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Investigations of freezing and cold storage for the analysis of peatland dissolved organic carbon (DOC) and absorbance properties

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Although measured rates of biological degradation of DOC are typically low under dark conditions, it is assumed that water samples must be analysed soon after collection to provide an accurate measure of DOC concentration and UV-visible absorbance. To examine the impact of storage on DOC quality and quantity, we took water samples from an ombrotrophic peatland, and stored them in the dark at 4 °C for 138–1082 days. A median of 29% of DOC was lost during storage, but losses of absorbance at 254 nm were less. DOC loss followed a first-order exponential decay function, and was dependent on storage time. DOC half-life was calculated as 1253 days. Specific absorbance at 254 nm suggested that samples containing more aromatic DOC were more resistant to degradation, although time functioned as the primary control. Samples from two fens showed that loss of absorbance was greater at 400 nm rather than 254 nm, after 192 days storage, suggesting that non-aromatic DOC is preferentially degraded. These results suggest that samples can be stored for several months before losses of DOC become detectable, and that it is possible to back-calculate initial DOC concentrations in long-term stored samples based on known decay rates. Freeze/thaw experiments using samples from a range of peatlands suggested that DOC concentration was mostly unaffected by the process, but DOC increased 37% in one sample. Freezing had unpredictable and sometimes strong effects on absorbance, SUVA and E ratios, therefore freezing is not recommended as a method of preservation for these analyses.

Environmental impact

Dissolved organic carbon (DOC) concentrations are routinely monitored in the waters of organic soils, particularly peatlands. This is because DOC affects the functioning of aquatic ecosystems and water treatment costs, as well as being a component of the carbon cycle. If there is to be a delay between collecting a water sample and analysing, it is typical to preserve the sample by acidification or freezing. Here, we demonstrate that that loss of DOC in untreated filtered samples is dependent primarily on storage time, with ~5% being lost in 3 months. Freezing samples had little effect on DOC concentration, but appeared to strongly influence the composition of DOC. We therefore do not recommend freezing as a preservation technique.

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1. Introduction

Dissolved organic carbon (DOC) is an important fluvial component of the global carbon cycle. DOC is converted to carbon dioxide (CO_2) during transport through rivers, lakes and the ocean, thereby providing a source of atmospheric carbon.[1] Additionally, DOC forms complexes with toxic metals[2] and can affect autotrophic and heterotrophic processes in aquatic ecosystems.[3,4] High DOC concentrations also result in increased water treatment costs[5,6] and human health risks from potable water due to the formation of harmful trihalomethanes when chlorinated.[7] Wetlands, and particularly peatlands, are a major source of DOC,[8] and there is a strong relationship between peat cover and DOC concentration for both lowland and upland catchments.[9] Peatland DOC dynamics are therefore well-studied in relation to various environmental factors and disturbances.

Considering the large volume of research concerning peatland DOC, it is notable that there is only partial guidance concerning sampling and analysis protocols, which limits the production of standardised results. One consensus is that DOC in a water sample is defined as the organic carbon that passes through a 0.45 μm filter,[10] although it should be noted that other filter sizes are sometimes used.[11,12] The Disinfectant/Disinfection By-products Rule (D/DBPR) of the US Environmental Protection Agency requires that water samples for DOC analysis must be analysed within 48 hours; if not, then

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active steps (*e.g.* sample acidification) must be taken to preserve the sample[11] in an attempt to limit biological, chemical, or physical degradation. It is common for peatland research projects to analyse DOC concentrations as soon as possible; *e.g.* within one day,[13] two days,[14] or one week,[15] even though it is often asserted that biological degradation is minimal.[16] Despite this, the stability of peatland DOC under long-term storage is not well quantified. In contrast, the stability of marine DOC during storage has been studied: Norrman[17] noted no change in DOC following cold storage for eight weeks, whilst Yoshimura[18] found that DOC stability in samples stored for up to four hundred days at room temperature depended somewhat upon the bottle type that the sample was stored in. Preservation techniques such as acidification have also been examined for marine DOC samples.[19] It should be noted that marine DOC concentrations are typically in the range 30–80 μM (ref. 20) and are therefore small compared to peatland DOC concentrations. There are also compositional differences in DOC from marine and terrestrial environments.[21]

UV-visible (UV-vis) absorbance is often used as a surrogate for direct DOC measurement.[22] Like DOC concentrations, the D/DBPR states that UV-vis absorbance must be measured within 48 hours,[11] and it is often measured as soon as possible after sampling in peatland experiments; *e.g.* within one day.[23] It has been shown that absorbance in water samples from an ombrotrophic peatland stored for twelve weeks in the dark at 4 °C showed no change across the UV-vis spectrum,[24] suggesting that DOC concentrations might also have remained unchanged after storage. Others have examined the stability of water samples following storage to check that delayed analysis will not affect the accuracy of results. For example, Ekström *et al.*[25] noted no significant change in DOC concentration in pore waters from a podzol following storage in the dark at 4 °C for two weeks. Similarly, Carter *et al.*[26] recorded only a 5% change in DOC concentration in samples from a variety of surface water sources (including peatland catchments) after 50–120 days storage at 5 °C. Proctor[27] states that surface water samples from an ombrotrophic bog showed a slight decrease in absorbance at 320 nm after two months of storage, but provides no raw data to consider.

Taken together, these observations suggest that DOC concentrations in samples stored in the dark at 4 °C are stable over extended periods. If true, this could have implications for sampling programmes and analysis techniques. For instance, it would negate the need for freezing or acidifying water samples that sometimes takes place,[28–32] and which risk affecting DOC concentrations in marine and freshwater samples,[19,33–35] as well as absorbance and fluorescence properties.[36,37] Changes in pH can cause the flocculation and coagulation of DOC,[38] whilst freezing can result in DOC loss through abiotic particle precipitation.[35] Preservatives such as sodium azide are also sometimes used, although DOC losses can still occur when such biocides are used.[34] To address this issue we used samples from UK peatlands to investigate how DOC concentrations change during storage at 4 °C and when frozen.

