Response to comments for the manuscript bg-2016-296 ‘Temporal changes in photoreactivity of dissolved organic carbon and implications for aquatic carbon fluxes from peatlands’

Amy E Pickard et al.

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We thank the referees for their thorough reading of the manuscript. We address their points and those of the Associate Editor (shown in italics) below.

In addition to making the corrections advised by the two referees and associate editor, we have changed how photoreactivity is quantified in the Loch Katrine samples, where minimal net DOC changes upon irradiation were observed in the sample aliquots. Photoreactivity (mg C / mg DOC) is now expressed as the sum of gaseous photoproduction divided by the pre-irradiation DOC concentration (eq. 1). For example, in the Loch Katrine May 2014 sample:

\[
\text{Photoreactivity} = \frac{(0.07 \text{ mg CO}_2 \text{-C L}^{-1} + 0.02 \text{ mg CO-C L}^{-1})}{5.05 \text{ mg DOC L}^{-1}} = 0.02 \text{ mg C /mg DOC} \quad \text{(eq. 1)}
\]

This is in contrast to Black Burn water samples, where the sum of gaseous photoproduction is added to net DOC loss (expressed as a positive value) and divided by the pre-irradiation DOC concentration (eq. 2). For example in the Black Burn May 2014 sample:

\[
\text{Photoreactivity} = \frac{(1.62 \text{ mg CO}_2 \text{-C L}^{-1} + 0.13 \text{ mg CO-C L}^{-1} + 2.02 \text{ mg DOC L}^{-1})}{50.9 \text{ mg DOC L}^{-1}} = 0.07 \text{ mg C /mg DOC} \quad \text{(eq. 2)}
\]

This method means that no negative photoreactivity values are produced for Loch Katrine (which may have been explained in large part by the limited resolution of the PPM LabTOC instrument at very low DOC concentrations). Photoreactivity data in figure 4b have been adjusted accordingly and text explaining the revised photoreactivity calculations has been added to the data analysis section 2.6 (lines 256-262). The revised method for calculating the photoreactivity of the Loch Katrine samples meant that the mean spring absorbance values shown in Figure 3b are now very similar to those for winter and autumn. Consequently the text referring to the previously apparent difference has been removed from Discussion section 4.1.

Associate Editor comments

Your manuscript has been reviewed by two referees. Both referees agreed that the manuscript represents a substantial contribution to scientific progress within the scope of this journal, and that the measurements discussed are novel and provide new insights on the chemical composition and photochemical reactivity of peatland DOM. At the same time, the reviewers recommended a number of improvements. Both referees were concerned about the choice of irradiation source, how relevant this study’s findings are to photochemical transformation processes in natural waters and how comparable the results are, in quantitative terms, with those of other studies. The implications of the choice of irradiation source would need to be discussed in detail in the revised manuscript, also in the context of previous studies discussing the dependence of absorption and fluorescence photobleaching on the spectral quality of exposure.

We thank the editor for their positive comments and for their suggestions to improve the manuscript. In alignment with these comments, we have added significant text to the discussion to highlight that the UV exposure used here allows only for relative differences over time and between our sites to be elucidated (lines 412-420). Furthermore we suggest that the spectral dependence of absorption may
mean that changes in for example the E4:E6 ratio may not be comparable to other studies and that its use in correlation analyses should be interpreted with caution (lines 439-448).

In addition to the discussion changes we have also added a spectral weighting function table to the methods section to compare the weighted exposure used in this study to standard weighting functions (Table 1).

In addition, the reviewers recommended to show additional parameters to get more insights on the composition and transformation of DOM in the study region. Additional information, from ancillary measurements or existing literature, on downstream water composition and light attenuation/optical depth could also help address the question raised by the reviewers regarding downstream DOC processing/turnover rates.

We have added additional parameters that provide insights to DOM transformation to our paper, including changes to absorbance at 350 nm and the spectral slope of absorbance. These have been included in the correlation analyses shown in Table 3. In terms of additional parameters to estimate downstream DOC processing rates, we believe that without significant further data collection obtaining a reasonable estimate is beyond the scope of this study. Instead we have expanded the discussion of the uncertainties in downstream DOC processing and have suggested that future studies should focus attention on potential hotspots for DOC processing (lines 529-558), which include mixing zones of freshwaters with different pH, conductivity and metal concentrations as identified by Palmer et al. (2015) in their study of peatland headwaters.

As mentioned by Reviewer #2, filtration through 0.22 um does not completely remove bacteria from the sample. The authors should report in their revised manuscript whether during their experiments they observed any changes (with time) in CDOM or DOC in their "control" samples (0.22 um, not exposed to light). On line 286, the manuscript mentions that: "dark control samples showed a greater drop in absorbance upon irradiation than light exposed samples". More discussion is needed on these results.

We thank the editor for highlighting this point. We have amended our text to make clear that the absorbance increases occurred in the irradiated samples relative to the initial absorbance values measured prior to the experiment (lines 305-306). Control samples showed no change relative to the initial samples. We have added a section to the discussion which reviews the potential effect of bacterial DOC production in the UV exposed samples in summer and suggest that carbon isotope data may help to resolve such uncertainties (lines 475-482).

Referee 1

Dissolved organic matter (DOM) has been sampled from Scottish peatlands, examining both the upper end stream drainage from a peat bog and from a lake as the lower end receiving basin. This material was experimentally exposed to UV radiation in order to understand DOM photoreactivity and address the hypotheses that photoreactivity is seasonally variable, linked to rainfall events and an important loss term of carbon from the peatland system. The report is a good contribution to on going efforts within the aquatic biogeochemistry community to better understand the significance of photoreactions to carbon and mineral flows. This is the first study addressing this topic for Scottish peatlands. The sampling and approaches have merit in regard to characterizing seasonality, response to rain events and comparing the systems. Clear patterns of response are resolved for the high DOM site at Black Burn with the interesting result of highest photoreactivity in early winter. On the other hand, DOM in Loch Katrine is much less photoreactive, and a seasonal pattern was not evident although resolution became an issue at the level of responses observed.
We thank reviewer 1 for their positive comments. We believe the edits described below will significantly improve the original manuscript. In particular we have added more discussion about the potential influence of the irradiation source upon the measured results.

The authors have made an unusual choice as to irradiation source in the experimental exposures with consequences for the environmental relevance of the production rates and their relation to environmental factors. Unlike any other similar study that I am aware of, the authors chose to a primarily UVB (280-315 nm) emitting fluorescent lamp. This lamp has comparable UVB output as solar irradiance at noontime, on a summer solstice, clear day but much lower UVA (315-400 nm) and PAR (400-700 nm). Thus, most studies on this issue use Xe lamp based solar simulator (example stream study – Porcal et al. 2013) or lamps with primarily UVA output (example Lu et al. 2013). Spectral distribution is important because in most aquatic environments in situ, CDOM will absorb much more UVA than UVB. For relatively "fresh DOM" (using brackish tidal marsh CDOM as an example), about 90% of the absorbed irradiance at the surface is UVA and only 5% UVB (rest PAR). Thus the treatment described could substantially underestimate actual rates exhibited by a sample that experienced an equivalent period of full sun (the experimental 8 h exposure to 1.8 W m$^{-2}$ is about the same as the cumulative incident UVB on a 14 h cloudless day at solstice, ca. 53 vs 49 kJ m$^{-2}$ respectively). Potentially, some adjustment for comparability to other studies could be made for this by considering the general shape of the apparent quantum yield spectrum for CO and CO$_2$ photoproduction from DOM comparing the lamp spectrum to solar irradiance (cf. the cited Stubbins et al. and Koehler et al. studies) and in addition by expressing results as a rate constant vs a simple change over the incubation period.

The irradiation source was selected as UVB is the most effective source of radiation in producing photochemical effects (Häder et al., 2007; Zepp et al., 2007), however we agree with the reviewer that this choice makes comparison with both previous studies and natural photochemical responses more difficult. In order to allow clearer comparison with previous studies using UV lamp sources, we have now included in the manuscript the following table of weighted action spectra responses for commonly used spectral weighting functions including CH$_4$ from pectin (McLeod et al., 2008), plant growth function (Flint and Caldwell, 2003), general plant action spectrum (Green et al., 1974) and DNA damage (Setlow, 1974). We include this in the methods section where details of irradiance regime and output are stated (lines 154-160).

Table 1. Photosynthetically active radiation (PAR) and ultraviolet irradiances during 8 h exposures to Q-Panel 313 fluorescent lamps filtered with 125 µm cellulose diacetate.

<table>
<thead>
<tr>
<th>Irradiance W m$^{-2}$</th>
<th>Total UV (280-400 nm)</th>
<th>UV-A (315-400 nm)</th>
<th>UV-B (280-315 nm)</th>
<th>PAR (400-700 nm)</th>
<th>CH$_4$</th>
<th>GEN (G)</th>
<th>PG</th>
<th>DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.52</td>
<td>4.63</td>
<td>2.89</td>
<td>0.92</td>
<td>2.50</td>
<td>1.25</td>
<td>1.05</td>
<td>0.98</td>
</tr>
</tbody>
</table>

We have also added to the discussion a section on the potential influence of the UVB313 lamps on the results of the study and the potential implications the methodology may have for any upscaling attempts (lines 412-420).

