conductivity and pH increased between
247 t_0 and t_{70} in both treatment (dark data not shown), suggesting an increase in the bicarbonate
248 concentrations over the course of the experiments. There was a slight increase in the absorbance at
249 400 nm, and decreases in both the POC and DOC concentrations.
250
251 **Changes in DOC concentrations**
252 For nearly every month of measurement the DOC concentration in both treatments decreased
253 (Table 2). The average DOC concentration over time showed a steep initial decline, although the
254 rate of decline was still not zero even after 70 hours (Figure 2). The average decline in the ambient
255 treatment was from 42 to 17 mg C/l (64% loss), whereas the decline in the dark treatment was from
256 42 to 36 mg C/l (6% loss), over the 70 hours. Of all the experiments run, there were 61 experiments
257 from both treatments (out of a total of 398 experiments) where an increase in DOC concentration
258 was observed at any time during the experiment, relative to the initial DOC concentration. In six of
259 the cases there was a higher DOC_{70} concentration than DOC_0. Given that no raw water samples
260 were filtered prior to inclusion in the experiment it was possible that particles or the microbial
261 population within the sample generated DOC over the course of the experiments. The Anderson–
262 Darling test showed that neither the distribution of DOC concentration nor relative DOC
263 concentration for the ambient or dark treatments, met the condition of normality, therefore all

subsequent ANOVA were performed on log-transformed data: re-application of the Anderson-Darling test indicated that no further transformation was necessary.

When the relative concentration data for both treatments (ambient and dark) were considered without covariates, all single factors were found to be significant (Table 3). The least important single factor was time (explaining only 7% of the variance in the original dataset). The most important factor was treatment, explaining 28% of the original variance.

One of the reasons for using relative DOC concentration was to minimize the difference between 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, even though there was no clear relationship between month and initial DOC concentration. Overall the ANOVA of the relative DOC concentration explained 68% of the variance in the original data. The error term represented 15% of the variance, which represents the unexplained variance in the model, 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. Possible variables that could not be included are the river discharge and the water chemistry at the start of each experiment – these data are not readily available for Cottage Hill Sike. The ECN water chemistry samples were taken on different days to this experiment, and so the data were not directly comparable.

Including covariates in the ANOVA (ANCOVA) showed the most important covariate was the t_0 specific absorbance, followed by DOC_0 concentration. This suggests that degradation rate was concentration and composition dependent. The absorbance at 400 nm showed a slight relationship with month, with the absorbance values being higher in the summer than in the winter. 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 the ambient treatment:

$$\Delta DOC = -1550(\pm 454)Abs_0 + 16.4(\pm 2.8)lnDOC_0 + 2.31(\pm 0.5)ln(t) - 39.5(\pm 11.4)$$

$p<0.0001$, $n=180$, $r^2=0.36$ (Eq. 1)

where Abs_0 is the specific absorbance at t_0, DOC_0 is the DOC concentration at t_0 (mg C/l), and t is the time since the start of the experiment (hours). Only variables that were found to be significantly different from zero at least at a probability of 95% were included. The values in brackets (e.g. ±454) give the standard errors on the coefficients and the constant term. This equation showed that the initial DOC concentrations and composition are significant in determining the change in DOC. Visual assessment of the data (Figure 2) suggested that the regression of the ambient treatment may benefit from using two regression lines, one for the initial rapid decrease during the first day and

one for the remaining time of the experiment, as there may be linear relationships for the two sections separately. Analysing the change in DOC concentrations for two time sections separately found an r^2 of 0.47 for the first 10 hours (Eq. 2), and 0.33 for the last 60 hours of the experiment (Eq. 3). The equations had three factors in common: the initial DOC concentration, the cumulative PAR and reciprocal of the cumulative temperature, however the parameter estimates suggest that both of these latter two parameters were more influential in the first 10 hours. It is interesting to note that neither equation found time of the experiment to be a significant parameter, however both the cumulative PAR and temperature factors will reflect changes in both time and month, with the PAR and temperature relationships with month showing a peak in late spring/early summer and the lowest points in winter. Absorbance at 400 nm which was significant in Eq. 1 was not significant in either model, suggesting that the composition of the DOC is less important than the light exposure and temperature when the ambient samples are analysed independent of the dark samples.

