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Response to Reviewers:	Thank you for your suggestions to improve the manuscript.

	I have altered all of the grammatical errors. Regarding the first comment, the Evans et al. (in press) reference will also be appearing in the Special Issue, so can the exact details of this reference be supplied by the publisher during the proofing stage? I have included as much as is known so far in the reference list. Regarding the second comment, I have added the following sentence to the end of that paragraph - "Bacteria can consume DOC, but the increases we observed in Experiment A suggest this was happening at a slower rate than production processes." to acknowledge that the process of bacterial consumption may also have been happening.

Transformations in DOC along a source to sea continuum; impacts of photo-degradation, biological processes and mixing.

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Abstract

Peatlands export significant amounts of Dissolved Organic Carbon (DOC) to freshwaters, but the quantity of DOC reaching marine environments is typically less than the input to the fluvial system due to processing within the water column. Key removal processes include photo-chemical degradation, and heterotrophic bacterial respiration. In this study we examined these processes using ^{14}C-labelled DOC to quantify the extent of DOC breakdown and to determine its fate following irradiation under controlled laboratory conditions. We examined the influence of microbial processes occurring within the water column, the potential role of stream-bed biofilms, and the possible modifying effects of downstream mixing, as DOC in water from the peatland encounters runoff from upland mineral soils ("Mountain"), nutrient-rich runoff from agricultural soils, and seawater in an estuary. Our results demonstrated conservative mixing of DOC from Peatland and Mountain waters but interactive effects when Peatland water was mixed with Agricultural and Estuary waters and exposed to solar radiation. The mixing of Peatland and Agricultural waters led to net DOC production, suggesting that DOC was only partially degraded by solar radiation and that the products of this might have fuelled autotrophic microbial growth in the samples. The mixing of Peatland water with saline estuary water resulted in net DOC loss following irradiation, suggesting a role for sunlight in enhancing the flocculation of DOC to Particulate Organic Carbon (POC) in saline environments.

Keywords – Biodegradation, Carbon cycling, Dissolved Organic Matter, Humic substances, UV radiation

Introduction

Dissolved organic carbon (DOC) represents a significant flux of carbon (C) from terrestrial to aquatic environments. It is usually the dominant form of C within upland fluvial systems in temperate and boreal latitudes, especially where organic soils such as peatlands occur. In the UK, for example, DOC typically comprises 90% of total fluvial C in upland regions (Billett et al. 2006) and concentrations can be high (>20 mg L^{-1}) where peat is the dominant catchment soil type. Research into the processes regulating DOC in fluvial systems has increased in recent years, as greater emphasis is placed on understanding the role of freshwater systems in the global C cycle (Cole et al. 2007; Battin et al. 2009; Tranvik et al. 2009). Although undisturbed peatlands represent long-term C sinks, the DOC that they export to freshwater ecosystems represents a significant C loss, often on the order of half the net CO_2 uptake by the ecosystem (Roulet et al. 2007). On a global scale, rivers transport a large amount of this DOC to the world's oceans; 0.25-0.45 Gt C yr^{-1} (Hedges et al. 1997; Cole et al. 2007), however this figure is significantly lower than the total amount of DOC input to the fluvial system due to processing within the water column and hyporheic zone as it flows from headwater sources to the sea (Cole et al. 2007; Tranvik et al. 2009). Most of the available evidence suggests that the majority of this DOC will at some stage be emitted to the atmosphere as CO_2 (Evans et al., in press). Potentially, the most significant process occurring in rivers is photo-chemical degradation; the breakdown of DOC by solar radiation (Cory et al. 2014). DOC absorbs UV and visible radiation due to the presence of chromophoric structures comprised of conjugated double bonds (Zepp 1988). Such bonds can be broken down by the levels of solar radiation that typically reach the Earth's surface, and partial or complete mineralisation to CO_2 can occur (Osburn et al. 2001; Cory et al. 2014). Waters draining peatlands contain DOC which is typically rich in chromophoric structures, so peat-derived DOC is particularly susceptible to photo-degradation (Dehaan 1993). Sunlight may therefore be a significant driver of CO_2 emissions due to the abiotic mineralisation of DOC exported from peatlands in fluvial systems.

It has been reported in a number of studies that certain physicochemical factors can influence the rate of photo-chemical degradation of DOC. For example, DOC breakdown takes place more rapidly at low pH due to more favourable conditions for Fenton's reactions involving hydroxyl radicals (Zepp et al. 1992; Molot et al. 2005; Wu et al. 2005). The breakdown of DOC reduces the molecular weight/size and aromaticity of the DOC (Wetzel et al. 1995; Bertilsson and Tranvik 2000), which for DOC that does not completely mineralise may reduce its recalcitrance and make it more bio-available (Graneli et al. 1998). Photo-chemical breakdown of DOC is therefore of great importance to the biological functioning of aquatic systems (Moran and Zepp 1997; Amaral et al. 2013) but in terms of GHG emissions the fate of non-mineralised photo-degraded DOC is complex.

Microbial uptake of DOC may lead to a relatively rapid turnover of DOC to CO_2 (del Giorgio and Duarte 2002). Most of the CO_2 emitted from freshwaters in temperate regions due to respiration is the result of heterotrophic bacterial metabolism (Findlay et al. 1998) and recent studies have demonstrated the importance of DOC source and quality for influencing rates of bacterial metabolism (Berggren & del Giorgio, 2015). DOC is also consumed by biofilms (Meyer et al. 1987), which are a complex mixture of algae, bacteria, micro-fauna and exopolysaccharides that reside on bed sediments. Baldwin et al. (2014) demonstrated that the first-order rate constant for DOC uptake varies linearly with the amount of biofilm present in a stream. It is reasonable to assume that solar radiation also makes DOC more available to microorganisms and biofilms and both act together to lead to rapid turnover of DOC in fluvial systems.

During this study we used ^{14}C labelling to determine the fate of DOC degraded by solar radiation. ^{14}C labelling is a technique used frequently in soil science to examine the cycling of organic matter in soils (e.g. Hill et al. 2008) but to our knowledge this is the first study to use the technique to assess the impact of solar radiation on DOC cycling. We aimed to quantify the rate of photo-chemical breakdown of ^{14}C-DOC derived from peatlands, in comparison to DOC from other water sources, under controlled laboratory conditions. We also examined the influence of a range of factors on photo-chemical degradation rates and fate of DOC, including the influence of microbial processes occurring within the water column; the potential role of stream-bed biofilms; and the possible modifying effects of downstream mixing, as DOC in water from the peatland encounters firstly runoff from upland Mountain soils, then nutrient-rich runoff from agricultural soils, and finally seawater in an estuary.

Materials and Methods

Site selection

The experimental work was undertaken on water samples collected from the Afon Conwy catchment, North Wales, UK. The Conwy drains a typical mixture of UK upland and lowland soils and land-use, and is the subject of intensive ongoing research into controls on water quality, including DOC transport from terrestrial to fluvial systems (e.g. Austnes et al. 2009; Cooper et al. 2014). It was one of the four UK river catchments surveyed extensively by Palmer et al. (in press) to assess *in-situ* organic C processing in fresh and estuarine waters. We performed experiments on four contrasting types of water that commonly occur along a source-to-sea continuum; from streams draining either (i) upland peat, (ii) upland mineral ("Mountain") and (iii) lowland agricultural soils, or (iv) from the estuary. The streams draining Peatland and Mountain soils were located in the headwaters of the Conwy catchment and were both sampled during the study described by Palmer et al. (in press). The

"Peatland stream" drains a catchment comprised entirely of blanket bog whilst the "Mountain stream" drains slowly permeable acid upland soils with a shallow organic horizon over podzolic mineral soil. The "Agricultural stream" joins the Afon Conwy approximately 30 km downstream of the source. It drains freely draining acid loamy soils that are used for intensive livestock grazing, and consequently has some of the highest nutrients loads within the Conwy catchment. The Estuary sample was taken from a point close to the mouth of the Conwy estuary, where salinity is consistently high. This site is always downstream of the Estuarine Turbidity Maximum. Table 1 presents basic properties of the 4 sampling sites.