However, there is a larger issue, which is that, as the authors state, due to the effects of bank shading and short transit time of water within the immediate catchment, light driven instream
DOC processing is unlikely to be significant for the high DOM Black Burn. Instead, they suggest that the actual processing may occur considerably downstream, in unshaded streams or lentic systems. But the rates there will further depend on the residence time, transparency and optical depth of those systems which are basically unknown for this material. So in the end, I would be very cautious in making any estimate even of an upper bound in the carbon loss rates from these systems given the very substantial methodological bias and involvement of unknown factors. I do agree that given the demonstrated photoreactivity of fresh peatland DOM more work should be done to obtain such an estimate, in particular, if it could somehow be scaled up to a catchment or regional scale.

We agree with the reviewer’s comment that there are significant uncertainties in downstream DOC turnover and have adopted more cautious wording in our discussion section ‘Implications for photochemical turnover of DOC in aquatic systems’. We have also removed the potential evaded photochemical CO$_2$ estimate and instead suggest that given the significant volume of DOC produced by the catchment, in-stream photo-processing may be an important term in carbon budgets of peatland draining aquatic systems (lines 529-539).

The choices of irradiation source may also influence the correlation of photoreactivity with other factors, particularly optical characteristics. Several studies have demonstrated that the spectral dependence of absorption and fluorescence photobleaching depends on the spectral distribution of the irradiation source (Del Vecchio and Blough 2002, Tzortziou et al. 2007). UVB-313 fluorescent lamp-based exposure system could produce a distinctly different absorption difference spectrum than natural irradiance (incident or in water), however I do not know of any study that has made the comparison. The results could influence the correlation of photoreactivity and other variables with delta E4:E6, for example.

We thank the reviewer for highlighting this issue. We have added text to Discussion section 4.1 ‘Peatlands as a source of photochemically labile DOC’ which evaluates the possible influence that the irradiation source (UV313 lamps) may have had on the optical characteristics of water samples and suggests that results should be interpreted with caution (lines 439-448). Correlations between delta E4:E6 values and other variables in Table 2 (now Table 3) remain in the manuscript as we assert that because all samples were exposed to the same irradiation conditions, the relative differences in the values can provide interesting information pertaining to factors influencing carbon budget changes.

Finally, the spectral distribution of the irradiance source could influence which chromophores are contributing to the mineralization processes for example, which lignin phenols are involved. I do not know whether this is the case, but it is something that should be kept in mind when relating photoreactions to DOM composition.

We agree that this would be an interesting line of enquiry. However, given the lack of literature on the topic we feel that it would be difficult to discuss the potential influence of spectral distribution of the irradiation source on preferential degradation of phenol groups. In our study lignin phenol analysis was not conducted on irradiated samples and hence we could not support such discussion with any evidence.

I made several minor comments on the mss which I have annotated directly on the pdf. On the figures, it would be helpful in visualizing the irradiance induced changes shown in Fig. 4 if independent scales were used for the Black Burn vs Loch Katrine samples. The point that the L. Katrine photoreactivity is much lower won’t be lost if (like in the other figures), the difference in scale is called to the attention of the reader. A more important point, is the relative variation in time (or lack thereof) which is presently difficult to see for the L. Katrine results.

All the minor comments annotated directly on the pdf have been addressed. We have adjusted the y-axis scale in Fig. 4 for plots for the Loch Katrine samples, and noted the difference in scale in the figure caption. We have also adjusted the method for determining photoreactivity in Loch Katrine samples.
where net DOC gains were observed upon irradiation, as explained on pp.1 of the author comment above.

Referee 2

This study investigates the seasonal and spatial variability in the photoreactivity of DOM from Scottish peatlands. Novel observations are presented on the chemical composition of peatland DOM, the influence of precipitation events on DOM mobilization and the significance of water residence time on DOM photodegradation and export. Peatland systems export high concentrations of photoreactive DOM, and this study demonstrates solar radiation can play an important role in carbon gas fluxes from these systems.

We thank the reviewer for their positive comments and for their constructive criticism of the manuscript.

It is important to use lamps that provide a good simulation of sunlight (spectrum and intensity) when investigating photochemical alterations of natural organic matter in the environment. The UV-B 313 lamp used in this study emits short wavelength UV (below the 295 nm solar cutoff) that is particularly destructive of organic molecules. Therefore, while providing useful information about the relative photoreactivity of DOM among different seasons and locations, the results from this study should not be used to estimate rates of photodegradation in natural waters. In addition, comparisons of the results from this study with those of other studies should be of a qualitative, rather than quantitative, perspective.

The UV313 lamps were covered with a film of 125 µm cellulose diacetate (CD), as described in the methods section of the original manuscript. However we made a typographical error when stating the transmission properties of the CD film. CD provides a cut-off point at 290 nm, below which no light is transmitted (e.g. McLeod et al., 2008; Fraser et al., 2015). This has now been corrected in the manuscript (lines 144-146 and 416-420) and should assure the reviewer that there are very limited photochemical effects generated as a function of short wavelength UV which is not present in the natural solar spectrum.

In alignment with this comment and similar comments provided by the first reviewer, we have removed the potential evaded photochemical CO₂ estimate and instead suggest that, given the significant volume of DOC produced by the catchment, in-stream processing may be an important term in carbon budgets of peatland draining aquatic systems (lines 529-539).

Comparisons of percentage DOC losses to other photodegradation studies cited in the discussion section ‘Peatlands as a source of photochemically labile DOC’ have been retained in the text. We believe that the inclusion in the manuscript of figures from other studies will give the reader confidence that although a UV-B irradiation source was used in this study, the magnitude of photochemically induced DOC losses are comparable to previous studies which used a solar simulator to output a natural irradiation spectrum.

A couple of additional optical parameters can provide insights about the source, composition and alteration of DOM. The following parameters should be included: spectral slope (S) 275-295 nm, and the absorption coefficient at 350 nm (a350). The S275-295 is an indicator of DOM molecular weight and extent of photochemical alteration, and the a350 has been used as an indicator of lignin phenol concentrations (Helms et al., 2008; Fichot and Benner 2012).

Thanks for these suggestions. We have incorporated both parameters into Table 2 of the manuscript and have included a method description for the spectral slope calculation (lines 204-207).

Specific comments:

Line 48: include Miller and Zepp 1995
Included (see line 46 and 718-720).

Lines 140-141: Filtration (0.22 μm) does not exclude microbial activity, it reduces microbial activity (filtered samples contain some active bacteria)

Sentence corrected to: “…syringe driven pore size MCE filters 0.22 μm (Merck Millipore, UK) to reduce the effect of microbial activity” (lines 137-139).

Line 151 – report the wavelength range of light measured by the PMA2102 broad-band sensor

We have stated that the wavelength range covers both UVA and UVB and that the sensor is erythemally weighted which allows comparison with previous studies through use of spectral weighting functions (lines 148-160).

Lines 160-161 – estimation of the exposure time of DOM to solar irradiation needs to consider mixing processes and extinction coefficients for the solar spectrum

We believe that this information is more relevant in the discussion and have now explicitly alluded to difficulties in estimating DOM exposure due to mixing processes in the Discussion section 4.3 ‘Implications for photochemical turnover of DOC in aquatic systems’ (lines 529-539 and 552-558).

Lines 180-182: provide information about the GC column and chromatographic conditions

We have added further information pertaining to GC analysis, including sample size, needle penetration depth and analytical run length (lines 179-190).

Line 186: concentrations of DIC were not measured

Corrected to “DOC and TC were measured…” (line 191)

Line 189: give the pathlength, not volume, of the quartz (?) cuvette

Pathlength information provided. We used disposable PLASTIBRAND® UV-Cuvettes for our analyses. This information has also been added to the method (line 194).

Line 216: provide information about the column and chromatographic conditions

We have added further information pertaining to the GC column and chromatographic conditions (lines 225-231).


Thanks for these paper recommendations. We have referenced them in the text (lines 492, 504, 522, 595-597, 698-700).

The clarity of Figures 1, 3, 5, 6 and 7 would be improved by the use of different colors for different parameters

We have changed the figures and improved clarity by adopting a consistent colour palette.

Figure 3: present the change in absorbance as a percentage of the controls and only show the wavelengths starting at 250 nm

Adjusted as specified. We have chosen to show data from 250 – 400 nm in the revised figure, as percentage absorbance data became noisy for the Loch Katrine samples in the visible part of the spectrum (>400 nm).

Molar units are preferred for all chemical measurements
After consideration we have decided to retain the original units with concentrations expressed in mg L\(^{-1}\) or μg L\(^{-1}\), as this format allows comparison of results with both previous studies at the Auchencorth Moss catchment (Dinsmore et al., 2010, 2013) and other relevant research in the field of DOC processing (e.g. Moody et al., 2013; Palmer et al., 2015; Spencer et al., 2009).