Between t_0 and t_{10}:

$$\Delta DOC = 29.6(\pm 4.1) lnDOC_0 + 0.19(\pm 0.06) \sum PAR + \frac{10800(\pm 6280)}{T} + 4.50(\pm 1.2)\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) - 137(\pm 29.5)$$

$p<0.0001$, $n=76$, $r^2=0.47$ (Eq. 2)

Between t_{10} and t_{70}:

$$\Delta DOC = 16.8(\pm 3.2) lnDOC_0 + 0.03(\pm 0.01) \sum PAR + \frac{14100(\pm 3140)}{T} - 75.2(\pm 15.2)$$

$p=0.0003$, $n=96$, $r^2=0.33$ (Eq. 3)

where $\sum PAR$ is the cumulative photosynthetically active radiation experienced by the sample (W/m^2), T is the cumulative temperature (K), m is the month number and all other terms are as described above. The model was also calculated using an exponential relationship between the change in DOC concentration and time, however the r^2 was only marginally better than the linear relationship, and the results were less physically interpretable.

The difference between the dark and ambient treatment DOC concentrations in each experiment was taken as the estimate of the impact of photic processes (Figure 3). The extent of photo-induced DOC degradation could be estimated in 202 cases, and there were 18 occasions where the DOC concentration was higher in the ambient treatment than in the dark treatment (implying photo-induced production). Of the 18 occasions where an increase was observed, only

four 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 ANOVA shows that all single factors and all interactions were significant (Table 4). Two covariates were found to be a significant: the PAR and temperature variables. The month factor, although significant and explaining the highest proportions of the variance in the ANOVA was no longer significant in the ANCOVA. The other significant factor, time, and the significant interaction (time*month) all explain 17% and 11%, respectively, of the variance in the ANOVA. Given the results of the ANOVA it was possible to identify the best-fit equation for the loss due to photo-induced degradation:

$$\Delta DOC_{photo} = -3.66(\pm 1.02)\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) - 4.60(\pm 1.32)ln(t) - 4.59(\pm 3.18)lnDOC_0 - \frac{2690(\pm 2040)}{T} + 18.0(\pm 13.1)$$

p<0.0001, n=191, r^2=0.21 (Eq. 4)

where ΔDOC_{photo} is the difference between the dark and light DOC concentrations (mg C/l). The apparent quantum yields (AQYs) were estimated for the photo-induced DOC loss and was found to vary between 82 and -56 mmol C/mol photons, and an ANOVA found that there were significant differences between the month and time factors, and the interaction of month*time. A regression analysis showed that both month and time were significant:

$$AQY = -3.06(\pm 1.09)\left(sin\left(\frac{\pi m}{6}\right) + cos\left(\frac{\pi m}{6}\right)\right) + 2.81(\pm 0.72)ln(t) - 12.2(\pm 2.09)$$

p<0.0001, n=173, r^2=0.12 (Eq. 5)

The seasonal cycle exhibited a similar pattern to that described in Moody et al. (2013), 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 AQY varied with time, having the smallest yields at the beginning of the experiment (Figure 4), showing that exposure to light had the greatest effect on the DOC when it was freshest, early in the experiment. The AQY relationship with month is the opposite of the relationship with absorbance, which peaks in June and is lowest in the winter, suggesting that there could be a link between the photodegradability of the DOC and its composition.

The regression analysis on ΔDOC_{photo} (Eq. 4) showed that the DOC loss due to photo-induced degradation could be calculated from the seasonal cycle, sample time, DOC_0 and temperature; all

variables that can be easily measured, and therefore the equation is easily physically interpretable and easy to apply to other data sets.

Changes in POC concentrations

The average changes in POC concentration in the ambient treatment across all months are shown in Table 2; there was a decrease in the POC concentration in the ambient treatment, and an increase in the dark treatment. The Anderson-Darling test showed that the distribution of POC concentration did not meet the conditions of normality, and so the data were log transformed. An ANOVA on POC concentrations found that time and month were significant single factors, as was the interaction between them (Table 5). Month explained the highest proportion of the original variance (26%). An ANCOVA found no covariates were significant, and although a regression was attempted, no significant equation could be calculated, even using only the ambient treatment samples.