2. Materials and methods

2.1. Bog peat cold storage experiment

Samples of surface and pore water were taken from the Afon Ddu catchment on the Migneint blanket bog in Snowdonia National Park, Wales, UK (latitude 52.97°N, longitude 3.84°W). The site is approximately 490 m above sea level with a mean peat depth of 1.3 m (M. Peacock, unpublished data). Vegetation consists of *Calluna vulgaris* with *Eriophorum* and *Sphagnum* species. The site has been extensively drained by a series of ditches that run downslope. These ditches were blocked in February 2011, with four being left unblocked as controls. The samples for this experiment were collected between 16th December 2010 and 17th July 2013 as part of a project investigating the effect of ditch blocking on water chemistry. Samples were collected in 60 ml Nalgene® bottles from: (i) blocked and open ditches (20 samples), (ii) piezometers at 10 cm depth, adjacent to ditches (18 samples), and (iii) crest-stage tubes adjacent to ditches that collect overland flow (12 samples). For this experiment, samples were selected to include a range of DOC concentrations (minimum = 8.9 mg l^{-1}, maximum = 64 mg l^{-1}, mean = 33.9 mg l^{-1}) and sampling months (Dec–Feb = 10 samples, March–May = 12 samples, June–Aug = 13 samples, Sept–Nov = 15 samples). The long-term nature of the project therefore ensured that samples used in this experiment were collected across a range of environmental conditions (*i.e.* after storms or droughts, across a range of temperatures).

Electrical conductivity (EC) and pH were measured on unfiltered samples. Samples were then filtered through 0.45 μm filters and analysed for DOC (as non-purgeable organic carbon NPOC) using a Thermalox Total Carbon analyser (Analytical Sciences, Cambridge, UK). In NPOC analysis, the sample is acidified and sparged with oxygen to remove any inorganic carbon. To ensure that the 0.45 μm filters were not leaching DOC into samples, unfiltered and filtered standards and blanks were analysed; no difference was found, showing that filters were not contaminating samples. UV-vis absorbance was measured using an M2e Spectramax (Molecular Devices, Sunnyvale, USA) at wavelengths of 250 nm, 254 nm, 365 nm, 400 nm, 465 nm and 665 nm. These allowed DOC quality data to be calculated as E2 : E3 ratio (250 : 365 nm), E2 : E4 ratio (250 : 400 nm), E4 : E6 ratio (465 : 665 nm), and specific UV absorbance (SUVA, at 254 nm). The concentration of phenolic compounds was measured in some samples using a method modified from Box;[39] 0.25 ml of sample was added to a microplate well, followed by 12.5 μl of Folin-Ciocalteau reagent and 37.5 μl of 200 g l^{-1} Na$_2$CO$_3$. After 1.5 hours absorbance was measured at 750 nm and phenolic concentrations calculated from a standard curve. After all analyses had been completed, samples were stored in the dark at 4 °C. Following long-term storage (range 138–1082 days, mean = 719 days) the fifty samples were reanalysed on 2nd December 2013. The range of storage times is due to the fact that samples were originally collected as part of a long-term environmental monitoring project, and thus on a range of dates prior to the

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reanalysis. For this study, a selection of samples was chosen for reanalysis. For the reanalysis, DOC was measured as NPOC using a TOC analyser (Shimadzu) and absorbance at 254 nm was measured using a Helios Gamma (Thermo Spectronic).

2.2. Fen peat cold storage experiment

Surface water samples were taken on 18th March 2014 from two fen peatlands: Holme Fen (52.49°N, 0.23°W) and Woodwalton Fen (52.45°N, 0.19°W), both in East Anglia, UK. Holme Fen consists of deciduous woodland (dominated by *Betula pendula*), fen, and remnant raised bog. The area surrounding the fen has been historically drained for agricultural purposes, leading to peat subsidence. As such, Holme Fen is now 2.7 m below sea level, and maintained by active drainage of the surrounding agricultural land. Woodwalton Fen contains a mix of deciduous woodland, open water, and *Phragmites* reedbeds. It is 0 m ASL. At each site, five samples were collected from open water (ditches or lakes) in 60 ml Nalgene® bottles. Electrical conductivity (EC) and pH were measured on unfiltered samples. Samples were then filtered through 0.45 μm filters and analysed for DOC (as NPOC) using a TOC analyser (Shimadzu), and absorbance at 254 nm and 400 nm was measured using a Helios Gamma (Thermo Spectronic). After analysis, samples were stored in the dark at 4 °C for 192 days, before being reanalysed for DOC and absorbance.

2.3. Freeze/thaw experiment

Surface water samples were taken from six different locations. These were:

(1) Bog pools (both natural and those created during restoration), an upland stream, and overland flow in the Afon Ddu catchment (see Section 2.1) ($n = 11$).

(2) Ditches and lake at Holme Fen (see Section 2.2) ($n = 5$).

(3) Ditches on Woodwalton Fen (see Section 2.2) ($n = 5$).

(4) Walton Lake (52.03°N, 0.72°W), a small, shallow lake dominated by *Phragmites australis* reedbed, situated near to the Open University in Milton Keynes ($n = 4$).

(5) Ditches on Sedge Fen (52.31°N, 0.28°E), a relatively intact, deep peat lowland fen, and part of the wider Wicken Fen area. The fen contains *Phragmites australis* and *Cladium mariscus* reedbeds ($n = 8$).