References


Temporal changes in photoreactivity of dissolved organic carbon and implications for aquatic carbon fluxes from peatlands

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Abstract

Aquatic systems draining peatland catchments receive a high loading of dissolved organic carbon (DOC) from the surrounding terrestrial environment. Whilst photo-processing is known to be an important process in the transformation of aquatic DOC, the drivers of temporal variability in this pathway are less well understood. In this study, 8-h laboratory irradiation experiments were conducted on water samples collected from two contrasting peatland aquatic systems in Scotland; a peatland stream and a reservoir in a catchment with high percentage peat cover. The first system was a stream draining the Auchencorth Moss peatland with high DOC concentrations subject to strong seasonal and flow driven variability. The second was the low DOC reservoir, Loch Katrine, also situated in a catchment with a high percentage peat cover. Samples were collected monthly at both sites from May 2014 to May 2015 and from the stream system during two rainfall events. DOC concentrations, absorbance properties and fluorescence characteristics were measured to investigate characteristics of the photochemically labile fraction of DOC. CO2 and CO produced by irradiation were also measured to determine gaseous total photoproduction and intrinsic sample photoreactivity. Significant variation was seen in the photoreactivity of DOC between the two systems, with total irradiation induced changes typically two orders of magnitude greater at the high DOC stream site. This is attributed to longer water residence time in the reservoir rendering a higher proportion of the DOC recalcitrant to photo-processing. Over the irradiation period During the experimental irradiation, 7% of DOC in the stream water samples was photochemically reactive and direct conversion to CO2 accounted for 46% of the measured DOC loss. Rainfall events were identified as important in
replenishing photoreactive material in the stream, with lignin phenol data ([Ad:Al] and [P:V]) indicating mobilisation of fresh DOC derived from woody vegetation in the upper catchment. Using DOC-CO₂ conversion data from irradiation experiments, we estimate that the contribution of Auchencorth Moss catchment to photo-induced aquatic CO₂ production is up to 3.48 ± 2.02 kg CO₂ yr⁻¹. We have shown that peatland catchments produce significant volumes of aromatic DOC and that photoreactivity of this DOC is greatest in the headwater streams, however an improved understanding of water residence times and DOC input-output along the source to sea aquatic pathway is required to determine the fate of peatland carbon.

**Keywords:** Carbon budgets • Rainfall events • Lignin phenols

1. **Introduction**

DOC is transported from terrestrial environments to aquatic systems where it plays an important role in carbon (C) cycling. Biogeochemical transformations of DOC via microbial and photochemical pathways impact significantly on aquatic C cycles, with up to 55% of C exported as DOC to freshwaters estimated to be lost to the atmosphere as CO₂ (Cole et al., 2007; Tranvik et al., 2009; Cory et al., 2014). These estimates suggest that the C sink strength of the land surface globally has been overestimated, as the role of freshwater systems in the biogeochemical processing of DOC and the subsequent production of greenhouse gases had not been considered. Understanding of the rate of turnover of DOC in aquatic systems remains incomplete and further efforts are required to quantify the extent to which biogeochemical processes in aquatic systems are a source of C to the atmosphere.

Photochemical reactions in aquatic systems are induced by the absorption of solar radiation, particularly in the UV region of the spectrum, and preferentially affect aromatic, high molecular weight (HMW) molecules derived from allochthonous sources. Upon radiation, HMW DOC is converted to microbially available low molecular weight (LMW) carbon substrates (Opsahl and Benner, 1998; Sulzberger and Durisch-Kaiser, 2009). Photodegradation of DOC also results in the production of C₂-based gases, primarily CO₂ and CO (Miller and Zepp, 1995; Stubbins et al., 2011). Whilst it is understood that input of photochemically labile terrigenous DOC can regulate C cycling in
aquatic systems (Cory et al., 2014; Koehler et al., 2014), the significance of DOC photodegradation processes in these cycles remains poorly constrained over time and space (Franke et al., 2012; Moody et al., 2013). Due to low temperatures and short residence times limiting autochthonous (in situ) DOC production in headwater systems of northern peatlands, photochemical processing may be a proportionately more important process.

A key control on DOC concentrations in headwater systems is rainfall events which flush young, less degraded plant material within the catchment into streams (Evans et al., 2007; Austnes et al., 2010). Rainfall events have been shown to contribute significantly to annual C export from peatland headwater streams (Clark et al., 2007), yet the degree to which they replenish photolabile material within the aquatic environment is less certain. Stormflows in northern catchments have been associated with increased contribution of humic like material (Fellman et al., 2009), suggesting that DOC photoreactivity may also increase during these events. Several studies have explored seasonal variation in intrinsic DOC photoreactivity in northern aquatic systems (Vachon et al., 2016; Franke et al., 2012; Vachon et al., 2016) yet, to our knowledge, the contribution of rainfall events to the seasonal cycle of photolabile material has not been previously investigated.

Further uncertainty remains in understanding the variation in DOC photolability at different positions within a watershed (Franke et al., 2012). The increasing residence time of downstream aquatic systems, as headwater streams drain into rivers, lacustrine and marine environments, may mean that photo-processing becomes a more important control on overall C budgets with distance downstream. Conversely, the extent to which the material has already been degraded in the upstream aquatic environment may mean that further processing is limited (Catalán et al., 2016; Vähätalo and Wetzel, 2008; Catalán et al., 2016). Investigating the susceptibility of DOC to photo-processing in different types of aquatic environments will allow the overall contribution of photochemical processes to C cycling to be understood on a catchment scale.

The primary aim of this study was to assess temporal variation in the photochemical lability of DOC from two contrasting aquatic systems draining peatlands and to understand how this variation may
impact aquatic C budgets. Controlled UV irradiation experiments were conducted on water samples collected from the two contrasting aquatic systems, one a stream and the other a reservoir. Water from both systems was sampled on a monthly basis over a 1-year period and also from the high DOC stream system during two rainfall events to characterise short term variability in DOC concentration and composition. After experimental exposure, optical, spectroscopic and biogeochemical analyses of the water samples were conducted to explore DOC photoreactivity and the resultant production of C-based gases. The results were used to test the following hypotheses:

H1: Both aquatic systems will exhibit seasonality with regards to the supply of photochemically labile DOC, with highest photolability detected in the winter due to limited processing in the aquatic environment.

H2: Photochemical degradation of DOC will be a more significant loss term of C in the high DOC aquatic system.

H3: Rainfall events in the high DOC system will replenish the supply of photolabile material.

2. Methods

2.1 Study sites

Water samples for the irradiation experiments were collected from two aquatic systems located in peatland catchments. The Black Burn (55°47’34” N; 3°14’35” W; 254 m a.s.l.) is a small headwater stream draining Auchencorth Moss, an ombrotrophic peatland located in central Scotland covering 3.35 km² (Billett et al., 2010). The stream is fed by a number of small tributaries from the surrounding peatland, part of which is used for peat extraction. Low density sheep grazing is the primary land use within the catchment and vegetation comprises a Sphagnum base layer and hummocks of Deschampsia flexuosa and Eriophorum vaginatum, or Juncus effusus. In the upper catchment shrubs are present, including Calluna vulgaris, Erica tetralix and Vaccinium myrtillus (Dinsmore et al., 2010; Drewer et al., 2010).
The Black Burn stream hydrograph is characterised by a steady base flow and rapid ("flashy") response to rainfall events which typically produce high flow accompanied by elevated DOC concentrations. Annual mean stream water DOC concentrations determined by weekly sampling over a 2-year period were high, at 28.4 ± 1.07 mg L\(^{-1}\) (Dinsmore et al., 2013), with a marked seasonal pattern, characterised by low DOC in winter and high concentrations in summer. In this study, water samples were collected from an established sampling site where DOC concentrations have been recorded for >9 years as part of the Centre for Ecology & Hydrology (CEH) Carbon Catchments project (https://www.ceh.ac.uk/our-science/projects/ceh-carbon-catchments).

The other sampling site was Loch Katrine (56°25′25″ N; 4°45′48″ W; 118 m a.s.l.) in the Loch Lomond and Trossachs National Park, Scotland. Loch Katrine has a surface area of 8.9 km\(^2\) and is fed by >80 tributaries which predominantly drain a catchment of upland blanket bog (SNH, 2005). Loch water DOC concentrations have been recorded by the Scottish Environment Protection Agency (SEPA) at Ruinn Dubh Aird, a peninsula located at the south eastern end of the loch, which was also selected as the sampling point for this study. DOC concentrations measured approximately six times a year from 2009–2014 were low at 3.68 ± 0.56 mg L\(^{-1}\) (SEPA, personal communication).

### 2.2 Sample collection

Water was sampled monthly from both sites from May 2014 to May 2015 inclusive (13 samples over the study duration) to characterise seasonal variation in DOC concentration and composition. Samples were collected at 20 cm below the surface of the water in a screw top sterile clear glass bottle. Upon return to the laboratory, samples were stored in the dark at 4°C and exposed to experimental conditions within a week of collection. Additional water sampling to characterise the effect of rainfall events focused on the Black Burn head water system. Intensive stream water sampling was conducted during two rainfall events, one in winter (defined as 1 October to 31 March) and the other during the summer (1 April to 30 September) (Gordon et al., 2004). An automatic water sampler (Teledyne Isco, USA) was programmed to collect a composite 1 L sample of water from the Black Burn into separate polypropylene bottles every 60 minutes (comprising two 500 mL samples collected each 30 minutes) throughout the rainfall events. Stream water sampling in the winter rainfall event was...
conducted from 11:00 on 9 December to 17:00 GMT on 10 December 2014, resulting in 31 samples across the event. Stream water sampling in the summer rainfall event started at 14:30 on 1 September and finished at 06:30 GMT on 2 September 2015, resulting in 17 samples. Water samples were transferred into glass bottles from the automatic water sampler for transport to the laboratory and irradiated within 5 days of collection.