Rates of DOC degradation

The minimum, maximum, mean and median rates of degradation in the ambient and dark treatment are shown in Table 2. The mean rate of DOC degradation in the ambient treatment was 1.7 mg C/l/hour, and 0.5 mg C/l/hour in the dark treatment. In each treatment, decreases or no change in DOC concentrations were observed in 88 cases out of 91, showing that in the majority of cases the DOC concentration decreased in both treatments (Figure 5). An extremely large maximum DOC loss rate of 37 mg C/l/hour would suggest that there was flocculation of the DOC in this sample, however there were no sub-daily samples analysed for POC and so this cannot be verified. The ANOVA of the rate of degradation for ambient samples showed that only the time factor was significant (Table 6). When included, no covariates were found to be significant, which means that the rate of degradation is not dependent on anything other than time of the experiment. Guided by the results of the ANOVA, the best-fit equation for the degradation rate was calculated:

$$lnrate_{ambient} = 0.08(\pm 0.8) - 0.79(\pm 0.1)ln(t) + \frac{277(\pm 228)}{T} + 0.0002(\pm 0.0005)\sum PAR$$

$p<0.0001$, $n=141$, $r^2=0.57$ (Eq. 6)

where $rate_{ambient}$ is the rate of DOC change in the ambient treatment, and all other terms are as described 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, suggesting that the DOC degradation was influenced by environmental factors,

such as the temperature and light levels during the experiments, both factors that change with time. The partial regression coefficients showed that the time variable was the most important variable in the model, with PAR and temperature accounting for only small proportions of the variation.

For the rate of degradation in the dark, the ANOVA and ANCOVA show that no factors or covariates were significant (Table 6); even so regression was attempted, but no significant variables were found. There were no significant differences between the rates at different times during the experiment. The processes controlling the degradation of DOC in the dark must therefore not be dependent on any of the measured variables, suggesting that biodegradation is not temperature or concentration dependent.

Although the rate of degradation in the dark was minimal, the rate of photo-induced degradation was calculated and analysed in the same way as the individual treatments. The ANOVA on the rate of the photo-induced DOC degradation found that Time was significant (Table 6). No covariates were found to be significant. Guided by the ANOVA, a regression was calculated:

$$lnrate_{photo} = 1.80(\pm 0.2) - 1.12(\pm 0.1) ln(t)$$

$p<0.0001$, n= 59, $r^2=0.7$ (Eq. 7)

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.

Rate of DOC degradation during each day and night

This analysis showed that there were times when there was net DOC production, such as in the dark treatment during both Night 1 and 2, and in the ambient treatment during Night 2 (Figure 6). This is likely to be due to release from POC or production of DOC in the quartz glass tubes being of greater magnitude than the degradation of DOC. The ANOVA found all three factors significant (Table 7), as well as three interactions: treatment*stage, treatment*month, and stage*month. Stage explains the largest proportion of the variance (27%) followed by the interaction of stage*month (14%), 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 and stage showed the significant differences between the average rates per stage for treatments, with the night rates being not significantly different from zero (Figure 6). There were no significant covariates.

The rates of degradation in the ambient 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.

Initial rates of DOC degradation

The average rate of DOC degradation in first hour of the experiment in the ambient treatment was 11.6 mg C/l, and 3.6 mg C/l in the dark treatment. An ANOVA on the rates had two factors, treatment and month. The ANOVA found all factors and interactions were significant (Table 8). The month factor explained the largest proportion of the variance (38%), closely followed by the interaction of month*treatment, 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. Once covariates were added, the DOC_0 concentration was significant, and the month factor was no longer significant. This shows that the initial rate of DOC degradation is dependent in the initial concentration of DOC, and the monthly differences found in the ANOVA are likely due to the monthly differences in the DOC_0 concentration.

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

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

$p<0.0001$, n= 18, $r^2=0.5$ (Eq. 8)

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).

Photo-stimulated bacterial DOC degradation

The average night time rates of DOC degradation for the two treatments were -0.2 ± 0.13 mg C/l/hour in the dark treatment and 0.1 ± 0.07 mg C/l/hour in the ambient treatment. An ANOVA based on the night time rates, using treatment and month as factors, found no significant differences in the rate of degradation. Secondly, a single sample t-test was used which showed that the mean ratio was 2.15 (95% ci = 0.31 – 3.98) i.e. not significantly different from 1 at the 95% probability. Therefore it was concluded that there was no net photo-stimulated bacterial

degradation, however, as the DOC concentrations measure the net changes, it was possible that there was a decrease in the DOC concentrations due to photo-stimulated bacterial degradation, but this was masked by other biological processes.