Experimental design

Experimental procedures were divided into two components based on the nature of the water samples; A) Filtered water from a single source and B) Unfiltered water mixed from multiple sources. The principal differences between the experiments were that A) used filtered samples and tested each water type separately, whereas B) used unfiltered samples and included treatments which were mixtures of the different water types. Experiment A was undertaken to assess rates of DOC photo-degradation only, as sample filtration should exclude most of the microbial communities that would be expected to contribute to DOC processing. Experiment B was undertaken to assess the rate of DOC processing under more natural conditions, where photo-degradation would be expected to contribute but not necessarily be solely responsible for DOC processing, and to determine whether the mixing of contrasting water types affected rates of DOC breakdown.. All experiments were undertaken in the laboratory using a SunTest CPS+ (Atlas, Linsengericht, Germany) sunlight simulator to expose samples to a controlled level of UV and visible radiation similar to that received on the Earth's surface. The continuous dose used during all experiments was 765 W/m^2, using a wavelength range of 300-800 nm. A refrigeration unit ensured that temperatures within the chamber did not exceed approximately 10°C, the approximate annual mean water temperature across the sites.

A) Single-water, filtered samples

The first set of experiments were undertaken on filtered samples. 100 ml of water sample from each of the four sites was collected in acid-washed plastic bottles, filtered within 24 hours through 0.45 µm syringe filters (Avonchem, Macclesfield, UK) and 22 ml placed inside a 25 ml capacity 87 mm x 25 mm custom-made quartz tube. Two tubes containing Peatland stream solution were used, with one wrapped in foil to exclude radiation but treated identically to the other tubes and used as a control. Two ml of sample was removed as the 'initial' (0 MJ m^{-2} dose) sample and the vessels were sealed and placed inside the chamber of the SunTest CPS+. Two ml subsamples were taken on 3 further

occasions, with the last sample being taken after a total dose of 66 MJ m^{-2} had been reached. This is equivalent to the dose received at mid-latitude UK site over 2-2.5 clear mid-summer days, which was considered a realistic maximum light dose for water in a typical, short residence time UK river system. The experiment was repeated five times for samples collected on separate occasions between May to December 2012. Samples were stored at 4°C until analysis for DOC and UV-Vis absorbance. As samples were collected during different seasons and weather conditions, the initial DOC concentrations varied, especially for those with a greater mean concentration (Table 1).

Experiment A was performed to test the response of each water type to simulated solar irradiation in the absence of biological processes, as well as particulates, which might have attenuated some of the radiation. The results were therefore intended to indicate the upper extent of DOC photo-degradation that could occur in these samples under near optimal conditions.

B) Mixed-water, unfiltered samples

This set of experiments formed the core component of the study and were designed to quantify the effects of photo-degradation, downstream mixing of contrasting water types, contact with bed sediment/biofilm and in-stream biological processes on overall rates of DOC removal, and on the fate of this DOC. The experiments were performed using the same SunTest CPS+ and quartz tubes but with the addition of a custom-built recirculation system to allow for the use of larger sample volumes. Samples were held in 500 ml amber bottles in a water bath set to field temperature (10°C) outside of the SunTest CPS+ box. Peristaltic pumps were used to recirculate sample from each bottle through the quartz tubes inside the SunTest CPS+ and back to the external sample bottles (1 amber bottle and quartz tube per sample). The system operated as a closed loop but samples could be taken whilst the experiment was running by temporarily disconnecting the tube returning to the external bottle. The flow rate was 2 ml/min. In addition to the inflow and outflow of water, the gaseous headspace of the quartz tubes was continually pumped to an external sampling point (see below for more details).

To provide more detailed information on the breakdown and conversion of DOC into other forms of C, such as mineralisation to CO_2 and uptake by the biofilm, we added ^{14}C-labelled DOC to the Peatland stream solution. The labelled DOC was prepared by exposing a *Calluna vulgaris* plant to a high dose of $^{14}CO_2$ during the growing season, so that the ^{14}C tracer became incorporated into the plant biomass during photosynthesis, as described by Hill et al. (2007). The plant was then ground to a fine powder, incorporated into a sample of peat soil, and left to decompose for 6 months. Soil porewater was collected from the peat using a 10 cm Rhizon sampler (Rhizosphere Research Products, the Netherlands) and the DOC was found to be highly ^{14}C-enriched (ca. 4000 DPM/ml).

Further testing indicated that this labelled DOC had very similar structural characteristics to natural DOC in peat soil solution and stream water and behaved similarly when exposed to solar radiation (see supplementary material).

The overall design of the mixing experiments is depicted in Figure 1. For each experiment four treatments were employed:

1) $DO^{14}C$-enriched Peatland water sample only, irradiated
2) 'Other' (Mountain/Agricultural/Estuary) water sample only, irradiated
3) Mixed (i.e. 'Peatland/$DO^{14}C$' + 'Other') sample, irradiated
4) Mixed (i.e. 'Peatland/$DO^{14}C$' + 'Other') sample, not irradiated (dark).

These experiments were performed in the autumn of 2012, using freshly collected samples. The ^{14}C-labelled DOC was mixed with fresh Peatland stream solution to provide a final DOC concentration of approximately 25 mg C L^{-1} (the approximate seasonal maximum DOC concentration in the Peatland stream from which samples were collected). The mixed treatments were 50:50 mixtures of $DO^{14}C$-enriched Peatland water and the other water type. Four replicates were run for each treatment, so 16 quartz tubes were employed inside the SunTest CPS+ during each experiment. The amber bottles contained 100 ml of unfiltered water. The non-irradiated treatment was run in exactly the same way as the other treatments, except the four quartz tubes inside the SunTest CPS+ were wrapped in foil to block exposure to solar radiation. Following the addition of all water samples to the amber bottles, the system was allowed to run for one hour without exposure to radiation to allow for thorough mixing of the 'Mixed' treatments (this would ensure that any changes in water chemistry measured during the experiment would be due to exposure to radiation rather than any initial effect of mixing). A set of samples were then taken from each bottle, corresponding to time zero (i.e. 0 MJ m^{-2}), which involved taking 5 ml sample in a syringe and filtering immediately, as previously described. As only a small proportion of each sample (approx. 7 ml) was exposed to radiation at any one time, the experiment was run for four days to provide a sufficiently large cumulative light dose. Samples were taken at 3 further time points, although for data analysis only the difference between the first and last time points has been used, to assess the impact of a 28 MJ m^{-2} radiation dose (approximately equivalent to one clear summer day at the study site). Samples were analysed for UV-Vis absorbance, DOC and DIC concentrations. At the end of the experiment the volume of sample remaining was measured to determine if any evaporative losses had occurred and the samples were analysed for the same determinants as above and also pH, conductivity and major anion and cation concentrations.

For the 'Peatland + Mountain' and 'Peatland + Agriculture' experiments we performed an additional set of mixing experiments, following an identical procedure, except for the additional

inclusion of biofilm-coated material intended to mimic stream bed sediment. The biofilm was prepared by extracting sediment from each of the three stream beds, scraping the biofilm from the surface and placing it onto inert glass beads (0.6-0.8 mm diameter, Waterco, United Kingdom) inside a shallow tray. The glass beads were then submerged in water derived from each of the respective streams and the biofilm allowed to grow outside for several months. For the experiments, 20 g of glass beads/biofilm were removed from the tray, rinsed with distilled water to remove biofilm not adhered to the beads (as this would lead to inconsistent quantities in each bottle) and placed in the bottom of the amber bottle. This quantity was used to provide a level covering on the bottom of the bottle. For the 50:50 'Peatland + Other' mixes we used 10 g of biofilm-coated beads from each of the 'Other' source water types.

To quantify the conversion of ^{14}C labelled peat DOC to $^{14}CO_2$, the headspace within each vial was continually pumped through two vials in series, each containing 3 mL 1M NaOH, to capture CO_2. On each occasion when water samples were collected, the NaOH was combined into a 6 mL composite sample and stored in 20 mL scintillation vials until analysis. Fresh NaOH was then put into the CO_2 traps. The 0.45 µm filters used to filter the sample remaining in the amber bottles at the end of the experiment were used to determine $PO^{14}C$, although it was not possible to determine actual POC concentrations as the filter was destroyed during the $PO^{14}C$ analysis and therefore not available for combustion. For determination of ^{14}C incorporated into biofilm, the glass beads were collected from the bottom of the amber bottles, allowed to dry naturally and placed inside 20 mL scintillation vials for analysis.

Analytical techniques

Sample pH and conductivity were determined on SevenEasy and FiveGo (both Mettler Toledo, Leicester, UK) pH and conductivity meters, respectively. DOC analysis was performed using the NPOC method on a Thermalox TC/TN (Analytical Sciences Ltd, Cambridge, UK) analyser and UV-Vis absorbance using a Spectramax M2*e* (Molecular Devices, Winnersh, UK) spectrophotometer. Anions and cations were determined on a 850 Professional IC (Metrohm, Runcorn, UK).