Throughout the year of sampling, the Black Burn water depth was measured at 15 minute intervals approximately 2 km downstream from the sampling site using a Level Troll pressure transducer (In Situ Inc., USA) with atmospheric correction from a BaroTroll sensor (In situ Inc., USA) located above the water surface. Water depth readings from the pressure transducer were converted to discharge at the sampling site using rating curves ($R^2 > 0.90$) based on flows measured by dilution gauging (Dinsmore et al., 2013). Equivalent hydrological data were not available for Loch Katrine.

### 2.3 Sample preparation

Prior to experiments, water samples were degassed under a vacuum pressure system for 20 minutes to remove dissolved gas from the water and then filtered using syringe driven pore size filters 0.22 μm MCE filters (Merck Millipore, UK) to reduce the effect of microbial activity. 15 mL of filtered sample was immediately transferred into 21 mL quartz vials (Robson Scientific, UK) which were sealed with aluminium crimp tops and rubber butyl plugs (Speck and Burke Chromacoal, UK). All samples were prepared at room temperature in oxygenated conditions.

### 2.4 Irradiation experiments

Experiments providing both UV-A and UV-B irradiation were conducted using UV313 lamps (Q-Panel Company, USA) covered with 125 μm cellulose diacetate (A. Warne, UK) to exclude UV-C (≤280 nm) and short wavelength UV-B (≤290 nm). Irradiation experiments were conducted using UV-B 313 lamps (Q-Panel Company, USA) covered with 125 μm cellulose diacetate (A. Warne, UK) to exclude UV-C (≤280 nm) and short wavelength UV-B (≤2980 nm), and providing both UV-A (315-400 nm) and UV-B (315-2980 nm) exposure. Lamps were mounted inside quartz tubing (Robson Scientific, UK) beneath the water surface in a water bath maintained at 16°C and vials were irradiated...
sideways while submerged. UV irradiance of the samples was modulated to remain constant throughout the 8-h exposure by measurement with an erythemally weighted UV-B broad-band sensor with a dimmer (Model PMA2102; Solar Light Inc., USA). The sensor was held beneath the water surface behind a quartz window of the same thickness as the vials. The UV exposure was calibrated with a double monochromator scanning spectroradiometer (Irradian™, UK), itself calibrated against a secondary deuterium lamp quartz halogen standard (FEL Lamp, F-1297) operated by the NERC Field Spectroscopy Facility, Edinburgh (http://fsf.nerc.ac.uk/). Total unweighted irradiance was 2.89 ± 1.81 W m$^{-2}$ in the UV-B, 4.63 W m$^{-2}$ in the UV-A, and photosynthetically active radiation (PAR) was 0.92 W m$^{-2}$ (Table 1; Supplementary Information Figure S1). These conditions reflect twice the UV-B irradiance that could be expected over two cloudless summer days in the UK and a significant underestimation of summer time daily ambient UV-A and PAR radiation. Weighting functions derived for a range of photochemical processes were applied to the spectral output (Table 1) and were determined to be within the range of global irradiance values within globally realistic values (Table 1). The time duration of the experiment (8-h) was selected to represent a conservative estimate of the exposure time of surface water during transit through a headwater peatland catchment to a marine outlet. Water temperatures of ~16°C were measured in both field sites in May 2014 prior to commencement of the year-long sampling programme and was employed in the experiments to represent summer time conditions. Controls comprising quartz vials containing water samples and wrapped in aluminium foil to exclude radiation were kept in the water bath for the experiment duration, with four replicates of each of the UV-exposed and control samples.

To select water samples from the Black Burn for irradiation experiments, POC concentrations, A$_{254}$ values and E4:E6 ratios were measured within 24 h in all samples (using the methods described below) and, from these results, eight stream water samples were selected from each rainfall event which represented the minimum, maximum and median values of these parameters (Supplementary Information Table S1).
2.5 Analytical methods

On each monthly sampling occasion the water dissolved oxygen (DO), conductivity, pH and temperature were measured on site with a handheld Hach HQd multimeter (Hach, USA). Measured volumes of water samples were filtered within 24 h of collection through pre-ashed (8 h at 450°C), pre-weighted Whatman GF/F (0.7 μm pore size) filter papers. POC was determined using loss-on-ignition, following the method of Ball (1964).

Following irradiation, partitioning of dissolved C gases from the liquid into the vial headspace was encouraged through use of a wrist action shaker for 30 seconds. An Agilent gas chromatography (GC) system (Hewlett Packard 6890; Agilent Technologies, USA) equipped with an autosampler (HTA, Italy) and a flame ionisation detector (FID) held at 250°C was used to analyse samples for headspace CO₂, CO and CH₄ concentration within 8 h of irradiation. Needle penetration depth was set to a standard depth and 1.5 mL of headspace sample was automatically injected into the sample loop.

Analytical runs lasted for 10.5 minutes and the column carrier gas was N₂ at a constant flow rate of 45 mL min⁻¹. CO₂ and CO measurements were made possible by a methaniser fitted between the column and FID. One standard 7-gas mixture (BOC Special Gases, UK) was used for daily detector calibration prior to sample analysis (detection limits: CO₂ 78 ppm; CO 1.6 ppm; CH₄ 0.8 ppm).

Dilutions of 50 and 75% were made from this standard using Zero Grade N₂ to produce a 3-point calibration series for each gas. Post-run peak analysis and integration were performed using Clarity software (DataApex, Czech Republic).

DOC and total carbon (TC) concentrations were measured using a PPM LABTOC Analyser (Pollution and Process Monitoring Ltd., UK) in UV treatment and control samples after exposure. Dissolved inorganic carbon (DIC) was calculated as the difference between total carbon (TC) and DOC. UV-visible absorbance of UV treatment and control samples contained in 3.5 mL PLASTIBRAND® UV-Cuvettes with a path length of 10 mm was measured at room temperature between 200 and 800 nm at increments of 1 nm using a Jenway spectrophotometer (Model 7315, Bibby Scientific, UK). Deionised water controls were used between each sample. Absorption coefficients aₐ were calculated as:
\[ a_\lambda = 2.303 \times \left( \frac{A_\lambda}{L} \right) \]  

where \( A \) is the absorbance at each wavelength and \( L \) is the path length (m) of the cuvette (Green and Blough, 1994). Specific UV absorbance (SUVA\(254\)) values, a measure of DOC aromaticity, were determined by dividing the UV absorbance measured at \( \lambda = 254 \) nm by the DOC concentration (Weishaar et al., 2003). E4:E6 ratios were estimated using the absorbance values at 465 and 665 nm, respectively (Peacock et al., 2014). Spectral slope (\( S \)) was calculated using a nonlinear fit of an exponential function to the absorption spectrum in the ranges of 275–295 and 350–400 nm, where \( S \) is the slope fitting parameter. The spectral slope ratio (\( S_{275-295} \)) was calculated as the ratio of \( S_{275-295} \) to \( S_{350-400} \) (Helms et al., 2008; Spencer et al., 2009).

Fluorescence intensity in water samples filtered to 0.2 \( \mu \)m was measured using a FluroMax-4 spectrofluorometer (Horiba Jobin Yvon Ltd., Japan). The instrument was programmed to scan across excitation wavelengths 200–400 nm (5 nm increments) and emission wavelengths 250–500 nm (2 nm increments) with a 1 cm path interval. Data were obtained at room temperature and were blank corrected using deionised water. Intensity ratios derived using these data allow discrimination between different sources of DOC. Here, the fluorescence index (FI), \( F_{450}/F_{500} \), the ratio of fluorescence intensity at the emission wavelength 450 nm to that at 500 nm at excitation wavelength 370 nm, was calculated to help identify dissolved organic matter (DOM) source material. Values around 1.8 suggest autochthonous organic material, whereas values around 1.2 indicate terrestrial derived material (Cory and McKnight, 2005).

Lignin phenol concentrations in unirradiated Black Burn water samples were measured using the CuO oxidation method (Benner et al., 2005; Spencer et al., 2008). After filtration to 0.2 \( \mu \)m, 45 mL of water sample was freeze dried to produce lyophilised DOM which was transferred to stainless steel pressure bombs with 1 g of CuO and 100 mg of \( \text{Fe(NH}_4\text{)}_2\text{(SO}_4\text{)}_2\cdot\text{H}_2\text{O} \). Under anaerobic conditions, 8 mL of NaOH was added to the bombs before they were sealed. Samples were then oxidised at 155°C for 3 h. Following oxidation, samples were acidified to pH 1 with \( \text{H_2SO}_4 \) extracted with ethyl acetate three times, and then passed through \( \text{Na}_2\text{SO}_4 \) drying...
columns. Samples were dried using a flow of N\textsubscript{2} and kept frozen prior to GC analysis. After redissolution in \( \approx 200 \) µL pyridine, lignin phenols were derivatised with bis-trimethylsilyl-trifluoromethylacetamide (BSTFA) at 60ºC for 30 minutes and quantified on a GC (Agilent 5890 MkII with twin FID). Specifically, a twin-column split-injection method was used with Agilent DB1 and DB17014 (both 30 m x 0.25 mm diameter x 0.25 µm film thickness) flow being split in the injection liner with a twin-hole ferrule. Column flow was 1 mL minute\(^{-1}\) with a split ratio of 20:1. The chromatographic conditions were 100ºC at T=0 held for 1.25 minutes, followed by a heating rate of 4ºC min\(^{-1}\) until 270ºC, then holding held for 15 minutes.