Discussion

Removal rates of fluvial carbon reported in the literature for similar environments range from 21% (Battin et al. 2009) to 70% (Gennings et al. 2001), so the loss of 64% from this study is not unprecedented, however it is towards to higher end of the literature ranges. Recent work by Cory et al. (2014) found DOC losses of 55%, of which approximately 75% was photodegraded, and Jones et al. (in press) indicated 50-80% of DOC is mineralised to CO_2 by photodegradation. The results show that photodegradation is responsible for the majority of the DOC loss, and are similar to this study.

The general trends in the data were that the DOC and POC concentrations decreased in the ambient treatment, while a smaller decrease was observed in the dark treatment. However, there were some cases were the DOC concentrations did not decrease: experiments where there was an increase in DOC over the course of the experiment were not removed from the analysis, as the study was interested in the conversion of POC to DOC and the average fate of DOC. The 61 occasions where an increase in DOC concentration was measured occurred in eight of the 11 months, with the largest numbers occurring during the spring months. The six occasions where there was a higher DOC_{70} concentration than at DOC_0 all occurred in the dark treatment, in March, May, August and October. Jones et al. (in press) also found increases in DOC occurred, especially in their dark treatment, suggesting aphotic production of DOC.

Even when using the relative DOC concentrations, there were still some significant differences between the months; 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.

The model results suggest that the physical process of DOC removal is controlled by time, light exposure, air temperature, composition of the DOC (absorbance at 400 nm) and a seasonal factor, but most importantly by the initial DOC concentration with higher concentrations leading to higher rates of DOC loss. This shows that waters with high natural DOC concentrations, such as peat-sourced rivers, will have higher rates of DOC removal. Comparing these models to those in Moody et al. (2013), the same factors were found to be significant (initial DOC concentration, time, seasonal factor, absorbance at 400 nm, PAR and temperature); however the models presented in

this paper are generally simpler and more physically interpretable, showing the benefits of sub-daily sampling.

Moody et al. (2013) found 73% DOC removal over 10 days. If this rate of loss were constant, it would result in a 21% loss in 70 hours. This is a lower estimate than found in this study (64%), although the former experiment was conducted over 10 days rather than 70 hours, and presuming a constant rate of loss is unrealistic, 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 rivers across a wide range of flows, and so will not provide a reliable estimate of the in-river loss of DOC. The more frequent sampling of this study 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.

The model of the initial rate of DOC degradation, which was estimated using the DOC concentration change during the first hour of the experiments, found that the factors affecting the initial rate are the initial DOC concentration and a seasonal factor. This method of analysis would suggest that in the ambient treatment, 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 rate and order of each of the contributing processes.

An advantage of recording the PAR and temperature throughout the experiment was the possibility of estimating the AQYs and the activation energy of the DOC degradation, which were calculated to be -4.99 ± 1.10 mmol C/mol photons, and 0.19 ± 0.16 kJ/g C respectively. The range of the AQYs found in this study are larger than the range found by Moody et al. (2013) of 9.6 to -1.7 mmol C/mol photons, and the literature values cited therein (Osburn et al. 2009). The activation energy is considerably lower than the value found by Moody et al. (2013) of 2.6 ± 1.2 kJ/g C, suggesting that the degradation for DOC from the headwater site is much less sensitive to changes in temperature than the average of the four sites used in Moody et al (2013). This could be a benefit of using only one site, where the temperature range is known, rather than four sites which are likely to have more variable temperatures.

This studies' sub-daily sampling meant that the DOC concentration changes during the night could be calculated, as previous research suggested that there would be an effect of prior light exposure on the dark bacterial degradation rates (such as Tranvik and Bertilsson, 2001 and Cory et al., 2013). However, this study found no difference in the night time rates of DOC degradation between those samples exposed to day light during the day and those kept in the dark. Cory et al. 2014 found photo-stimulated bacterial respiration consumed more oxygen than bacterial respiration

in the dark, and Judd et al. (2007) found evidence of a long-term benefit to bacterial respiration based on photo-oxidation of DOM.