Samples collected for the analysis of ^{14}C concentration were measured on a Wallac 1404 liquid scintillation counter (Wallac EG&G, Milton Keynes, UK). $DO^{14}C$ content of the water samples was measured by mixing 4 mL of sample with 16 mL ScintiSafe 3 scintillation cocktail (Fisher Scientific, Leicestershire, UK) in a 20 mL scintillation vial. For the NaOH, which contained captured $^{14}CO_2$, 14 mL of scintillation fluid was added to 6 mL of sample. $PO^{14}C$ was determined according to the method described in Uselman et al. (2007); filters were placed into separate scintillation vials and 1 mL 2 M HCl was added to remove any inorganic C. Subsequently, 20 mL of scintillation fluid

was added and the filters were allowed to dissolve for 48 hours. For the biofilm, 12 mL of scintillation fluid was added to the vials containing the glass beads and the biofilm allowed to dissolve for 24 hours before analysis. Following the addition of scintillation cocktail to sample, the vials for all sample types were capped and vortexed for 3 seconds to ensure thorough mixing. All scintillation counts were performed for 1000 seconds.

Data analysis

In Experiment B, by mixing the contrasting water types, we tested whether the combination of Peatland stream water with different sources of water (to mimic mixing occurring naturally in a fluvial system) changed the propensity for DOC breakdown by solar radiation (i.e. non-conservative behaviour). The responses for these mixed treatments were compared to a hypothetical 5th treatment, designated a 'Conservative Mix', which was calculated by averaging the responses of the 'Peatland only' and 'Other only' treatments, The response of this treatment would be that expected if the pools of DOC from both streams behaved independently i.e. no interactive effect of mixing. For example, if Streams A and B had DOC concentrations of 10.0 mg/L and 5.0 mg/L respectively at the start of the experiment and concentrations of 8.0 and 4.0 mg/L respectively after irradiation, then a simple, non-interactive (i.e. conservative) mix of these 2 streams should result in concentrations of 7.5 mg/L before and 6.0 mg/L after irradiation.

We focused our statistical analysis on treatment effects, rather than examining whether there were significant differences in our measured parameters from day 0 to the final sample. Differences between treatments were determined using one-way ANOVA and Tukey's HSD post-hoc test in R v2.15.1. Prior to running ANOVA analyses, all data distributions were tested for normality using the Shapiro-Wilk test and for variance heteroscedasticity using the Bartlett test. Data that did not conform to the assumptions of ANOVA were log transformed (excluding pH as it is already on a log scale).

Results

A) Single-water, filtered samples

Figure 2 shows changes in mean DOC concentration and absorbance at 254 nm (Abs_{254}, an indicator of the concentration of coloured, 'chromophoric' DOC) over the course of five repeated irradiation experiments. Initial DOC concentrations and absorbance of the samples used were consistently in the order Peatland > Mountain > Agricultural > Estuary, reflecting the different characteristics of the soil types they drain and, in the estuary, the influence of marine DOC inputs. Peatland water had the

highest ratio of Abs_{254}/DOC, also referred to as specific UV absorbance ($SUVA_{254}$), reflecting the highly coloured (and by inference higher molecular weight and aromaticity; Volk et al. 2002) nature of DOC from this source. In comparison, DOC in the Agricultural and Estuary samples had a very low $SUVA_{254}$ and was thus relatively transparent to solar radiation.

During the experiments, on average 43% of the original DOC was lost in the Peatland samples exposed to light. Peatland samples kept in the dark showed no change, indicating that all of this loss could be attributed to light exposure. For the Mountain stream samples, DOC also declined, on average by 27%. The Agricultural and Estuary waters, on the other hand, both demonstrated increasing DOC concentrations with increasing light dose; 27% on average for the former and 56% for the latter.

In all five tests, measured Abs_{254} of the Peatland, Mountain and Agricultural samples declined, towards similar values at the end of the experiment in all cases (~0.1 absorbance units at 66 MJ m^{-2}). The absorbance values of the Estuary water were already at this low level, and did not decrease further. For the Peatland, and to a lesser extent the Mountain streams, the loss of absorbance was rapid and extensive; in the Peatland around three quarters of the chromophoric DOC had been lost by the end of the experiment. Initial $SUVA_{254}$ correlated positively with the change in DOC induced by exposure to solar radiation (Figure 3), indicating that if DOC contains a greater proportion of chromophoric structures then the DOC is more likely to be lost by photo-degradation. Similarly if the $SUVA_{254}$ is sufficiently low then the DOC is not likely to be degraded by solar radiation and net DOC increases can occur.

B) Mixed-water, unfiltered samples

Response of single water types

For all three mixing experiments, and in the absence of bed sediment and biofilms, the DOC concentration of the Peatland sample decreased by 14-17% for the cumulative light dose of 28 MJ m^{-2} (Figure 4a). The DOC concentration increased by 21% for the Mountain 14% for the Agricultural and 69% for the Estuary samples. The $DO^{14}C$ count decreased for the Peatland sample for all three experiments (by 11-22%), providing evidence that peatland DOC was transformed to other forms of C (Figure 5a). The presence of biofilms did not significantly alter the rate of DOC or $DO^{14}C$ processing for any of the three single water types (ANOVA; $p > 0.05$) (Figures 4b and 5b).

Effects of mixing water from upland peatland and Mountain streams

The DOC concentration decreased in the 'Peatland + Mountain' irradiated treatment, but was not significantly different to the 'Conservative Mix' calculation (ANOVA; $p > 0.05$), indicating that the DOC from the two contrasting waters effectively behaved as two independent pools. The 'Peatland + Mountain' dark treatment recorded an increase in DOC by 7-9%, which was statistically significant compared to the previous two treatments (ANOVA, $p < 0.05$), implying that aphotic production of DOC was taking place. The presence of biofilms reduced the magnitude of the DOC response (whether this was an increase or a decrease) for all 3 treatments; these effects were not significant but the significant treatment effect reported above without biofilm (i.e. DOC production in dark conditions) was also observed with biofilm.

The $DO^{14}C$ response was comparable for the 'Peatland only', 'Peatland + Mountain' irradiated treatments and the 'Conservative Mix' calculation, again suggesting no interactive effects of mixing. In the absence of biofilms, the 'Peatland + Mountain' dark treatment lost approximately 50% less $DO^{14}C$ than the 'Peatland + Mountain' irradiated treatment, although the difference was not significant (ANOVA, $p > 0.05$).

The presence of biofilms increased the loss of $DO^{14}C$, but only significantly for the 'Peatland + Mountain' dark treatment. For this treatment the presence of biofilm increased $DO^{14}C$ loss by 149%, such that the reduction in $DO^{14}C$ was comparable to the 'Peatland + Mountain' irradiated treatment in the absence of biofilm.

Effects of mixing water from upland peatland and lowland agricultural streams

In the 'Peatland + Agricultural' irradiated treatment there was very little change in DOC concentration in the absence of biofilm and an 11% increase when biofilm was included. This contrasts with the 'Conservative Mix' calculations, which predicted -11% (without biofilm) and -8% (with biofilm) changes if the two pools of DOC responded independently. These treatment effects were significant (ANOVA, $p < 0.05$). For the 'Peatland + Agricultural' dark treatment including biofilm, the DOC concentration increased by 18%, significantly greater than the small increase for the 'Peatland + Agricultural' irradiated treatment. With biofilm present, the final DOC concentration of the two treatments was almost identical.

The $DO^{14}C$ data revealed that the non-conservative DOC increase for the irradiated 'Peatland + Agricultural' treatment is unlikely to be due to changes in the degradation of the peatland-derived DOC; in the absence of biofilms the loss of $DO^{14}C$ behaved conservatively and was almost identical between the 'Peatland only', 'Peatland + Agricultural' irradiated and 'Conservative Mix' treatments. The inclusion of biofilm resulted in a significant 57% increase in $DO^{14}C$ removal in the 'Peatland + Agricultural' irradiated treatment, whereas no increase in $DO^{14}C$ removal was observed for the

'Peatland only' treatment. As was observed with the 'Peatland + Mountain' experiment, the presence of biofilms affected the turnover of $DO^{14}C$ in the dark treatment. In the absence of biofilms just 5% of $DO^{14}C$ was lost in the 'Peatland + Agricultural' dark treatment, but when biofilm was included this figure increased significantly to 23%.