Eleven lignin phenols were measured, including three p-hydroxybenzene phenols (P): p-hydroxybenzaldehyde, p-hydroxyacetophenone, p-hydroxybenzoic acid; three vanillyl phenols (V): vanillin, acetovanillone, vanillic acid; three syringyl phenols (S): syringaldehyde, acetosyringone, syringic acid; and two cinnamyl phenols (C): p-coumaric acid and ferulic acid. Blank controls, taken through the method from CuO oxidation onwards, were quantified and subtracted from sample concentrations. Quantification was achieved through use of cinnamic acid as an internal standard. In addition to total concentration of lignin phenols (Σ\textsubscript{11}) and carbon normalised yields (\( \chi \)), the ratio of syringyl to vanillyl phenols (S/V), the ratio of cinnamyl to vanillyl (C/V) phenols, the ratio of p-hydroxybenzenes to vanillyl phenols (P/V) and the ratio of acids to aldehydes (Ad/Al\textsubscript{v,s}) were calculated to aid interpretation of the data. Lignin phenols for Loch Katrine samples were not measured due to insufficient production of lyophilised material using the stated method.

### 2.6 Data analysis

Data collected in the irradiation experiments were tested for normality using the Shapiro-Wilks test and were found to be normally distributed. Unpaired t-tests were conducted between irradiated and unirradiated samples to assess differences in spectral properties, DOC and DIC concentrations, lignin phenol concentration and gaseous production. Pearson correlation coefficients were used to test the potential role of DOC composition and site conditions in regulating photochemical lability, measured as total DOC loss, production of DIC and C gases (CO and CO\textsubscript{2}) and change to a\textsubscript{254} and E4:E6 ratios.
Carbon species DOC, DIC, CO$_2$, and CO measured each month at the Black Burn and Loch Katrine were included in C mass budgets calculated for irradiated and unirradiated samples. By converting all data to mg L$^{-1}$, the difference in C budget between treatment and control samples could be determined (see Supplementary Information Table S2 for example calculations). To obtain a standard error value for differences between irradiated and control samples, the mean control value was determined and subtracted from each of the irradiated replicates.

Photoreactivity (mg C / mg DOC) was determined as total change to C species (DOC, CO$_2$, and CO) upon irradiation normalised for initial DOC concentration. For the Loch Katrine samples, where minimal net DOC changes upon irradiation were observed in the sample aliquots, photoreactivity (mg C / mg DOC) is expressed as the sum of gaseous photoproduction (CO$_2$, and CO only) divided by the initial DOC concentration. This is to avoid production of negative photoreactivity values for Loch Katrine which may have been explained in large part by the limited resolution of the PPM LabTOC instrument at very low DOC concentrations.

Correlation coefficients were also calculated between intrinsic sample photoreactivity, measured as total change to C species upon irradiation normalised for initial DOC concentration, and lignin phenol data. The Durbin-Watson statistic was used to test for the presence of autocorrelation in residuals of lignin phenol analyses of stream water samples collected during rainfall events and showed no correlation between the samples. Minitab v.16 (Minitab Inc., USA) was used for all statistical analyses.

3. Results

3.1 Climate and water chemistry conditions at time of sampling

Total rainfall measured at the European Monitoring and Evaluation Programme (EMEP) supersite at Auchencorth Moss (Torseth et al., 2012) for the 13 month sampling period was 1015 mm. It varied from lowest monthly values in September and April to the highest in October (Figure 1a). The mean air temperature of the study period was 7.7°C, similar to the 8 year average of 7.6°C, and reached a maximum of 27.6°C in July 2014 and a minimum of -7.9°C in January 2015.
At Comer meteorological station, located 10 km from the Loch Katrine sampling site, rainfall was considerably higher, totalling 2368 mm over the sampling period (Figure 1b) (Met Office, 2012). Seasonal variation in rainfall was clear, with >40% of rainfall falling from December to February. Air temperatures were higher than at the Black Burn, with a mean of 10.2°C.

Water chemistry differed considerably between the two aquatic systems over the year-long sampling period (Table 2). The water temperatures reflected the difference in air temperature between the sites, with higher mean values at Loch Katrine than at the Black Burn. Mean pH at the Black Burn was 5.4, compared to 6.7 at Loch Katrine. Conductivity was more variable at the Black Burn and was on average 53 μS cm⁻¹ higher than at Loch Katrine, although values at both sites were low. POC concentrations at the Black Burn were over double those at Loch Katrine. FI values were slightly higher at the Black Burn, but at both sites were low and stable, indicative of terrestrially derived DOC material (Cory and McKnight, 2005).

DOC concentrations at the Black Burn ranged from 14.2 to 50.9 mg L⁻¹ (Figure 2) and showed a similar seasonal pattern as described in Dinsmore et al. (2013). Concentrations were lowest in late winter and highest in autumn; the latter consistent with increased organic matter inputs to the stream from flushing of soils during autumn rainfall events. At Loch Katrine, DOC concentrations were low and consistent, ranging from 3.10 to 5.82 mg L⁻¹. Concentrations were lowest in spring and highest in summer. SUVA₂₅₄ values at the Black Burn were higher than at Loch Katrine, suggesting that the DOC pool was comprised of a greater percentage of aromatic material (Weishaar et al., 2003). The E₄:E₆ ratio at the Black Burn varied considerably over the sampling period, ranging from 1.0 to 10.2. At Loch Katrine, the E₄:E₆ ratios were lower and less variable, but are a less meaningful parameter in the low DOC concentration Loch Katrine samples due to minimal absorbance in wavelengths greater than 400 nm.

3.2 Optical changes in water samples upon irradiation

Absorbance coefficients typically decreased upon irradiation of water samples relative to dark controls, with the strongest decrease occurring in the UV part of the spectrum at ~225 nm, and...
smaller inflection at ~300 nm (Figure 3). The maximum percentage loss of absorbance upon irradiation was 5% a factor of 4 higher in water samples from the Black Burn than from compared to Loch Katrine samples when averaged across wavelengths 250-400 nm. In the Black Burn, decreases in absorbance were greater in the summer and autumn in the summer, whereas at Loch Katrine the decreases in absorbance were greater in the winter and spring.

Positive values Percentage values consistently >100% (where dark control UV exposed samples showed an increase in greater drop in absorbance upon irradiation than relative to dark control light exposed samples) were recorded for summer water samples from Loch Katrine. E4:E6 ratios decreased by a mean of 1.52 in irradiated Black Burn water samples, indicating accumulation of E4:E6 ratios decreased by a mean of 0.21 upon irradiation.

3.3 Carbon budget changes upon irradiation

Typically, DOC concentrations in Black Burn water samples decreased after light exposure compared to unirradiated controls (Figure 4a). Mean change in DOC in irradiated samples from the Black Burn for the whole sampling period was -2.14 mg C L⁻¹ (ranging from 0.06 to -4.35 mg C L⁻¹ for individual months). DOC decreased after irradiation in all Black Burn samples with the exception of September 2014, indicating a photolabile DOC pool for most of the year. In contrast, in water samples from Loch Katrine irradiation induced DOC losses occurred in 56 of 13 samples and small gains were observed in 87 of 13 samples (Figure 4b). Whilst these results should be interpreted with caution as small differences in DOC concentrations (<0.5 mg C L⁻¹) are below the instrument detection limit, they suggest that the DOC pool in Loch Katrine was largely recalcitrant to photochemical degradation.

Irradiation resulted in notable photoproduction of DIC, CO₂ and CO from Black Burn samples. DIC concentration increased by a mean of 0.77 mg C L⁻¹ for the whole sampling period, although production across the samples was highly variable between months. CO₂ was the most abundant photoproduction and was produced at a mean rate of 1.2 mg C L⁻¹ across all monthly samples. At Loch Katrine, CO₂ production was two orders of magnitude lower than in the Black Burn, produced at a
mean rate of 0.06 mg C L\(^{-1}\). In all monthly water samples from both sites CO concentrations increased
in the irradiation experiments, with mean production rates of 0.07 and 0.01 mg C L\(^{-1}\) observed for
Black Burn and Loch Katrine samples, respectively.
Carbon mass budgets for DOC loss and photoproduct accumulation (DIC, CO₂ and CO) in water samples were calculated for all the irradiation experiments. Budgets for all monthly water samples from the Black Burn were balanced to within ± 5.1% of the total measured C concentration. For Loch Katrine water samples, budgets were balanced to within ± 11%. The lower accuracy of budget closure in the Loch Katrine samples is likely due to lower overall C concentrations, which are more susceptible to measurement error. CH₄ was detected in all samples at very low levels, with mean concentrations of 0.63 and 0.57 μg L⁻¹ detected at the Black Burn and Loch Katrine, respectively, and thus were not included in the mass calculations.

Intrinsic photoreactivity of C in the Black Burn ranged from 0.02 to 0.15 mg C/mg DOC L⁻¹ and was highest in August (Figure 4a). Photoreactivity peaked again in November and remained elevated until January. Lowest sample photoreactivity was detected in September. At Loch Katrine, mean C photoreactivity was 0.0194 mg C/mg DOC L⁻¹, with a maximum of 0.0259 mg C/mg DOC L⁻¹ detected in July-November.