To scale up the DOC loss from the Tees to the whole UK, 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 the UK of 312-2178 Gg C/yr (Worrall et al. 2014b) were used, in conjunction with the 13% loss of POC and the 64% loss of DOC loss from this study. Applying the 64% loss of DOC to this would suggest the DOC flux at the source would have been 1542-3508 Gg C/yr. Loss of DOC to the atmosphere would be 987-2245 Gg C/yr, or 3619-8231 Gg CO_{2eq}/yr (14.86-33.79 Mg CO_{2eq}/km^2/yr from the UK). The 13% loss of POC observed in this study would equate to a POC flux at the source of 359-2503 Gg C/yr, and loss of POC to the atmosphere would be 47-325 Gg C/yr, or 171-1194 Gg CO_{2eq}/yr (0.70-4.90 Mg CO_{2eq}/km^2/yr from the UK). These CO_2 emission values assume that 100% of the DOC and POC lost from a catchment is lost to the atmosphere. It is likely that they will be over-estimates as the DOC losses from peat-sourced water bodies are higher than from other water bodies, and peat DOC is more photo-reactive than other forms of DOC (Jones et al. in press).

The total man-made 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 8.2 Tg CO_2/yr from rivers in the UK is equal to 1.4% of the UK total anthropogenic emissions; however the emissions from DOC would count as 'natural' rather than 'man-made'. Maximum CO_2 from POC losses equates to 1.2 Tg CO_2/yr; it increases the total greenhouse gas contribution from UK rivers to 9.4 Tg CO_2/yr.

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 (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 9.4 Tg CO_2/yr from this study for UK is 0.52% of the total CO_2 emissions from inland waters from Raymond et al. (2013), or 0.94% of the estimate from the 2013 IPCC, implying than 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 because 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 study were from low-order streams, potentially resulting in over-estimates of loss as CO_2. The higher contribution from the UK inland waters to the global CO_2 flux relative to the land area of the UK suggests it could also be due to the high

565 percentage of land covered by deep peat in the UK. This is linked to high and increasing DOC fluxes,
566 and therefore high losses of organic carbon as CO_2, especially in peat-sourced streams.

567 The water samples in this study were deliberately kept outside to be exposed to natural light
568 and air temperatures; however these would not have been exactly the same conditions as in the
569 stream. The light exposure and penetration experienced by the samples in the quartz glass tubes
570 would have been similar to in the stream when it was a small and shallow headwater, but once it
571 joined with the larger stream and rivers the conditions would have been quite different, such as the
572 depth, light absorbance and turbidity, conditions which could not be replicated in this experiment.

573 This study shows the importance of the diurnal cycle in flux calculations. Previous estimates
574 of flux that do not account for the diurnal cycle of in-stream processing are prone to under/over
575 estimation, due to the times of day at which the majority of samples are taken. Residence times of
576 rivers are rarely an exact multiple of 24, and so estimates of fluxes based on measurements during
577 the day and extrapolated to represent the whole 24 hours will overestimate the flux, as the night
578 time flux is unlikely to be the same as the flux during daylight. Worrall et al. (2013) developed a
579 'correction factor' dependent on the residence time of the water body and the day:night ratio of the
580 biogeochemical process being investigated. They applied their model to the flux on the River Tees
581 and found that fluxes could have been overestimated by between 5 and 25%. Using their model and
582 the median first day and first night rates found in this study for the ambient treatment, it was
583 calculated that sampling at 9am would have underestimated the flux of DOC by 46%, compared to
584 sampling at every hour on every day. This demonstrates the need to take the diurnal cycle into
585 account when scaling up fluxes.

586 In this study, as Moody et al. (2013) and Jones et al. (in press), the DOC concentration does
587 not become zero during the experiment, suggesting that something other than time is limiting the
588 DOC degradation; for example, the nutrient concentration of the river water, non-degradable DOC,
589 or autochthonous production of DOC in nutrient-rich waters, that means over all concentration does
590 not decrease but reaches a position of quasi-equilibrium.

591

592 ## Conclusion

593 This study found the average loss of DOC in ambient conditions was 64% over 70 hours with the
594 majority of the loss occurring within the first 10 hours of daylight. The study found a strong diurnal
595 cycle, with the average rates of headwater DOC degradation during the daylight being approximately
596 30 times higher than those during the night for the same treatment. The analysis of the initial rates
597 of DOC degradation in the ambient treatment found that that a 2^{nd} order, or a mixed order reaction,
598 best explains the process.