Effects of mixing water from an upland peatland stream and estuary water

This mixing experiment was undertaken without the inclusion of biofilm. The DOC concentration of the 'Peatland + Estuary' irradiated treatment decreased by 18%, significantly more than the 'Conservative Mix' decrease of 7% (ANOVA, $p < 0.05$). The DOC concentration in the 'Peatland + Estuary' dark treatment increased by 22%, significantly different to the decreases observed for the other two treatments. Conversely, the loss of $DO^{14}C$ in the 'Peatland + Estuary' irradiated treatment was only that observed in the 'Peatland only' treatment, although this result was not significant (ANOVA, $p > 0.05$).

Recovery of lost $DO^{14}C$

The lost $DO^{14}C$ was recovered as $^{14}CO_2$, $PO^{14}C$ and as biofilm-^{14}C and the percentage recovery into each form of C is presented in Tables 2-4. Over the full set of experiments, between 3.8 and 12% of ^{14}C lost from DOC was recovered as CO_2, 5.6 to 41% as POC, and (where included in the experiment) 4.3 to 9.4% in biofilms. For all experiments and treatments most (61 to 86%) of the lost ^{14}C was not recovered; the possible reasons for this are discussed later.

For the 'Peatland + Mountain' mix, no significant differences in $DO^{14}C$ recoveries were recorded between treatments and when comparing the presence and absence of biofilms. However, the most notable difference between treatments was for the dark treatment in comparison to the other treatments, for which a greater proportion of $DO^{14}C$ was converted to $PO^{14}C$ (15.2%) compared to captured $^{14}CO_2$ (3.8%). For the irradiated treatment the conversion was 5.6% to $PO^{14}C$ and 12.9% to $^{14}CO_2$. Less $DO^{14}C$ was incorporated into biofilm C (6-9%) than converted to $PO^{14}C$ (9-10%) for all treatments.

For the 'Peatland + Agricultural' mix, 34% of the $DO^{14}C$ lost in the dark treatment in the absence of biofilms was recovered as $PO^{14}C$, significantly higher than the $PO^{14}C$ recoveries in the other treatments (ANOVA, $p < 0.05$). In comparison to the 'Peatland + Mountain' irradiated treatment, for which 12.9% of $DO^{14}C$ was converted to $^{14}CO_2$, the figure was far less for the 'Peatland + Agricultural' irradiated treatment, at just 3.7%. With biofilm present the percentage recoveries into the three pools of C were similar between the irradiated and dark treatments and the high recovery of $PO^{14}C$ recorded for the dark treatment in the absence of biofilms was not replicated when biofilms were

present. Biofilms captured about 4-5% of the lost $DO^{14}C$ in all experiments where they were included, which was lower than the 'Peatland + Mountain' experiment.

For the 'Peatland + Estuary' mix the greatest effect was again for $PO^{14}C$ but this time for the irradiated mix treatment (rather than the dark treatment for the 'Peatland + Agricultural' experiment); 41% of the lost $DO^{14}C$ was recovered as $PO^{14}C$, significantly higher than the $PO^{14}C$ recoveries for the other treatments and the greatest $DO^{14}C$ percentage recovery for any of the experiments. The $PO^{14}C$ recovery was much less in the dark treatment (13.2%). The conversion of $DO^{14}C$ to $^{14}CO_2$ for the irradiated treatment was 9.5%, which was less than the comparable treatment in the 'Peatland + Mountain' experiment but more than the 'Peatland + Agricultural' experiment.

Effects of filtering samples

To evaluate the effects of sample filtration on responses to simulated radiation we compared the rates of DOC degradation for the Peatland samples derived from Experiments A and B. To enable better comparison between the two data sets, we also included two Mountain sample data points from Experiment B (the two with the lowest initial DOC concentration for the unfiltered samples) as we did not use a Peatland stream solution with an initial DOC concentration lower than 25 mg l^{-1} in this experiment. Results showed that DOC losses approximately followed first order reaction kinetics for both sets of experiments, with higher loss rates at higher initial concentrations. We used this relationship to calculate the loss of DOC at a specific radiation dose of 28 MJ m^{-2}, and then calculated the loss of DOC per MJ m^{-2} of solar radiation. The results (Figure 6) indicate that loss rates were 2-3 times higher in filtered versus unfiltered samples.

Discussion

Susceptibility of contrasting waters to photo-degradation

Both experiments provided clear evidence that solar radiation can break down large amounts of DOC, but that this is strongly dependent on the type of DOC present. The greater loss of Abs_{254} than DOC concentration in Part A shows that it is the highly coloured component of DOC that is most readily degraded by solar radiation. Furthermore, we demonstrated that when DOC has a higher proportion of chromophoric structures (as indicated by a high $SUVA_{254}$) it is more susceptible to being completely mineralised to CO_2. This supports findings from previous studies (Dehaan 1993; Koehler et al. 2014) and highlights how DOC leaching from peatlands is particularly susceptible to photo-degradation. The 14-17% reduction in DOC concentration of the unfiltered peatland stream

solution after exposure to a dose of solar radiation equivalent to one sunny mid-summer day demonstrates that a significant proportion of DOC may be photo-degraded during transport down the river system, despite the relatively short residence time. This finding agrees with that of Moody et al. (2013), that the loss of DOC in rivers due to photo-degradation can be rapid and extensive.

Comparing DOC losses across the experiments for the DO^{14}C/Peatland solution only from Experiment B, the rate of DOC degradation by solar radiation was dependent on the DOC concentration of the sampled solution, which varied due to seasonal and climatic factors. The loss was more rapid for higher initial DOC concentrations. It would be expected that DOC loss would continue until all photodegradable DOC has been removed, which for the Peatland stream water would correspond to a final DOC concentration of approximately 5 mg/L. The rate of DOC loss was, however, around three times lower for unfiltered versus filtered samples, although we acknowledge that this comparison must be treated with caution as we included data for Mountain stream samples, which is less reactive to sunlight than Peatland stream DOC. Several possible mechanisms could explain this, including i) as discussed in the next section, autotrophic production of DOC leading to smaller net DOC losses; ii) attenuation of light by particles within the water; and iii) conversion of POC to DOC by abiotic or biotic processes (although it is unlikely that there was sufficient POC in the initial samples to explain all of the observed differences in DOC loss rates in all experiments).

Biologically-mediated DOC production

DOC production was observed for unfiltered Mountain samples, and all filtered and unfiltered Agricultural and Estuary samples. Measured DOC increases for single water types in Experiment B suggest that production of DOC outweighs photo-degradation under certain conditions when particulates and microorganisms are present. The size of the DOC pool in natural waters can increase by lysis of plankton cells and excretion of photosynthates by algae and cyanobateria (Brock and Clyne 1984; Malinsky-Rushansky and Legrand 1996; Ye et al. 2011). These products are considered to be a major source of DOC, particularly in marine environments (Lee and Henrichs 1993) and to be a significant driver of heterotrophic bacterial growth (Brock and Clyne 1984; Teira et al. 2001). Although DOC release during these processes is considered to be continuous, past studies have found that under high light intensities, large excretions of DOC by algae can occur as a stress response (Hellebust 1965; Zlotnik and Dubinsky 1989). The light intensity used in these experiments (765 W/m^2) was similar to that used in those two previous studies, therefore it is likely that we observed the release of DOC from phytoplankton and algae, and possible that this may have occurred at a higher rate than would occur naturally.

The DOC increases were greater for the Agricultural and Estuary waters. As the concentration of inorganic nutrients was higher for these two waters than the Mountain stream, this would suggest that the nutrients are driving greater photosynthetic production of DOC. It is important to consider that the initial concentration of photodegradable (coloured) DOC in the Mountain, Agricultural and Estuary samples was much lower than the Peatland water, so there would have been much less photo-degradation to mask any DOC increases. This was demonstrated in Experiment A, where there was minimal loss of absorbance at 254 nm for Agricultural and Estuary water. DOC produced by the photosynthetic activity are generally of low molecular weight/size, highly labile (Brock and Clyne 1984), and transparent, such that they are not susceptible to photo-mineralisation. The loss of DOC for the Mountain water when the sample was filtered, versus the increase when the sample was unfiltered, as well as the greater DOC increases for the unfiltered versus filtered Agricultural and Estuary waters, supports our view that autotrophic release of DOC was probably the cause of the measured increases. However, small DOC increases were also recorded for the filtered Agricultural and Estuary waters. Past studies have suggested that a "bottle effect" may occur with natural water samples stored in plastic or glassware, where contact with such media can artificially increase microbial numbers in water samples and lead to experimental artefacts (Bischofberger et al. 1990). On the other hand, Hammes et al. (2010) found no evidence of significant bottle effects across a range of bottle sizes and surface area to volume ratios. Although we cannot discount the possibility that the artificial conditions used in this experiment may have elevated microbial abundance, and thereby enhanced DOC concentrations to some extent, a full experimental run using samples of only deionised water (without having rinsed out the bottles beforehand, so some microbes would still have been present) did not lead to increases in DOC, providing some evidence that our results were not due to an experimental artefact.