(6) Ditches on Baker's Fen (52.30°N, 0.29°E), also a part of the Wicken Fen area. Baker's Fen was historically drained and converted to agricultural use. For the past two decades the fen has been undergoing restoration through hydrological management and grazing with cattle and horses ($n = 8$).

EC and pH were analysed on unfiltered samples, then samples were filtered at 0.45 μm. Samples were analysed for DOC and absorbance at 250, 254, 365, 400, 465 and 665 nm using the same methods as Section 2.2. This allowed E2 : E3, E2 : E4, E4 : E6 and SUVA to be calculated as in Section 2.1. Samples were then frozen in the dark for a minimum of 48 hours. Following this, samples were transferred to a refrigerator and allowed to thaw, and DOC and UV-vis analyses were repeated.

2.4. Statistical analysis

Statistical analysis was carried out using SPSS v16.0.1 (IBM Corporation). The Shapiro–Wilk test was used to check whether data were normally distributed. For the bog peat experiment, neither DOC concentration, absorbance at 254 nm, nor SUVA were normally distributed. Although it was possible to normalise DOC concentration and absorbance at 254 nm using square root transformation, SUVA data could not be normalised. For the fen peat experiment, DOC and absorbance were not normally distributed, and transformations failed to normalise the data. Because of this, the non-parametric Wilcoxon signed rank test was used to test for differences in samples before and after storage for all determinands, and for both bog, fen and freeze/thaw samples, thus providing statistical consistency throughout.

Linear regression was used to search for relationships between determinands, and a model developed to predict changes in DOC during cold storage based solely on original DOC concentration and storage time. To create the model for bog samples, a random selection of 23 data points were selected. DOC concentration was predicted as:

$$\text{DOC}_{\text{storage(modelled)}} = \text{DOC}_{\text{original(measured)}} \times 0.5^{(\text{storage time}/t^{1/2})}$$

where 'DOC$_{\text{storage(modelled)}}$' is the predicted concentration of DOC after storage, 'DOC$_{\text{original(measured)}}$' is the measured concentration of DOC before storage, 'storage time' is the number of days a sample is stored for, and '$t^{1/2}$' is the number of days it takes for DOC concentration to reduce by half (half-life). $t^{1/2}$ was calculated in Microsoft Excel using the Goal Seek function to minimise the root mean square error (RMSE) of the model, followed by manual calibration to lower RMSE and obtain a slope equation closest to a 1 : 1 line between measured and modelled DOC.

This model was then tested on the remaining 24 samples by rearranging the above equation so that the original DOC could be predicted from DOC measured after storage. Three samples where DOC apparently increased during storage were excluded. This may have been analytical error, or it may be that not all particulate organic carbon (POC) was removed during filtration; this POC could then have been converted to DOC through biotic processes.[40] Rearranging the equation gives:

$$\text{DOC}_{\text{original(modelled)}} = \text{DOC}_{\text{storage(measured)}} \times 2^{(\text{storage time}/t^{1/2})}$$

where 'DOC$_{\text{original(modelled)}}$' is the predicted concentration of DOC before storage, 'DOC$_{\text{storage(measured)}}$' is the measured concentration of DOC after storage, 'storage time' is the number of days a sample is stored for, and '$t^{1/2}$' is the number of days it takes for DOC concentration to reduce by half. The results of this equation were tested using the Nash–Sutcliffe efficiency,[41] calculated as:

$$\text{NS} = 1 - \frac{\sum \left(\text{DOC}_{\text{measured}} - \text{DOC}_{\text{modelled}} \right)^2}{\sum \left(\text{DOC}_{\text{measured}} - \text{DOC}_{\text{mean}} \right)^2}$$

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where 'DOC$_{measured}$' is the measured concentration of DOC before storage, 'DOC$_{modelled}$' is the concentration of DOC before storage predicted by the model, and 'DOC$_{mean}$' is the mean of the DOC measurements before storage. A NS value of 1 indicates a perfect model fit, and as the NS value decreases this indicates a poorer model fit, with a value of 0 implying that the model performs no better than a simple mean of the data.

3. Results

3.1. Bog peat experiment

The median loss of DOC during storage for all 50 samples was 9.0 mg l^{-1}, equating to 28.9%. The change in DOC concentration was significant ($p < 0.001$) (Fig. 1). Three samples showed increases in DOC concentration during storage. There was little obvious difference in pore water and surface water; median loss rates of DOC were 0.039% per day for surface water and 0.042% per day for pore water (with respective median storage times of 749 and 807 days).

There was no linear relationship between the percentage loss of DOC during storage and the length of time a sample had been stored (linear regression $R^2 = 0.0004$, $n = 50$). However, a significant relationship was found by normalising DOC loss by storage time (Fig. 2). This relationship suggests that DOC loss is relatively rapid to begin with, but then slows over time. A second significant relationship was determined between normalised DOC loss and SUVA (Fig. 3), if three samples that had been stored for a much shorter time compared to the other samples (138 days *versus* >350 days for the remainder of the dataset, see 4.1 for Discussion) were removed. If both storage time (log 10 transformed) and SUVA were used in a multiple regression against DOC loss there was some improvement. This regression used 34 data points (UV-vis analysis was not conducted for all samples), and involved the exclusion of the three aforementioned samples. For this reduced data set, R^2 between time and normalised DOC loss was 0.55 ($p < 0.001$) (DOC loss = $(-0.11x$ log time$) + 0.36$). With the inclusion of SUVA R^2
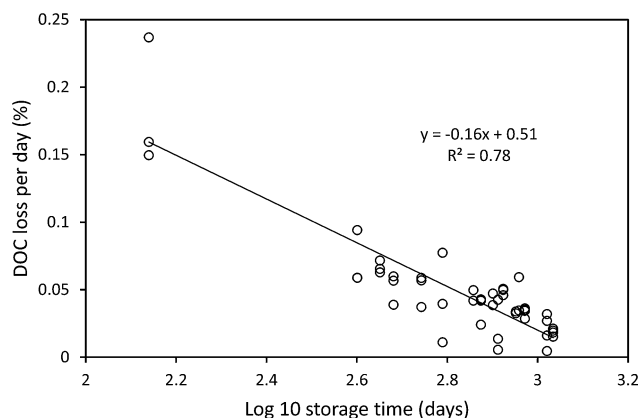