### 3.4 Factors influencing carbon budget changes

Factors influencing irradiation induced changes to C species and spectral properties in Black Burn water samples were investigated using Pearson correlations (Table 3). Loss of DOC, absorbance at 254 nm and production of both CO₂ and CO were significantly positively correlated with initial DOC concentration. Initial E₄:E₆ ratios had positive coefficient values with all light induced changes to the DOM pool, whilst FI values were all negative, although most of these correlations were not significant.

Of the meteorological and discharge variables investigated, air temperature and PAR were significantly negatively correlated with changes to E₄:E₆ ratios. Total monthly rainfall had positive coefficient values with irradiation induced changes to the DOM pool. Correlations between C species changes and discharge were less consistent, although mean monthly discharge was significantly positively correlated with changes to E₄:E₆ ratios.
3.5 Effect of rainfall events on carbon photo-processing in Black Burn water samples

The Black Burn was sampled hourly during a winter rainfall event, with collection commencing 6 h before peak rainfall (Figure 5a). Total rainfall during the event, which we define here as the water sampling period, was 19.6 mm, with an hourly maximum of 3.3 mm and rainfall recorded in 22 of the 31 sampling hours. Stream discharge peaked at 391 L s$^{-1}$ although a separate smaller peak of 266 L s$^{-1}$ also occurred during the sampling period.

During the event, an initial dilution of stream DOC concentrations was followed by recovery to pre-event levels (Figure 5a). DOC was most photoreactive at 06:00, with DOC concentration reduced after irradiation by 6.72 mg L$^{-1}$. DOC loss in this sample was greater than at any time through the year-long study (Figure 4a), even though the DOC concentration (44.4 mg L$^{-1}$) was within the range of measured monthly concentrations. The greatest irradiation induced increase in CO$_2$ concentration (2.25 mg L$^{-1}$) occurred in the first event sample at 11:00, collected prior to rainfall input. Photoreactivity was lowest at 12:00, and was similarly low in the sample collected at 17:00, which coincided with peak rainfall.

In the late summer rainfall event occurring at the end of an extended period of base flow in the Black Burn (Supplementary Information Figure S2), 3.2 mm of rainfall was recorded with a maximum hourly total of 2.2 mm. Samples were collected from 14:30 to 06:30, with rainfall only occurring between 16:30 and 18:30. Discharge remained low and relatively stable throughout the event, with a mean flow of 6.14 L s$^{-1}$. Rainfall marginally diluted the stream DOC concentrations (Figure 5b). Photo-induced changes were much smaller than in the winter event and maximum DOC losses were a factor of 2.5 lower than the mean DOC reduction observed in the Black Burn monthly water sample experiments (Figure 4a). Photoreactivity was lowest in the initial sample collected at 14:30 prior to rainfall and coinciding with the highest discharge during the sampling period. Photoreactivity was highest in the 19:30 sample collected 3 h after peak rainfall.
To understand the effect of DOM composition on photolability, lignin phenols were measured in all Black Burn monthly and rainfall events water samples prior to the irradiation experiments.

Dissolved lignin concentrations ranged from 15.3 to 108 μg L⁻¹ (mean = 52.8; n = 28) and were significantly positively correlated with sample DOC concentration (Pearson = 0.831; p < 0.01) (Supplementary Information Figure S3). Carbon normalised yields were between 0.71 and 2.66 mg (100 mg OC)⁻¹. The contribution of individual phenol groups to the lignin signature varied between monthly samples of the year-long study and the rainfall events (Figure 6). In the monthly samples, the P phenols were most abundant, followed by V phenols (Figure 6a). Samples in the winter rainfall event contained higher and more variable mean yields for each phenol group, with S phenols most abundant, followed by V phenols and P phenols.

Overall yields were significantly lower (1-way ANOVA, p < 0.01) during the summer rainfall event. As in the year-long samples, P phenols were the most abundant, followed by S phenols and V phenols. Across all three sampling regimes, the contribution of C phenols to the overall lignin signature was smallest.

P:V ratios, an indication of Sphagnum derived DOC (see section 4.2), ranged from 0.83 to 1.69 across all samples, indicating significant temporal variability in DOM source material. Photoreactivity was significantly negatively correlated with P:V ratios when all samples were combined in a correlation analysis (-0.523; p < 0.01) (Figure 7a). This suggests that the relative abundance of P versus V phenols contributed considerably to sample photoreactivity. The lowest P:V ratios were in winter rainfall event samples, where photoreactivity was highest.

Ad:Al, ratios, which are an indicator of sample degradation, ranged from 0.58 to 1.26, towards the lower end of reported values in the literature (Winterfeld et al., 2015). Photoreactivity was also significantly negatively correlated with Ad:Al ratios (-0.492; p < 0.01) (Figure 7b) and again lower ratios typically occurred in winter rainfall event samples.
4. Discussion

4.1 Peatlands as a source of photochemically labile DOC

Photo-processing resulted in considerable DOC loss from water samples from the Black Burn. Mean DOC loss in the 8-h irradiation experiments conducted on the monthly water samples was 76% relative to initial concentrations. Percentage DOC losses determined here are similar to those reported from irradiation experiments conducted over similar timescales using stream water draining a boreal watershed (3–10% DOC loss over 10 h; Franke et al., 2012 and 11% TOC loss over 19 h; Köhler et al., 2002). It should be noted that percentage loss rates reported in this study are not directly comparable to those reported in other studies due to the use of UV-B 313 lamps. These lamps emit short wavelength UV that is particularly destructive of organic molecules so possibly that the magnitude of photo-processing may have been over-estimated. Use of a cellulose diacetate filter ensured that no wavelengths below 290 nm were emitted, however the lamp output is not comparable to a solar spectrum.

However, photochemical processes are dependent on the spectral composition of irradiation sources and direct comparison of percentage loss rates in this study with those of other experimental studies using different lamp types or ambient sunlight is not possible.

The irradiation source used in this study was selected as UV is the most effective source of radiation in producing photochemical effects (Hader et al., 2007; Zepp et al., 2007). The UV313 lamps used in this study provided both UVB and UVA exposures (2.89 and 4.63 W m\(^{-2}\) respectively) which were an appropriate UVB exposure but a lower proportion of UVA and visible wavelengths than ambient sunlight. Cellulose diacetate filters were used in this study to remove wavelengths <290 nm (which are absent in sunlight) but lamp outputs used in the irradiation experiments are not directly comparable to the solar spectrum. Consequently, the magnitude of photo-processing determined in this study allows relative comparison of temporal changes and between our sites but do not provide an accurate value of ambient photo-processing.
Photochemical transformations were low in the Loch Katrine samples, with minimal losses to the DOC pool (0.4303% mean from year-long study). Whilst our sites were not located within the same watershed, it seems likely that position within the catchment plays a role in determining the photolability of DOC. The Black Burn headwater stream at Auchencorth Moss receives fresh inputs of DOC from the surrounding peatland catchment and material has less time for light solar irradiation exposure in the water column relative to the DOC in the reservoir system. DOC losses may occur in Loch Katrine soon after water entry into the loch but, due to long water residence times, DOC may have become recalcitrant to photo-processing by the time of sample collection. Catalán et al. (2016) observed a negative relationship between organic carbon decay and water retention time, resulting in decreased organic carbon reactivity along the continuum of inland waters. SUVA$_{254}$ data suggest that DOC in Loch Katrine samples was less aromatic than in the Black Burn (Table 21), with values indicating an approximate humic content of 30% based on the findings of Weishaar et al. (2003). As humic molecules are more labile to photo-processing, irradiation had a greater effect on the stream samples relative to the reservoir samples.

Strong seasonal fluctuations in DOC concentration and composition occurred in the Black Burn, in agreement with patterns observed in the same system by Dinsmore et al. (2013). DOC concentrations were highest in the late autumn, consistent with a flushing effect whereby soil organic material produced over the summer is mobilised and delivered to aquatic environments by more intense rainfall after a prolonged, relatively dry period (Fenner et al., 2005). Positive correlation between the irradiation induced change in the E4:E6 ratio and mean monthly discharge suggest that hydrological conditions in the month prior to sampling significantly influence the reactivity of the sample, with high flow delivering more reactive carbon to the stream. Change in the E4:E6 ratio correlated significantly with several other variables, however spectral dependence of absorption photobleaching depends on the spectral distribution of the irradiation source (Del Vecchio and Blough 2002, Tzortziou et al. 2007). UVB-313 exposure may have produced a distinctly different absorption difference spectrum than ambient irradiance, though there is a lack of literature to test this assertion.
Hence, whilst the correlations are significant and can be explained theoretically, they should be interpreted with caution.

Overall the magnitude of photo-induced C losses was significantly positively correlated with DOC concentration in the year-long Black Burn dataset. However, despite low DOC concentrations, photoreactivity remained elevated in January. This suggests that even when lower DOC concentrations are detected in aquatic systems, the DOC may be intrinsically more photoreactive due to its aromatic content and minimal light exposure history.

Lowest DOC concentrations were observed in the late winter and early spring, due to depletion of soil organic C within the catchment by autumn and winter rainfall events. Low rainfall inputs limit the recharge of fresh, photolabile material to the stream and may account for the reduction in DOC photoreactivity detected in September. Furthermore, due to longer residence time in the water column, these samples may have already been degraded by natural light. A previous study at the Black Burn reported 13C enrichment of stream water DOC in September, consistent with increased in-stream processing at this time of year (Leith et al., 2014). Reductions in intrinsic DOC photolability during summer have similarly been reported in northern lakes (Vachon et al., 2016) and a boreal watershed (Franke et al., 2012). Another minimum in photoreactivity occurred in April, where SUVA254 data indicate decreased contribution of aromatic material to C within the stream. Although algal abundance was not measured during this study, production of DOC from such sources would account for the reduction in photolability (Nyugen et al., 2005).