The increases may be due to the imperfect removal of microorganisms by filtration media. A number of recent studies have demonstrated that some bacteria are able to pass through commonly used filters sizes (Wang et al. 2007; Fedotova et al. 2013). For the filter pore size used in this study for Experiment A, 0.45 µm, Wang et al. (2007) demonstrated with lake water that on average 50% of total bacterial populations passed through the filter. It seems clear that for Experiment A to have assessed the effects of photo-degradation only, the samples needed to have been filtered through a much smaller pore size to ensure removal of cyanobateria. However, even a pore size of 0.1 µm could have allowed some bacteria through (Wang et al. 2007), but would likely have excluded a significant part of the total DOC. The DOC increases observed in Experiment A must have been due to the incomplete removal of bacteria and their subsequent reproduction, although the exact mechanism responsible for the increase (exudation through growth or stress) remain unresolved.

The increases are not considered to be due to cell breakage and release of DOC during filtration, an issue raised in previous studies (Goldman and Dennett 1985), firstly because we did not vacuum-filter samples, and secondly because cell breakage would be expected to cause an initial flush of DOC, rather than the progressive changes we observed during the experiments. Bacteria can consume DOC, but the increases we observed in Experiment A suggest this was happening at a slower rate than production processes.

Impacts of mixing of contrasting water types

In the mixing experiments, we found no evidence that mixing Peatland and Mountain water types, both of which were derived from upland unimproved soils, led to any change in the degradation of peat-derived DOC. Conversely, mixing Peatland with Agricultural water led to apparently 'non-conservative' behaviour in terms of the balance of DOC degradation and production. To our knowledge, this is the first study to report the apparently interactive effects of solar radiation and mixing on DOC processing in freshwater. From the rates of $DO^{14}C$ loss, we infer that this was not the result of a change in photo-degradation of the peatland DOC (as % losses of $DO^{14}C$ in the mixed sample were similar to the 'Peatland only' treatment), but rather that more 'new' DOC was being produced *in situ*. As we observed this effect for the mixing experiment involving Agricultural and not Mountain stream samples, this suggests the higher nutrient content of the former is key to driving this process. These results imply that the DOC degraded but not completely mineralised by solar radiation could have fuelled autotrophic microbial growth in the samples (Wetzel et al. 1995), such that net DOC production occurred. It is also important to consider that solar degradation will degrade all components of dissolved organic matter, including organically-bound nitrogen (N) and phosphorous (P). This process will release inorganic N and P which (as the limiting nutrients for primary production in most aquatic ecosystems) could be driving autotrophic microbial growth (Vähätalo et al. 2003). It may also be that changes in pH or microbial communities led to greater in-stream DOC production following mixing. As the percentage loss of $DO^{14}C$ did not differ between the 'Peatland only' and 'Peatland + Agricultural' irradiated treatments, we did not find evidence that an increase in pH, which would be expected when water draining a peatland meets water draining agricultural land, would affect the continued photo-degradation of peatland-derived DOC down the fluvial system.

For the mixing of the Peatland and Estuary waters, the loss of DOC in the irradiated treatment was actually greater than if the two pools of DOC had behaved conservatively, and a high proportion (41%) of lost $DO^{14}C$ was converted to $PO^{14}C$. DOC can be removed from the water when fresh and saline waters mix due to flocculation of DOC to POC (Spencer et al. 2007; Asmala et al.

2014), but our results suggest that solar radiation aids this process. Helms et al. (2013) discussed the role of photochemical flocculation of terrestrially derived DOC in estuaries and their experimental work demonstrated a 7% conversion of DOC to POC in the presence of simulated solar radiation. The results of our study add weight to their conclusion that photochemically-induced flocculation may be an important DOC removal process in estuaries, and one that warrants more research.

Net DOC increases were recorded for all three mixtures incubated in the dark, in the order Estuary > Agricultural > Mountain. The 22% increase in DOC for the Peatland + Estuary mix strengthens our argument that photochemical flocculation must have played a role in the 18% decrease in DOC for the same treatment subject to irradiation. As discussed earlier, exposure of samples to a high dose of solar radiation may have increased microbial exudation of DOC, but it is also reasonable to assume that the absence of light would have had a similar effect by inducing senescence (Jack et al. 2002). The greater percentage increases in DOC for the Agricultural and Estuary samples may therefore simply reflect the greater abundance of autotrophic organisms in these samples and the decay of this living material to form DOC. However, this cannot be the only cause of the DOC increases, as there was insufficient initial POC (i.e. algae/phytoplankton biomass) in all three non-peatland samples to account for the magnitude of the DOC increases. For example, the DOC concentration increased by 2.85 mg L^{-1} in the 'Peatland + Agricultural' dark treatment, but the initial POC of the mixed sample was only 1.6 mg/L. Similarly, this senescence theory cannot explain why, particularly for the Agricultural mix, a large proportion of lost $DO^{14}C$ was recovered as $PO^{14}C$.

The role of stream bed biofilms

The inclusion of biofilms had a substantial effect on DOC processing. In both the 'Peatland + Mountain' and 'Peatland + Agricultural' experiments, removal of $DO^{14}C$ by biofilms in the dark was similar to the rate of $DO^{14}C$ removal in light-exposed samples without biofilms (Figure 5). This suggests that solar radiation and biofilms may 'compete' for peat-derived DOC in fluvial systems, and supports previous studies demonstrating biofilms may have a regulating influence on DOC transport through river systems (Freeman and Lock 1995). The combined solar radiation and biofilm treatments did not lead to an overall increase in $DO^{14}C$ removal in the 'Peatland + Mountain' mix, but resulted in around 50% more removal in the 'Peatland + Agricultural' mix. This suggests that biofilm activity may be nutrient-limited, and therefore that they are more able to utilise peat-derived DOC (either directly, or by utilising organic matter which has been partially broken down by photo-degradation) in the presence of elevated nutrients from agricultural runoff (Tank and Dodds 2003). It is worth noting that the amount of biofilm used in these experiments may represent upper

limits for field conditions, because biofilm surface area to water volume ratios were relatively high, and contact times relatively long. The eventual fate of peat DOC incorporated into biofilm organic matter is uncertain. DOC is released by biofilms and can be an important source of energy for heterotrophic activity (Ziegler and Lyon 2010). It is therefore unlikely to be subject to further photo-degradation but instead ultimately respired to CO_2 *in situ*. High discharge events prevent extensive build-up of biofilm in rivers and streams (Augspurger et al. 2008) and sloughed off biofilm transported down-river will be subject to biodegradation. Our expectation is therefore that most of this material will eventually be converted to CO_2, but further work would be needed to fully resolve this.

Use of the ^{14}C labelling technique

The conversion of DOC to CO_2 was demonstrated using ^{14}C labelling, and CO_2 production was higher in the treatments exposed to light compared to those incubated in the dark. However, the percentage of $^{14}CO_2$ captured in the NaOH traps was much smaller than the amount of $DO^{14}C$ lost in all cases. Since the amounts of ^{14}C recovered in POC and biofilm C were also usually fairly small, a significant proportion of the original ^{14}C label was not recovered. Although we cannot be certain as to the fate of this unrecovered tracer, we measured large increases in pH over the course of all experiments, which suggests an increase in bicarbonate concentrations, presumably following dissolution of CO_2 into the water. This was not reflected in the final DIC measurements, possibly because the CO_2 degassed during the analytical process, for example during sample filtration. If correct, this would imply that a much higher proportion of DOC was mineralised to CO_2, but that this was not successfully captured by the experiments. If we assume that all unrecovered $DO^{14}C$ did escape as CO_2, then our experiments would indicate that 50-80% of DOC is mineralised to CO_2 by photo-degradation.