Fig. 2   Linear regression between percentage DOC loss per day during long term storage, and storage time transformed by log 10. $n = 47$ as three samples where DOC increased during storage were removed from the analysis. The relationship is significant ($p < 0.001$).

increased to 0.62 (time $p < 0.001$, SUVA $p = 0.01$) (DOC loss = $(-0.078x$ log time$) + (-0.013 \times$ SUVA$) + 0.32$). No significant relationships were found between normalised DOC loss and the E4 : E6 ratio, E2 : E3 ratio, or E2 : E4 ratio ($n = 37$). A significant negative relationship was observed between the phenolic : DOC ratio and normalised loss of DOC ($n = 23$, $R^2 = 0.4$, $p = 0.001$) (DOC loss = $(-0.025x$ phenolic : DOC$) + 0.0078$).

The calculated value of $t^{1/2}$ was 1253 days, which was used to develop a model of DOC concentrations following storage (Fig. 4). This $t^{1/2}$ value was then used to calculate DOC concentrations before storage, simply using storage time and DOC concentration after storage. This produced a strong model fit between measured and modelled DOC concentrations, as indicated by a high Nash–Sutcliffe efficiency, although there was some slight overestimation (Fig. 4).

The mean loss of absorbance at 254 nm during storage was 5.6% (Fig. 5), and this was significant ($p < 0.001$). Only one sample showed a substantial increase in absorbance after storage. For absorbance, a simple linear relationship between the change in absorbance and the length of time a sample had
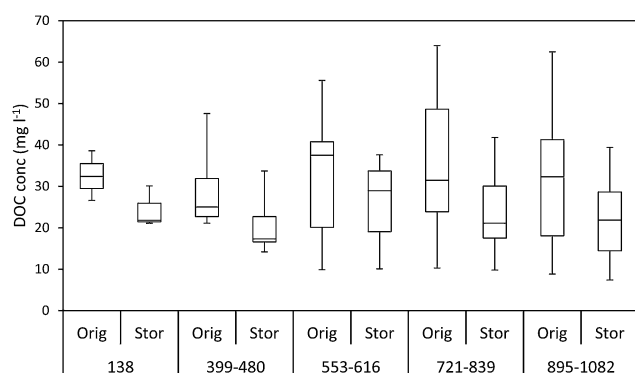

Fig. 1   Median DOC concentrations for bog samples measured before ("orig") and after storage ("stor"), and grouped according to storage time: 138 days ($n = 3$), 399–480 days ($n = 9$), 553–616 days ($n = 8$), 721–839 days ($n = 14$), 895–1082 days ($n = 16$). Limits of boxes represent first and third quartile, whilst the line within the box is the median. Whiskers represent minimum and maximum concentrations.
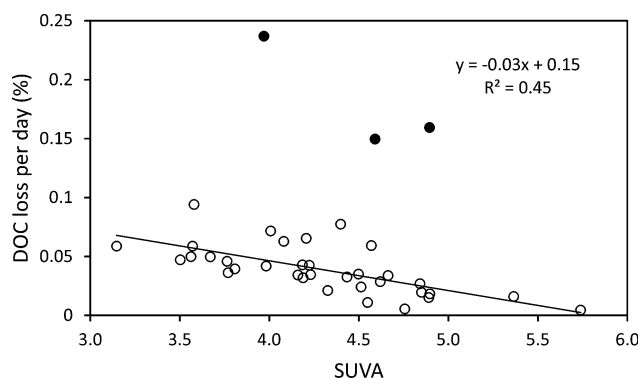

Fig. 3   Relationship between SUVA and normalised loss of DOC concentration. $n = 37$ (UV-vis analysis was not conducted on all samples). $p < 0.001$. Solid data points indicate three samples stored for 138 days that were not used to fit the regression line.
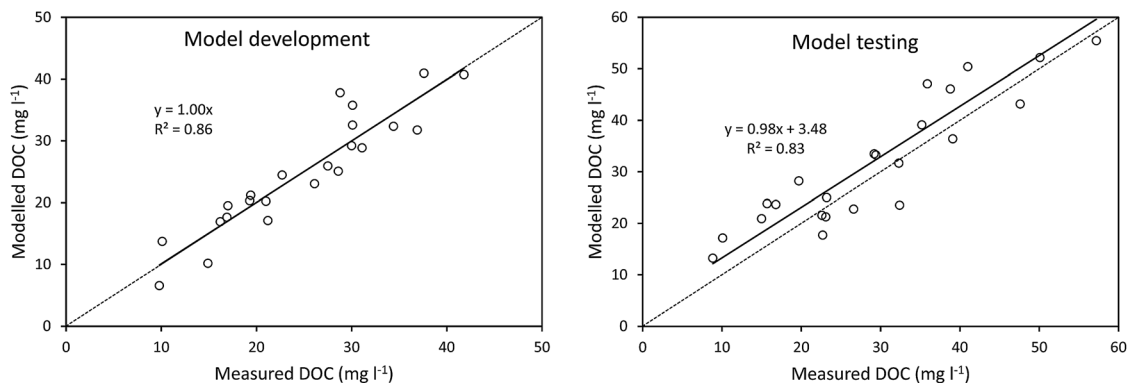
Fig. 4 Linear regressions between modelled and measured DOC concentrations following long-term storage. For model development $n = 23$, $p < 0.001$, RMSE = 3.43. For model testing $n = 24$, $p < 0.001$, RMSE 6.47, NS = 0.76. For both figures, dashed line is the 1 : 1 relationship.
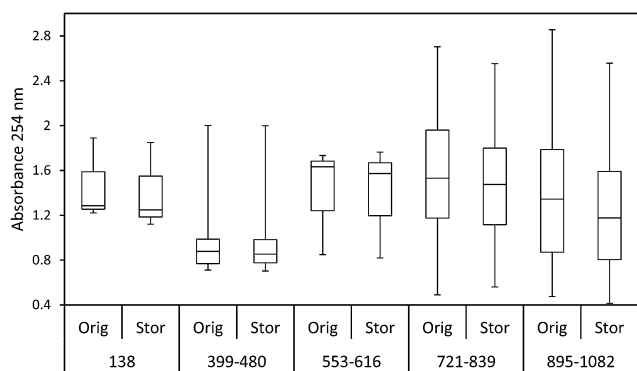