Whilst DOC losses from Loch Katrine water samples were minimal, the peak in photolability, indicated by the greatest absorbance reduction in the light exposure experiments, occurred in spring. Similar seasonal photolability peaks have been observed in northern lakes (Vachon et al., 2016) and boreal streams (Porcal et al., 2013) and are partly attributed to mobilisation of terrigenous material with high flows associated with spring snow melt. The magnitude of melt in the Loch Katrine catchment will be considerably less than in snow dominated northern catchments (e.g. Laudon et al., 2005).
although increased flow and stream water chemistry changes with spring snow melt have been reported in upland Scottish catchments (Abrahams et al., 1989; Gilvear et al., 2002).

Absorbance increased during irradiation of Loch Katrine samples in summer. Absorbance increased in light exposed samples during irradiation in summer. Loch Katrine samples, indicating production of microbial DOC production. A possible explanation for increased absorbance in the irradiated water samples is the formation of an iron (Fe)-DOC complex, since the reaction kinetics of Fe-DOC complexes are directly affected by light exposure (Maranger and Pullin, 2003). Whilst Fe concentrations were not measured in this study, in a long-term SEPA bimonthly measurement campaign (2009-2013) at Loch Katrine, peak Fe concentrations in August of up to 0.50 mg L\(^{-1}\) were detected, corresponding to the time of year when we found increased absorbance in irradiated water samples. As the data set does not cover the sampling period, the role of Fe-DOC complexes in producing the observed effect cannot be directly determined; however the role of micronutrients in peatland aquatic C cycling should be further investigated.

Prior filtration of samples to 0.22 µm means that the anomalous absorbance increases are unlikely to be the result of microbial DOC production. However, this cannot be entirely discounted as some bacteria can pass through 0.22 µm filters and lacustrine freshwater bacteria colonies are seasonally variable, which may explain why the effect was only observed in summer (Kent et al., 2004; Fortunato et al., 2012). In order to determine microbial effects in the samples, stable C isotope (\(\delta^{13}C\)) data could be used as it can distinguish microbial activity from photochemical effects due to preferential fractionation of DOC fractions of different molecular weights for each respective process (Opsahl and Zepp, 2001).

4.2 Importance of rainfall events in mobilising photolabile material

Dissolved lignin phenol composition indicates that different sources of plant material were mobilised as a result of rainfall in the Auchencorth Moss catchment. High P:V ratios have been used as an indicator of peatland inputs to aquatic systems, as *Sphagnum-derived organic acids Sphagnum acid*
typical of peatlands is converted into P phenols during lignin extraction (Fichot et al., 2016; Winterfeld et al., 2015; Fichot et al., 2016). Typically P phenols constituted the largest contribution to the total lignin concentration of the measured phenols, consistent with Sphagnum inputs. However, during the winter rainfall event where stream discharge was considerably higher than the year-long mean value, the largest contribution to total lignin concentration was from S and V phenols (Figure 6). The former are reported to be the most photolabile phenol (Opsahl and Benner, 1998; Benner and Kaiser, 2011) and are unique to woody angiosperms. This suggests that hydrological pathways within the catchment were activated upon rainfall, causing DOC release from soil profiles associated with angiosperm plant material. Potential sources within the Auchencorth upper catchment are Calluna vulgaris, Erica tetralix and Vaccinium myrtillus. Further evidence of the operation of variable source areas in the catchment was the observation of delayed input of water, containing high CO₂ concentrations, from the deep peat area in the upper catchment at Auchencorth Moss during a storm event (Dinsmore and Billett, 2008). Low P:V values and high lignin concentrations have been reported during peak flow in Arctic rivers, and the reverse during base flow (Amon et al., 2012). As samples with low P:V values were typically more photoreactive (Figure 7a), our data indicate that rainfall events are important in mobilising photolabile material from this catchment.

Elevated Ad:Al_v,s ratios have previously been interpreted as indicators of decomposition of organic matter resulting from preferential degradation of aldehydes relative to acids (Spencer et al., 2009; Lu et al., 2016; Spencer et al., 2009). In the Black Burn water samples, lowest ratios were measured in the winter rainfall event. This implies that DOC mobilised during rainfall is less degraded relative to base flow DOC, in agreement with previous studies of peatland high flow events which detected increased contribution of near surface flow and younger DOC (Clark et al., 2008). The form of the degradation, either microbial or photochemical, cannot be distinguished using these data. However, based on the higher measured photoreactivity of samples with lower ratios (Figure 7b), light exposure history may be one of the key moderators of Ad:Al_v,s ratios in the Black Burn. High flow events release fresh DOC from soils derived from recent plant material (Evans et al., 2007) and may have
significant implications for C processing rates in streams as they are recharged with labile material (Lapierre et al., 2013).

Whilst the samples collected during the winter rainfall event were clearly distinct in composition relative to samples from the year-long study, the summer rainfall event samples had similar P:V and Ad:Al ratios, but significantly lower photoreactivity and overall lignin yields (Figures 5b, 6c, 7). This could be attributed to the timing of sample collection in early September at the end of summer, where considerable degradation may have already occurred across all phenol groups so that the DOC pool remaining was more recalcitrant to further photo-processing. Discharge data indicate that there was no discernible flushing effect during the summer rainfall event, with slight decreases in DOC concentration attributed to dilution of the stream water by direct rainfall inputs or overland flow. The abundance of P phenols, which have been determined as the least photoreactive phenol (Benner and Kaiser, 2011), within the samples suggest that passive transfer of DOC from the riparian zone, which is dominated by Sphagnum and Juncus vegetation, to the stream was the dominant mode of stream DOC recharge at this time of year (Jeanneau et al., 2015). The summer rainfall event samples were notably depleted in V phenols, suggesting that these phenols exert an important control on sample photoreactivity in addition to S phenols.

4.3 Implications for photochemical turnover of DOC in aquatic systems

Our 8-h irradiation experiments found 7% of DOC to be labile to photo-processing, with and conversion to CO₂ was the main loss pathway. DOC loss from samples upon irradiation resulted in significant production of CO₂. The mass budget calculations for Black Burn water samples show that a mean of ~46% of DOC loss in the irradiation experiments was accounted for by production of CO₂. Dinsmore et al. (2010) estimate that 108 ± 62.7 kg DOC yr⁻¹ is exported to the Black Burn from the Auchencorth Moss catchment. Based on our finding that 7% of DOC is removed via photo-processing, and assuming that 46% of this loss is converted to CO₂ and also that UV-B irradiance was comparable to a clear sky summer day, we estimate a potential evasion loss of 3.48 ± 2.02 kg CO₂ yr⁻¹ to the atmosphere. Whilst this calculation makes significant assumptions in upscaling from 8-h exposure experiments, it highlights the potential importance of photo-processing in the turnover of
aquatic C and the need for more in situ studies. Given the significant volume of DOC produced by the

catchment, in-stream photo-processing may be an important term in carbon budgets of peatland

draining aquatic systems. Clearly, headwater peatland streams are important sources of photoreactive

material to the freshwater aquatic continuum and may contribute to the high CO2 efflux reported from

these systems (Billett et al., 2015). However, determining the volume of material photo-processed

both in the stream and in downstream environments relies upon a range of unknown parameters,

including optical depth and mixing processes in downstream aquatic environments which are

generally poorly understood in relation to photochemical DOC processing.

Due to the effects of bank shading and short transit time of water within the immediate catchment,

light driven instream DOC processing is unlikely to be significant. The river continuum concept

suggests that increased DOC processing will occur further downstream, where the channel widens

(Vannote et al., 1980), and will be partly controlled by the stream water mean transit time (McGuire

and McDonnell, 2006; McDonnell et al., 2010; McGuire and McDonnell, 2006). Based on mean

velocity (0.58 m s⁻¹) of a larger nearby river (Ledger, 1981), we estimate a mean water transit time

of ~19 h from the Black Burn at Auchencorth Moss to its coastal outlet in the River Esk, 34 km

downstream, considerably longer than the exposure time in our experiments. However, in a study of

1st to 4th order streams in Sweden no significant change to DOM composition as stream order

increased was detected and this was partly attributed to short transit times (<2 days) restricting DOC

processing (Kothawala et al., 2015). Peatland derived C is clearly photoreactive, but limited time for in-stream processing may render photo-processing unimportant in the C budgets

of some freshwater systems. freshwater aquatic C budgets.

Recent studies have determined hotspots of DOC processing within peatland draining systems, which

include mixing zones of freshwaters with different pH, conductivity and metal concentrations (Palmer

et al., 2015; Jones et al., 2016). In the context of this study, measuring DOC processing at the

confluence of the Black Burn, which largely drains peatland, and the River North Esk, which drains a

catchment of mixed land use including natural and plantation forestry, 4 km downstream of the point

from which our samples were collected would provide a logical starting point for quantifying in situ
DOC turnover. The optical depth and mixing processes in downstream aquatic environments are generally poorly understood in relation to photochemical DOC processing and further work is needed to reduce uncertainties of their influence.