Conclusions

This study emphasises the importance of photo-degradation in removing DOC in freshwaters, and through the use of ^{14}C labelling provides new information concerning transformation processes occurring naturally in rivers along the upland source to lowland estuary continuum. Photochemical breakdown depends primarily on the composition of the DOC, whilst biological breakdown within the water column and biofilm uptake on bed sediments appear to be constrained by low nutrient availability in peaty headwaters, and thus becomes more important as peat-derived DOC mixes with nutrient-enriched runoff from agricultural land. Biofilm uptake may nevertheless be constrained in

these downstream environments by short residence times and low sediment surface area to volume ratios in larger river channels (Battin et al. 2008). It is also likely that photochemical and biological processes interact, for example via the creation of bioavailable organic compounds during photolysis. Improved quantification of these interacting processes should lead to greater understanding of the processes that control carbon cycling in aquatic systems, their biological impact, and the contribution of inland waters to global CO_2 emissions.

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Table 1. Locations and summary chemical characteristics (minimum and maximum, with mean in parentheses) of the four contrasting natural waters used for irradiation experiments. Values of pH, DOC, POC and nutrient concentrations were collated from all data collected at these sites through the course of this project. There is only one measurement of POC for sites B, C and D as only one set of samples was used for Experiment B.

Site type	**Latitude, Longitude**	**Altitude** (m a.s.l.)	**pH**	**DOC** (mg L^{-1})	**POC** (mg L-1)	**Nutrient concentration** (mg L^{-1})
Peatland	52.991, -3.802	380-460	4.5-6.9 (5.5)	4.47-24.84 (13.45)	0.15-2.40 (1.45)	NO_3: 0.04-0.28 (0.13) PO_4: <0.01
Mountain	53.007, -3.804	340-400	5.1-5.8 (5.5)	1.47-9.45 (5.57)	0.15	NO_3: 0.02-0.42 (0.24) PO_4: <0.01
Agricultural	53.171, -3.798	80-220	7.6-7.7 (7.6)	2.28-5.33 (4.01)	1.40	NO_3: 5.92-7.63 (6.91) PO_4: 0.03-0.04 (0.03)
Estuary	53.294, -3.836	0	7.9-8.1 (8.0)	1.36-4.26 (2.80)	9.60	NO_3: 0.02-0.28 (0.10) PO_4: <0.02

Table 2. Mean percentage recovery of lost $DO^{14}C$ into different pools of C following exposure of the '$DO^{14}C$/Peatland + Mountain' solutions to a 28 MJ m^{-2} dose of simulated solar radiation. Values in parentheses show the range across the four replicates.

		Treatment			
		Peatland	Peatland + Mountain	Peatland + Mountain (DARK)	Peatland + Mountain (Conservative Mix)
Without biofilms	$^{14}CO_2$	7.6 (3.0-11.7)	12.9 (6.8-18.1)	3.8 (0.2-8.1)	7.6
	$PO^{14}C$	7.1 (6.0-9.1)	5.6 (5.1-7.2)	15.2 (12.4-19.8)	7.1
	Biofilm ^{14}C	N/A			
	Unaccounted	*85.3*	*81.2*	*81.0*	*85.3*
With biofilms	CO_2	7.7 (2.7-9.5)	8.3 (4.7-12.3)	5.0 (2.5-9.7)	7.7
	$PO^{14}C$	9.6 (7.4-11.8)	9.7 (8.1-10.8)	10.9 (5.7-15.6)	9.6
	Biofilm ^{14}C	5.8 (4.5-7.0)	8.1 (7.9-8.2)	9.4 (6.6-12.0)	5.8
	Unaccounted	*77.0*	*74.0*	*74.6*	*77.0*

Table 3. Mean percentage recovery of lost $DO^{14}C$ into different pools of C following exposure of the '$DO^{14}C$/Peatland + Agricultural' solutions to a 28 MJ m^{-2} dose of simulated solar radiation. Values in parentheses show the range across the four replicates.

		Treatment			
		Peatland	Peatland + Agricultural	Peatland + Agricultural (DARK)	Peatland + Agricultural (Conservative Mix)
Without biofilms	$^{14}CO_2$	7.3 (2.1-11.5)	3.7 (2.7-5.2)	4.8 (3.0-7.6)	7.3
	$PO^{14}C$	8.2 (6.8-9.7)	10.0 (7.5-12.7)	34.0 (24.5-43.7)	8.2
	Biofilm ^{14}C	N/A			
	Unaccounted	*84.5*	*86.3*	*61.2*	*84.5*
With biofilms	CO_2	9.2 (3.6-15.6)	6.8 (2.7-9.9)	5.5 (3.4-9.4)	9.2
	$PO^{14}C$	12.4 (3.9-19.1)	12.7 (10.3-16.2)	12.2 (9.5-14.7)	12.4
	Biofilm ^{14}C	4.3 (2.5-5.3)	5.4 (5.1-6.3)	3.8 (3.3-4.1)	4.3
	Unaccounted	*74.1*	*75.1*	*78.6*	*74.1*

Table 4. Mean percentage recovery of lost $DO^{14}C$ into different pools of C following exposure of the '$DO^{14}C$/Peatland + Estuary' solutions to a 28 MJ m^{-2} dose of simulated solar radiation. Values in parentheses show the range across the four replicates.

		Treatment			
		Peatland	Peatland + Estuary	Peatland + Estuary (DARK)	Peatland + Estuary (Conservative Mix)
Without biofilms	$^{14}CO_2$	9.3 (6.0-13.8)	9.5 (9.1-10.0)	11.9 (6.6-25.4)	9.3
	$PO^{14}C$	9.9 (4.2-20.3)	40.8 (27.3-54.6)	13.2 (13.1-13.4)	9.9
	Biofilm ^{14}C	N/A			
	Unaccounted	*80.8*	*49.8*	*81.5*	*80.8*

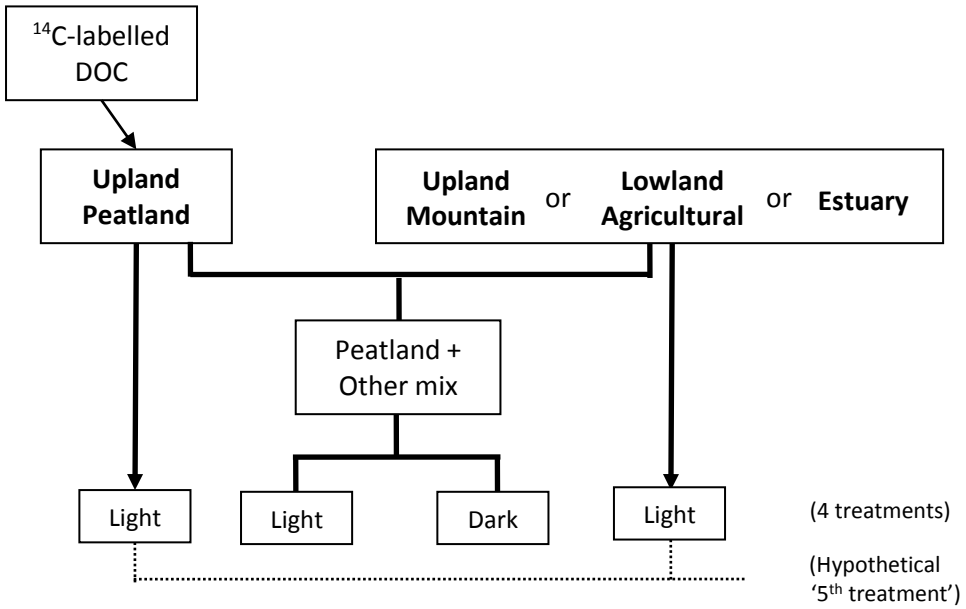

Figure 1. Schematic outline of the design of Experiment B. This experiment had four treatments and was run with and without the presence of biofilm for the Peatland + Mountain and Peatland + Agricultural experiments. The Peatland + Estuary experiment was run without biofilm only. Therefore five separate experiments were run in total.