Fig. 5 Median absorbance at 254 nm values for bog samples measured before ("orig") and after storage ("stor"), and grouped according to storage time: 138 days ($n = 3$), 399–480 days ($n = 6$), 553–616 days ($n = 3$), 721–839 days ($n = 10$), 895–1082 days ($n = 16$). Limits of boxes represent first and third quartile, whilst the line within the box is the median. Whiskers represent minimum and maximum concentrations.
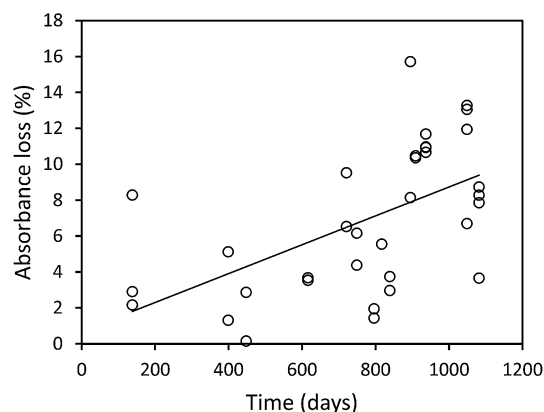

Fig. 6 Relationship between percentage absorbance loss at 254 nm and the amount of time samples had been stored for. $n = 34$ (four samples where absorbance increased were removed), $R^2 = 0.31$, $p < 0.001$, $y = 0.01x + 0.69$.

been stored for was found (Fig. 6). There was also a significant difference in SUVA following long-term storage; median value before storage was 4.36, increasing to 5.61 after storage ($p < 0.001$) (Fig. 7).

3.2. Fen peat experiment

The ten samples collected for this experiment were mainly alkaline, although two from Holme Fen were slightly acidic. Following 192 days of storage all samples showed a loss of DOC, with a median loss of 7% being observed, (Fig. 8) and a loss of absorbance at 400 nm, where a median loss of 16.8% was observed (Fig. 8). Nine samples displayed decreases in absorbance at 254 nm, and one sample showed a slight increase, with a median loss of 3.5% (Fig. 8). Wilcoxon signed rank tests showed that these losses were significant ($p < 0.01$ for DOC, 254 nm and 400 nm). When expressed as percentages, loss of absorbance at 400 nm was significantly greater ($p < 0.05$) than loss of absorbance at 254 nm. Following storage, six samples displayed increases in SUVA and four displayed decreases (Fig. 8).

3.3. Freeze/thaw experiment

After the freeze/thaw procedure, 13 samples showed a decrease in DOC concentration, whilst 28 showed an increase. Typically these changes were small (Fig. 9), with the median and maximum loss of DOC being 2.4% and 7.2%. The median gain of DOC was 4.4%, but one sample from the Afon Ddu increased from 3.46 mg l^{-1} to 4.75 mg l^{-1}; an increase of 37%. Nevertheless, the change was significant ($p < 0.05$).

Changes in UV-vis absorbance were evident after freeze/thaw. For 250 nm and 254 nm there was an even split, with approximately half of the samples showing increases in absorbance, and half showing decreases. From 365 nm upwards more samples displayed increases in absorbance, and this became much more pronounced in the higher wavelengths; at 465 nm and 665 nm, only 4 and 5 samples respectively showed decreases. Wilcoxon signed rank tests confirmed that these differences were significant for 465 nm and 665 nm only ($p < 0.001$). The magnitude of relative changes in absorbance also increased with increasing wavelength, so that the median increase in absorbance at 665 nm was 100% (Table 1).
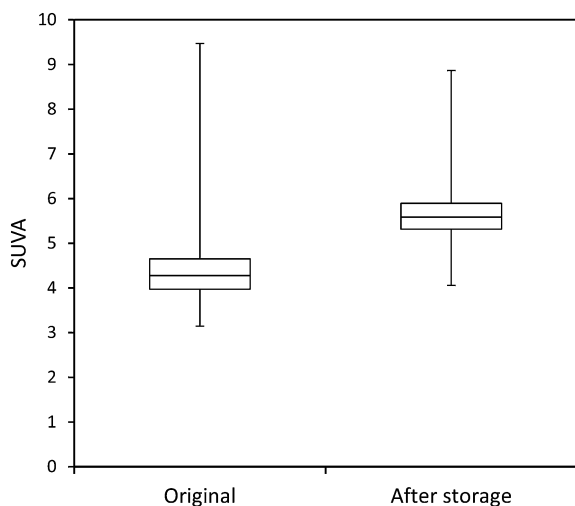
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Fig. 7 Median SUVA values for bog samples ($n = 38$), measured before and after storage. Limits of boxes represent first and third quartile, whilst the line within the box is the median. Whiskers represent minimum and maximum concentrations.