599

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603

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690 Quantifying in-stream losses and carbon sinks. Journal of Hydrology 519:611-625.
691

692 Table 1. Average and coefficient of variation (CV - %) of conductivity, pH, absorbance at 400 nm and
693 POC and DOC concentrations, across all months of the study, from Cottage Hill Sike in the ambient
694 treatment, for the initial (t_0) and end (t_{70}) concentrations.
695

Variable	t_0		t_{70}	
	Mean	CV (%)	Mean	CV (%)
POC (mg C/l)	6.99	31	6.11	14
Conductivity (µS/cm)	35.87	25	78.23	61
pH	4.57	14	6.34	5
DOC (mg C/l)	41.75	30	16.52	85
Abs_{400}	0.16	39	0.17	45

696
697

698 Table 2. The DOC and POC concentration changes, and the minimum, maximum, mean and median
699 overall and initial rates, in the two treatments, and the difference between the two treatments (the
700 photo-induced change).
701

	DOC concentration change		DOC rate (mg C/l/hour)			
Treatment	average change (mg C/l)	% change	min	max	mean	median
Ambient	42 to 17	64	-5.27	37.76	1.73	0.09
Dark	42 to 36	6	-5.10	27.63	0.48	0.01
Photo-induced	0 to -17	58	-13.05	36.44	1.25	0.07
	POC concentration change		Initial DOC rate (mg C/l/hour)			
Treatment	average change (mg C/l)	% change	min	max	mean	median
Ambient	7 to 6	13	-6.18	37.76	11.57	6.76
Dark	7 to 12	increase	-7.93	30.33	3.60	1.17

702
703

704 Table 3. Results of ANOVA for relative DOC concentrations for all experiments across both ambient
705 and dark treatments.
706

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
Abs_{400}/DOC_0	na		<0.0001	4.94
DOC_0	na		0.0161	0.67
treatment	<0.0001	27.93	<0.0001	33.31
time	<0.0001	6.67	<0.0001	3.65
month	<0.0001	10.62	ns	-
treatment*time	<0.0001	6.20	<0.0001	4.42
treatment*month	<0.0001	13.48	ns	-
time*month	0.0070	2.65	ns	-
Error		15.19		3.47

707
708

709 Table 4. Results of ANOVA for the difference in DOC concentrations between ambient and dark
710 treatments, attributed to photo-induced degradation.
711

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
1/T	na	-	0.0003	6.10
∑PAR	na	-	0.0059	3.35
time	<0.0001	16.60	0.002	12.10
month	<0.0001	36.59	ns	-
time*month	0.0008	10.83	ns	-
Error		21.87		1.98

712
713

714 Table 5. The results of ANOVA of the POC concentrations.

715

	Without covariates	
Factor	p	ω^2
time	0.0016	4.70
month	<0.0001	25.96
time*month	<0.0001	19.12
Error		24.32

716

717

718 Table 6. The results of ANOVA on the degradation rates of DOC

719

		Without covariates		
Variable	Factor	p	ω^2	Error
Ambient rate	time	<0.0001	35.21	5.98
Dark rate	-	ns	-	-
Photo rate	time	0.0206	11.19	8.00

720

721

722 Table 7. The results of the ANOVA on the rates of degradation in each stage.

723

	Without covariates	
Factor	p	ω^2
treatment	<0.0001	6.87
stage	<0.0001	27.15
month	0.0383	2.06
treatment*stage	<0.0001	11.76
treatment*month	0.0183	2.59
stage*month	<0.0001	13.91
Error		12.17

724

725 Table 8. The results of the ANOVA on the rates of degradation in the first hour.

726

	Without covariates		With covariates	
Factor (or covariate)	p	ω^2	p	ω^2
DOC_0	na	-	<0.0001	30.23
treatment	<0.0001	10.94	0.0065	9.84
month	<0.0001	38.29	ns	-
treatment*month	<0.0001	34.20	ns	-
Error		8.25		3.32

727

728

729 **Fig 1.** Location of the site and study catchment.

730

731 **Fig 2.** The main effects plot of relative DOC concentration change for ambient and dark treatments
732 over the course of the experiment. Error bars give the standard error.

733

734 **Fig 3.** The main effects plot of the change in loss due to photo-induced degradation of DOC over the
735 course of the experiment. Error bars give the standard error.

736

737 **Fig 4.** Main effects plot of the apparent quantum yield (AQY) over time in the experiment. Error
738 bars give the standard error.

739

740 **Fig 5.** Main effects plot of rate of DOC loss in ambient and dark treatments over time in the
741 experiment. Error bars give the standard error.

742

743 **Fig 6.** The main effects plot of average rates of DOC degradation per stage of the experiment for
744 both treatments. Error bars give the standard error.