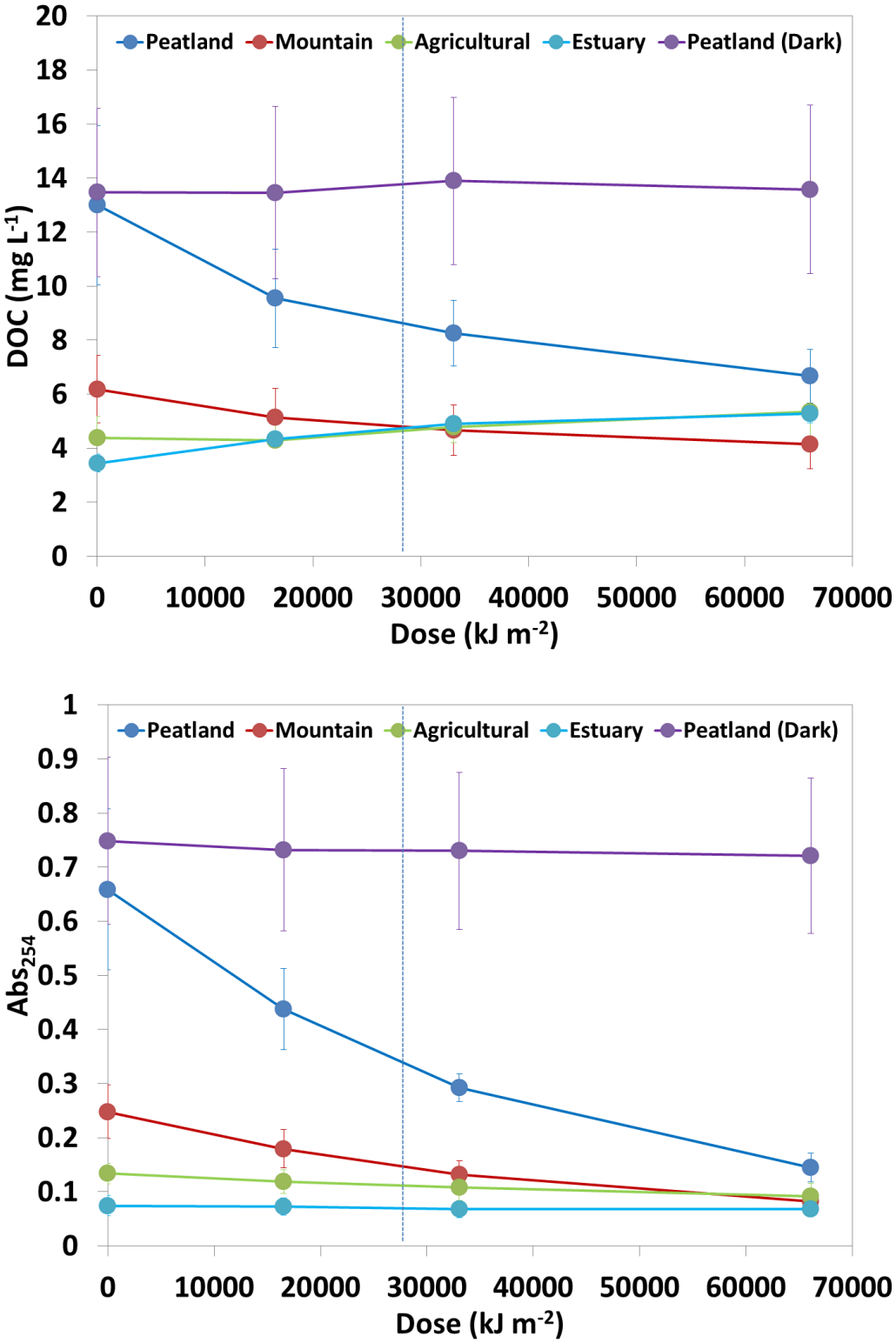

Figure 2. Mean a) DOC concentration (top) and b) 254 nm absorbance (bottom) of five sets of samples from the four source water types exposed to a cumulative light dose of 66 MJ m^{-2}.

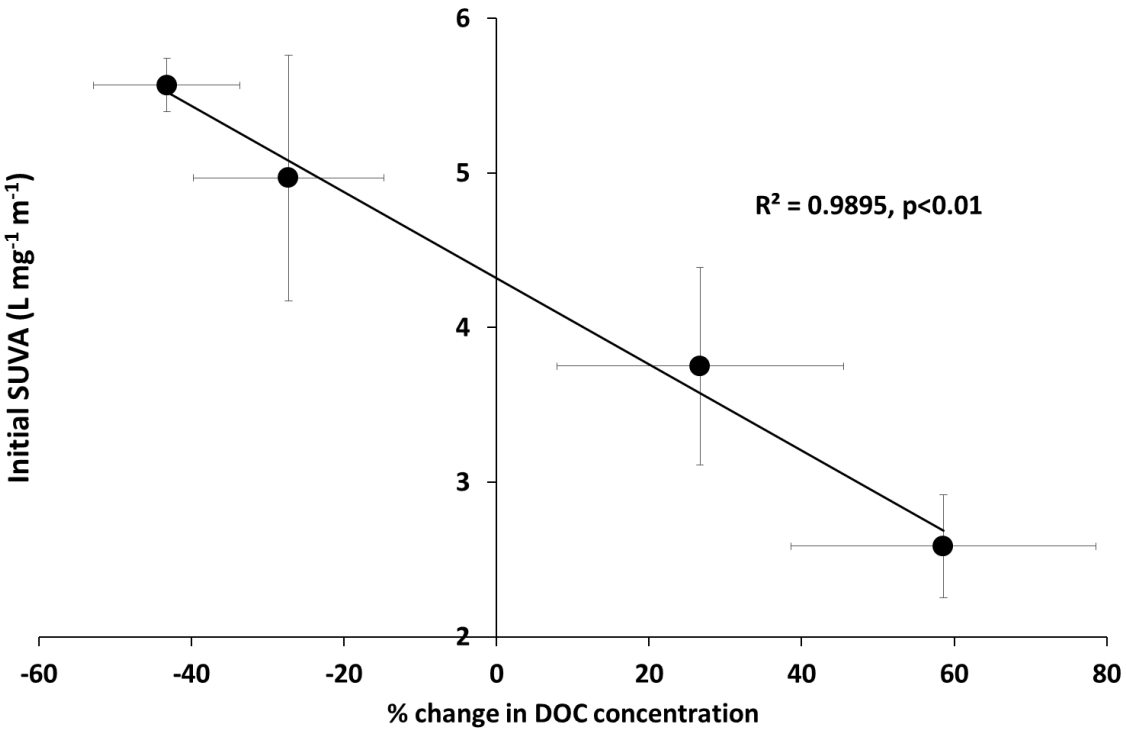

Figure 3. Mean initial SUVA vs mean % change in DOC of five sets of samples from the four source water types exposed to a cumulative light dose of 66 MJ m^{-2}