Despite the absence of a significant effect in the lower wavelengths across all samples, some drastic changes were observed. The same sample from the Afon Ddu bog that showed

a 37% increase in DOC also showed large increases in absorbance at all wavelengths: 99% at 250 nm, 102% at 254 nm, 233% at 365 nm, 283% at 400 nm, 550% at 465 nm, and 300% at 665 nm. Absorbance increased considerably across all wavelengths in three samples from Walton Lake: this was 40–57% at 254 nm. The same three samples had no absorbance at 665 nm upon collection, but after freeze/thaw had absorbance values of 0.018, 0.006 and 0.012. The various changes in absorbance caused significant ($p < 0.05$) changes in SUVA, E2 : E3, E2 : E4 and E4 : E6 (Fig. 10). Taken together, these findings suggest that the processes of freezing and thawing drastically alters the composition of DOC.

4. Discussion

4.1. Changes in DOC and absorbance during cold storage

Our results clearly show that DOC concentrations in both surface water and pore water samples taken from peatlands declined during short-term and long-term storage (138–1082 days). This storage was in the dark at 4 °C, following filtration at 0.45 μm. Kothawala *et al.*[42] (2012) stored water samples from Boreal lakes in the dark at 20 °C for three and a half years and recorded losses of DOC as 32–45%. Samples in our study that had been stored for approximately three years showed a mean
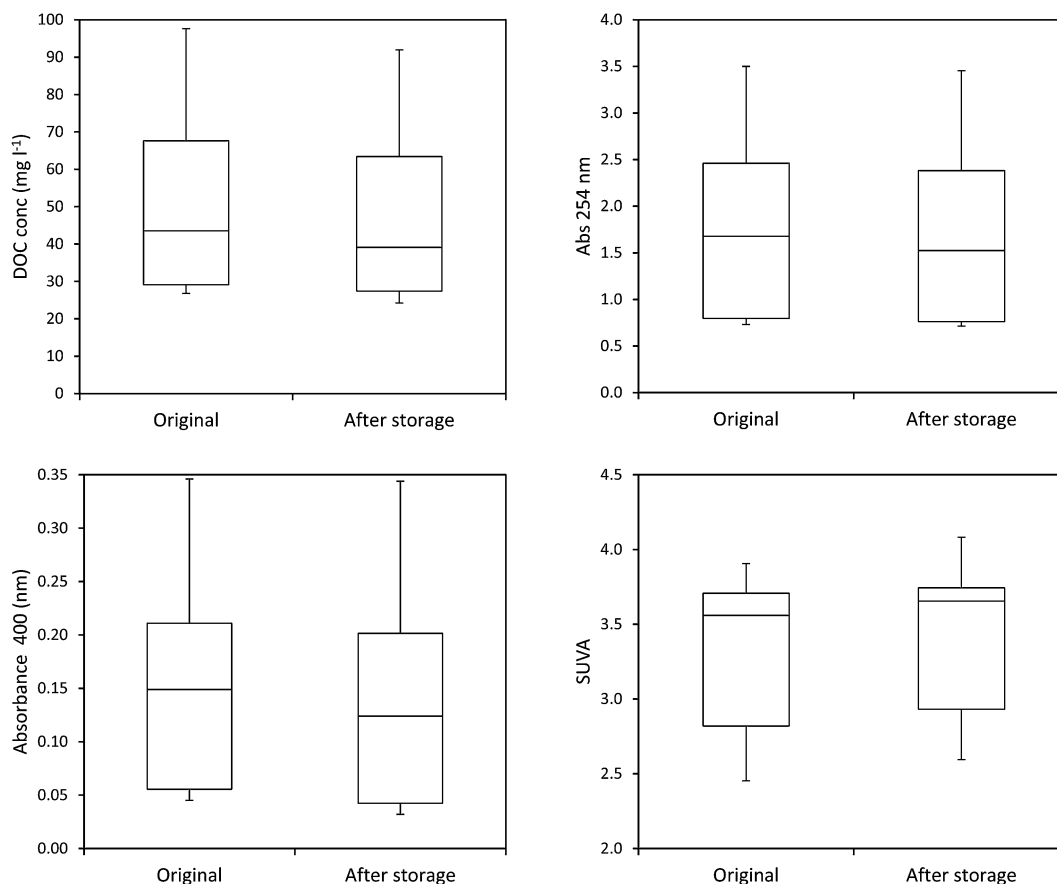

Fig. 8 Median values of DOC, absorbance at 254 nm, absorbance at 400 nm and SUVA for fen samples ($n = 10$), measured before and after storage. Limits of boxes represent first and third quartile, whilst the line within the box is the median. Whiskers represent minimum and maximum concentrations.
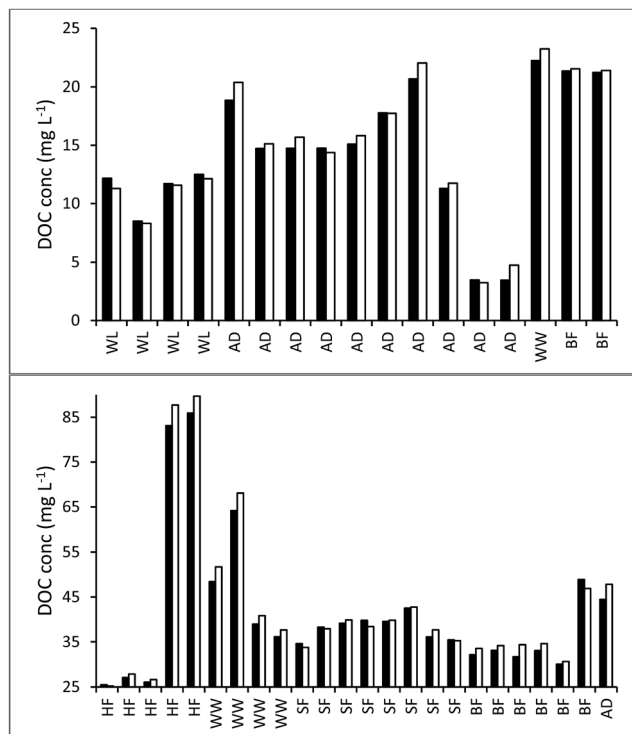
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Fig. 9 Differences in DOC concentration in individual samples analysed immediately after collection (black bars) and after samples have been frozen and thawed (white bars). For clarity the samples have been sorted into low DOC (<25 mg l⁻¹, top graph) and high DOC (>25 mg l⁻¹, lower graph). Sites are: WL = Walton Lake, AD = Afon Ddu, WW = Woodwalton Fen, BF = Baker's Fen, HF = Holme Fen, SF = Sedge Fen.