Determining the C cycling implications of this study is further complicated as the most photoreactive material was recorded during a heavy winter rainfall event. The potential for photochemical transformation of DOC within the freshwater aquatic environment would have been limited due to low light availability, extensive cloud cover and decreased stream water transit times associated with the event. During the year-long study period, 12 rainfall events occurred which resulted in similar flow conditions in the Black Burn (stream discharge exceeding 250 L s⁻¹), with a maximum discharge of 2059 L s⁻¹ in a late winter storm. Of these high flow events, 11 occurred during winter and one in summer and hence, whilst large quantities of photoreactive material may have been mobilised during heavy rainfall, the likelihood of in-stream processing would remain small.

Increases in precipitation, with more frequent and intense rainfall events, are expected with climate change (Capell et al., 2013; Edenhofer et al., 2014) with heavier summer downpours predicted in the UK (Kendon et al., 2014). Thus, although the contribution of rainfall events to freshwater aquatic photochemically induced C cycling in this study is likely to be minimal, they could become more significant if heavy rainfall events occur more frequently in summer.

Author Contributions

AEP collected field samples and undertook laboratory experiments, analyses, data analysis and writing of the paper. KVH, ARM and KJD provided guidance on the scope and design of the project, and contributed to the editing of the manuscript.

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phenol analyses and Andrew Addison for his contribution to fieldwork. We also thank Tony Dickinson and Jim Donnelly at the University of Central Lancashire for use of a Horiba FluroMax-4 spectrofluorometer.

References


Dinsmore, K. J., Billett, M. F., Skiba, U. M., Rees, R. M., Drewer, J., and Helfter, C.: Role of the


Table 1. Photosynthetically active radiation (PAR) and ultraviolet irradiances during 8 h exposures to Q-Panel UV313 fluorescent lamps filtered with 125 µm cellulose diacetate.

<table>
<thead>
<tr>
<th>Irradiance W m⁻²</th>
<th>Total UV (280-400 nm)</th>
<th>UV-A (315-400 nm)</th>
<th>UV-B (280-315 nm)</th>
<th>PAR (400-700 nm)</th>
<th>CH₄⁻</th>
<th>GEN</th>
<th>PG⁻</th>
<th>DNA⁻</th>
</tr>
</thead>
</table>
| CH₄⁻, idealized spectral weighting function for CH₄ production (McLeod et al., 2008) 
| weighted with a mathematical function of the general plant action spectrum (GEN G) (Green et al., 1974) 
| weighted with the plant growth (PG) function (Flint and Caldwell, 2003) 
| weighted with the DNA damage action spectrum (Setlow, 1974) | 
| 7.52 | 4.62 | 2.80 | 0.92 | 2.50 | 1.25 | 1.05 | 0.98 |
Table 2. Mean (n=13 ± 1 standard deviation) water temperature and chemistry parameters including pH, conductivity, POC concentrations, and fluorescence index FI values at the Black Burn and Loch Katrine (n=13 ± 1 standard deviation).
<table>
<thead>
<tr>
<th></th>
<th>Black Burn</th>
<th>Loch Katrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water temperature °C</td>
<td>8.32 ± 4.54</td>
<td>10.9 ± 5.16</td>
</tr>
<tr>
<td>pH</td>
<td>5.43 ± 0.945</td>
<td>6.74 ± 0.32</td>
</tr>
<tr>
<td>Conductivity μS cm⁻¹</td>
<td>78.2 ± 30.7</td>
<td>25.2 ± 4.04</td>
</tr>
<tr>
<td>POC mg L⁻¹</td>
<td>5.28 ± 2.28</td>
<td>3.02 ± 0.64</td>
</tr>
<tr>
<td>FI value</td>
<td>1.24 ± 0.13</td>
<td>1.19 ± 0.248</td>
</tr>
</tbody>
</table>
Table 3. Pearson correlation coefficients between irradiation induced changes to aqueous carbon species and spectral properties, and water chemistry of Black Burn water samples from the year-long sampling campaign prior to irradiation and site conditions at Auchencorth Moss (n=13).

<table>
<thead>
<tr>
<th></th>
<th>ΔDOC</th>
<th>ΔDIC</th>
<th>ΔCO₂</th>
<th>ΔACO</th>
<th>ΔΔE₂₅₄</th>
<th>ΔΔE₄:₈₆</th>
<th>ΔΔE₄:₈₆</th>
<th>ΔΔS₅₈</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC</td>
<td>0.708**</td>
<td>-0.074</td>
<td>0.773**</td>
<td>0.824**</td>
<td>0.766**</td>
<td>-0.168</td>
<td>0.095</td>
<td>-0.547</td>
</tr>
<tr>
<td>E4:E6</td>
<td>0.366</td>
<td>0.049</td>
<td>0.463</td>
<td>0.434</td>
<td>0.183</td>
<td>-0.579*</td>
<td>0.770**</td>
<td>-0.157</td>
</tr>
<tr>
<td>SUVA₂₅₄</td>
<td>0.228</td>
<td>0.460</td>
<td>0.232</td>
<td>0.129</td>
<td>0.231</td>
<td>0.157</td>
<td>-0.098</td>
<td>-0.059</td>
</tr>
<tr>
<td>FI</td>
<td>-0.438</td>
<td>-0.161</td>
<td>-0.318</td>
<td>-0.238</td>
<td>-0.115</td>
<td>0.492</td>
<td>0.485</td>
<td>-0.186</td>
</tr>
<tr>
<td>Air temp.</td>
<td>-0.032</td>
<td>-0.379</td>
<td>-0.029</td>
<td>-0.052</td>
<td>0.220</td>
<td>0.402</td>
<td>0.571*</td>
<td>-0.408</td>
</tr>
<tr>
<td>Rainfall</td>
<td>0.603*</td>
<td>0.061</td>
<td>0.537</td>
<td>0.445</td>
<td>0.365</td>
<td>0.3890</td>
<td>0.492</td>
<td>-0.226</td>
</tr>
<tr>
<td>PARc</td>
<td>-0.161</td>
<td>-0.459</td>
<td>-0.380</td>
<td>-0.267</td>
<td>-0.224</td>
<td>0.054</td>
<td>0.662*</td>
<td>0.482</td>
</tr>
<tr>
<td>Discharge</td>
<td>0.132</td>
<td>0.237</td>
<td>0.123</td>
<td>0.088</td>
<td>-0.139</td>
<td>0.2354</td>
<td>0.767**</td>
<td>-0.072</td>
</tr>
</tbody>
</table>

*p < 0.05
**p < 0.01
*Mean monthly air temperature
*Total monthly rainfall (mm)
*Mean monthly PAR (μmol m⁻² s⁻¹)
*Mean monthly discharge (L s⁻¹)
Figure 1. Mean monthly air temperature, total rainfall and mean discharge from May 2014 to May 2015 are shown for a) Auchencorth Moss, with discharge of the Black Burn shown on the left hand offset axis. Mean monthly air temperature and total rainfall are shown for the same period for Comer meteorological station, near b) Loch Katrine. Note inverted right hand y axes.
Figure 2. Time series at a) the Black Burn and b) Loch Katrine of DOC concentration and parameters for DOC quality: SUVA$_{254}$ and E4:E6 from May 2014 to May 2015. Note different y axis scales for DOC data.
Figure 3. Change in absorbance upon irradiation expressed as a percentage of the unirradiated control samples from 250 - 400 nm at a) Black Burn and b) Loch Katrine. Summer is the mean of June, July and August values, autumn is the mean of September, October and November values, winter is the mean of December, January and
February values, and spring is the mean of March, April and the combined mean of May ’14 and May ’15 values. Note different y axis scales.
Figure 4. Irradiation induced changes to carbon species DOC, DIC, CO₂ and CO in monthly water samples from panel Black Burn (panel a) and Loch Katrine (panel b). DOC normalised changes to all C species changes (photoreactivity, quantified as explained in the text) are shown on the bottom row. Data represent the difference between the mean of irradiated and unirradiated control samples. Error bars show the standard error of the mean (n=4). Note different y axis scales for Black Burn and Loch Katrine water samples.
Figure 5. Rainfall events sampled on 9-10 December 2014 (panel a) and on 1-2 September 2015 (panel b). Row one shows a time series of hourly rainfall, discharge and DOC concentrations for each event. Row two shows photo-induced C pool changes of irradiated samples expressed as a total change value per C species in vertical
bars (left y axis) and as a DOC normalised value in dots (right y axis). Data represent the difference between the mean of irradiated and unirradiated control samples (n=4). Note different x- and y-axis scales.
Figure 6. Boxplots of carbon-normalised yields of phenols groups for Black Burn water samples collected a) monthly in the year-long study (n=13), b) during the winter rainfall event (n=8) and c) during the summer rainfall event (n=7). P = p hydroxyl, V = vanillyl, S = syringyl and C = cinnamyl. The box spans from the first quartile to the third quartile, with the line showing the median value. Whiskers show the minimum and maximum values, with dots representing outlying values.
Figure 7. Pearson correlation between mg DOC lost upon irradiation per mg DOC and a) P:V ratios and b) Ad:Alv,s (derived from acids and aldehydes from vanillyl and syringyl phenol groups) ratios in all Black Burn water samples analysed (n=28). Lines of best fit for all water samples are also shown. The monthly samples in the year-long study and the winter and summer rainfall event samples are indicated.