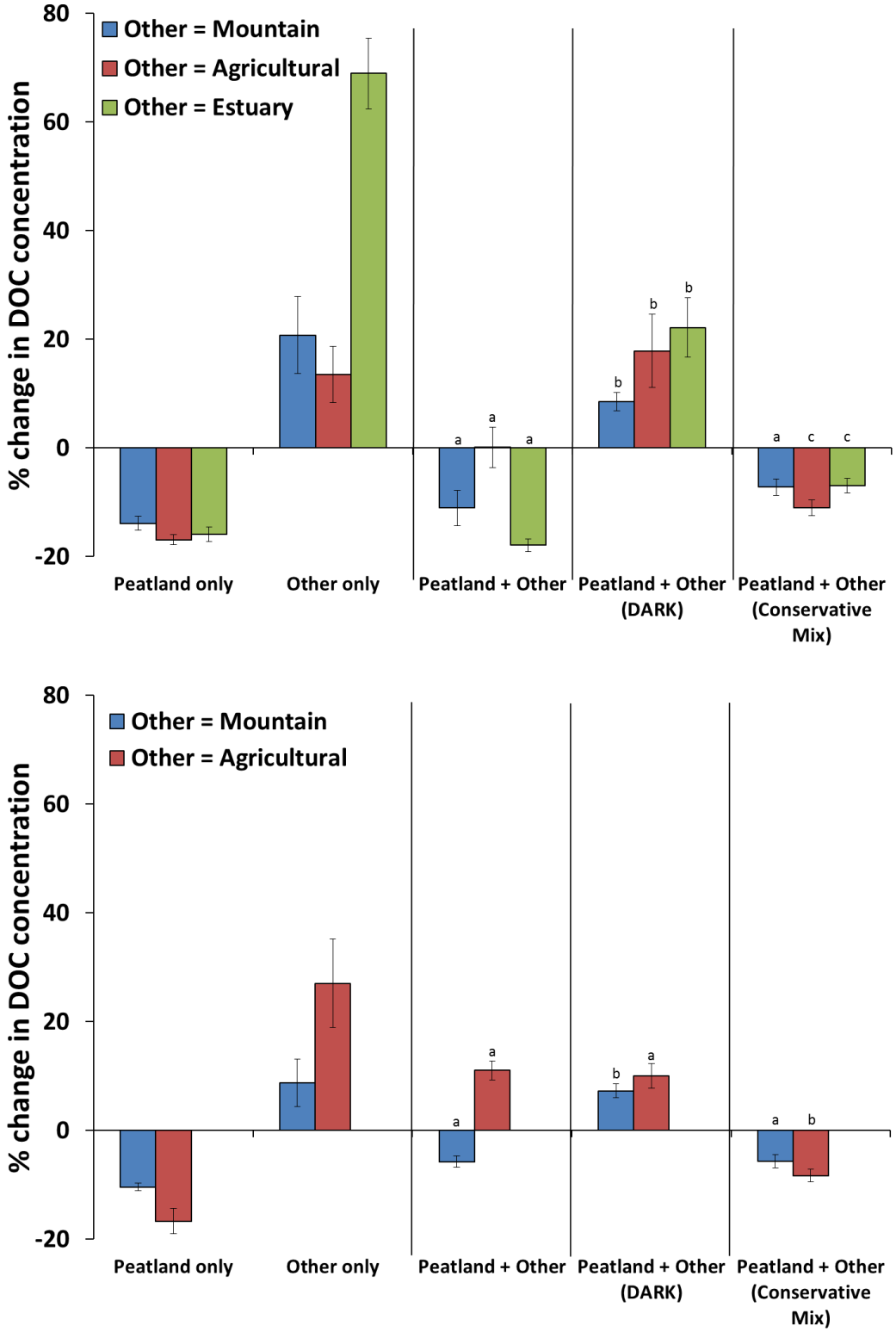

Figure 4. Percentage change in DOC concentration a) without biofilm (top) and b) with biofilm (bottom), for each treatment and for each mixing experiment for Experiment B, after exposure to a 28 MJ m^{-2} dose of simulated solar radiation.

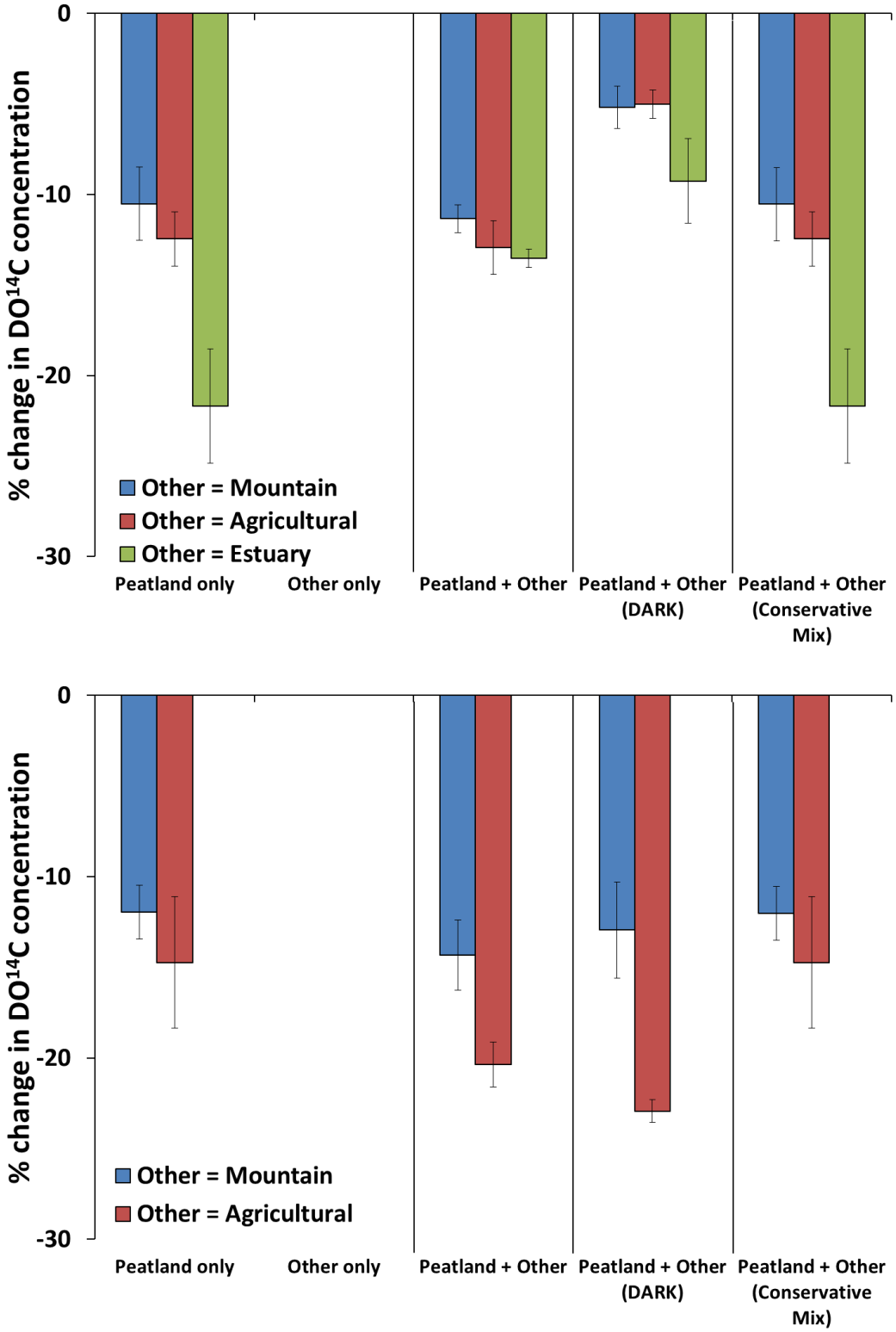

Figure 5. Percentage change in $DO^{14}C$ concentration a) without biofilm (top) and b) with biofilm (bottom), for each treatment and for each mixing experiment for Experiment B, after exposure to a 28 MJ m^{-2} dose of simulated solar radiation.

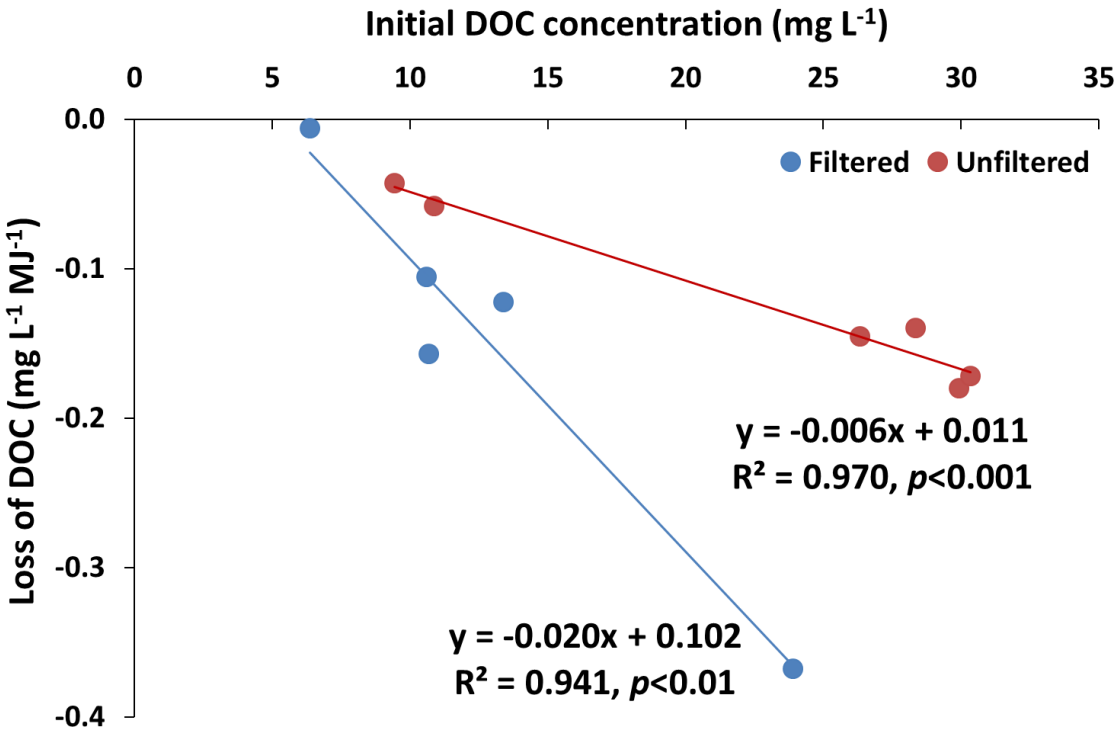

Figure 6. Loss of DOC against initial DOC concentration for unfiltered (Peatland and Mountain) and filtered (Peatland only) samples.