Table 1 Median percentage change in absorbance following sample freeze/thaw, and number of individual samples where absorbance decreased, increased, or didn't change for all wavelengths measured

Wavelength (nm)	250	254	365	400	465	665
Median decrease (%)	4.8	4.4	6.4	7.3	6.0	33.3
n	21	22	16	14	4	5
Median increase (%)	1.8	2.4	4.2	4.8	9.8	100.0
n	20	17	25	27	33	29
No change n	0	2	0	0	4	7

Fig. 10 Percentage changes in SUVA and E ratios for all samples following sample freeze/thaw. A positive number indicates an increase, negative indicates a decrease. Note that four samples are missing for the E4 : E6 ratio — the ratio could not be calculated for these as absorbance at 665 nm was 0.

loss of 20%, and so it seems likely that cold storage does reduce degradation rates.

We found that DOC loss was strongly related to storage time as an exponential function; it appears that a relatively high percentage (approximately 20–30%) of DOC is lost <138 days, and that as time increases the rate of DOC loss decreases. This first-order exponential decay is dependent solely on the original concentration of DOC, and such a relationship has been noted elsewhere (*e.g.* in brackish UK water samples filtered at 0.7 μm (ref. 43)). This finding suggests that some DOC will be lost, regardless of the molecular properties of the DOC, in any sample stored for several months. There was no difference in loss rates between pore and surface water. It may simply be that a larger sample size would have enabled differences to be
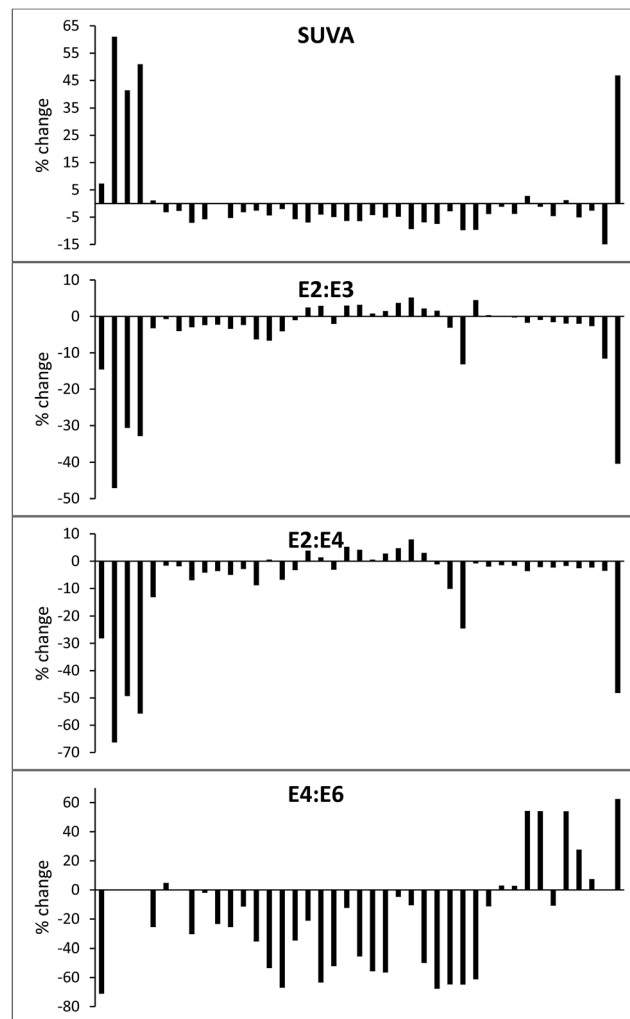
observed. For the bog samples, the strong relationship ($R^2 = 0.78$) between storage time and DOC loss allowed the half-life of DOC to be calculated as approximately 1250 days. It should be noted that the minimum storage time of any sample was 138 days, and the lack of any samples stored for less time could introduce some error into the half-life calculation.

Despite this apparently simple relationship between DOC concentration and time, there was also evidence that DOC composition influenced stability. For instance, DOC loss was reduced as values of SUVA and phenolic : DOC increased. The exceptions to this relationship were three samples that had been stored for the minimum amount of time (138 days); presumably degradation in these samples was still being primarily being controlled by time (*i.e.* they were still losing the most labile DOC). It can be hypothesised that if these samples were returned to storage for more time, then once the most labile DOC was depleted then the composition of the DOC

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would start to have a small but important role in regulating the rate of decomposition. As such, a multiple regression using SUVA as well as storage time produced a slightly higher R^2. Higher values of SUVA indicate that a larger percentage of the DOC is aromatic in nature,[44] as higher SUVA values signify the presence of humic and fulvic acids.[45] Phenolics are also aromatic so this result implies that samples with more aromatic DOC possess a greater degree of recalcitrance, as has been experimentally observed for arable soils.[46] It is known that phenolic compounds can inhibit biological processes,[3] and accordingly the less aromatic and more hydrophilic DOC compounds will be susceptible to biological decomposition.[47]

Adding further weight to this explanation is the observation that absorbance at 254 nm was considerably more stable than DOC concentration during sample storage in the long-term experiment. Absorbance at 254 nm is due to the presence of compounds with aromatic moieties and conjugated double bonds.[48] There was a positive relationship between storage time and loss of absorbance at 254 nm. This implies that the degradation of the aromatic components of DOC is, to some extent, a steady process. As DOC declined considerably during storage but absorbance at 254 nm showed less of a decrease, this suggests that it must have been non-aromatic compounds that were preferentially biologically degraded. It may have been that absorbance at 400 nm would have displayed a similar decrease as DOC concentration, but this metric was not re-measured after storage in the bog peat experiment. However, both 254 nm and 400 nm were remeasured in the fen peat experiment, and it was observed that loss of absorbance was significantly greater at 400 nm. Köhler et al.[16] also found that absorbance at 420 nm decreased more than absorbance at 254 nm in a dark incubation (15–20 °C) over 12 days, whilst Baldwin and Valo[49] posited that bioavailable DOC may not always absorb light in the UV region.

Measurements of SUVA before and after storage also support this explanation in the bog peat experiment; on average there was a significant increase in SUVA after storage (Kothawala et al.[42] also noted an increase in SUVA after dark storage at 20 °C), suggesting that the composition of the DOC changed, becoming more aromatic and possessing a higher molecular weight.[44,50] However, for the fen samples there was no consistent change in SUVA, with some samples showing increases after storage, and others showing decreases. Such results should be interpreted with caution however, as Weishaar et al. show that SUVA is a poor predictor of DOC reactivity.

It is noteworthy that neither E2 : E3, E2 : E4, nor E4 : E6 ratios were related to DOC loss. E2 : E3 has been shown to correlate well with the aromaticity and molecular weight of humics,[51] whilst E4 : E6 has been correlated with molecular weight,[52] although it has been suggested that E4 : E6 is not a useful metric to characterise freshwaters.[51,53] It has been proposed that E2 : E4 is a measure of humification.[54] Despite the widespread use of E ratios to characterise DOC, it remains the case that the exact compounds responsible for absorbance at different wavelengths are unknown.[55] Weishaar et al.[44] stress the fact that, although two water samples may return similar results when analysed for UV-vis, the chemical composition of

their DOC may be quite different. Baldwin and Valo[49] also found no relationship between the E2 : E3 and E2 : E4 ratios, and degradation of DOC, in samples stored at 20 °C for 28 days. As such, it may be that E2 : E3, E2 : E4, and E4 : E6 are too unrefined as metrics to predict the stability or degradation of DOC in stored samples.

It has been shown that peat-derived DOC is highly susceptible to photodegradation,[16] with upper estimates showing that 50% of DOC can be degraded within 2–3 summer (UK) days.[56] Our data, however, show that biological degradation is also significant, albeit over much longer time periods, and therefore might be important in lakes or reservoirs with large residence times, as well as in marine systems. As interest grows in the fate of peatland fluvial carbon dynamics and in situ processes[40] such knowledge is needed.

4.2. Modelling DOC loss and other practical applications

Considering the above controls on DOC degradation, we constructed a simple model using storage time and DOC concentration after storage, to predict DOC concentrations before storage. The discussion in Section 4.1 suggests that a more complex model (i.e. including variables such as SUVA) might perform better, and two-pool models have been used before (featuring a slowly degradable pool of DOC, and a rapidly degradable pool[57]). However, a simple model may offer the greatest practical value. For instance, our model would require no analysis before a sample be placed into storage, whereas a more advanced model would require UV-vis or phenolic analysis to be performed before storage. Such requirements may be difficult to fulfil in the circumstances where such a model may be applied (i.e. for calculating DOC concentrations from samples stored during remote fieldwork campaigns). As such, a simple model proved very accurate at predicting DOC concentrations from before storage, as demonstrated by high Nash–Sutcliffe efficiencies. It is important to note that samples used in the analysis were collected through all seasons over several years, and may therefore contain DOC that varies compositionally (as suggested by a wide range of SUVA values, minimum = 3.15, maximum = 9.47). This variability in samples did not appear to compromise the effectiveness of the simple model.

Data from the same site used for the long-term bog peat experiment concluded that the UV-vis spectrum was stable in water samples stored for 84 days.[24] However, it seems likely that losses of DOC are actually continuous, but small. Using the half-life equation for the bog samples suggests that 1% of DOC would be lost after 18 days of storage, and 5% would be lost after 92. Considering that studies have shown that DOC analysis typically has a precision of 2–5% (ref. 58–60) it seems that losses of DOC will not become detectable for several months. Furthermore, the level of accuracy required will depend on the nature of the study in question. If the aim of an experiment is to detect differences in DOC concentrations between sites then, although concentrations will decrease during storage, the relative differences between samples should remain similar. If the aim is to quantify fluxes or long-

term DOC trends, then degradation will become considerably more important.

Filters of 0.45 μm pore size are generally used when preparing water samples for DOC analysis.[11] However, if it is known that samples will be stored for long periods of time before analysis, a smaller pore size may prove beneficial in reducing losses of DOC by excluding bacteria, thus limiting DOC degradation. The use of a smaller pore size does mean that approximately 10% of DOC will be lost during filtration,[61] and there is evidence to suggest that some bacteria may pass through such filters anyway.[62] For samples that have been filtered at 0.45 μm and stored for long time periods, additional estimates of DOC concentration may be made using absorbance at 254 nm as a proxy, as our data show this to be more stable. Additionally, a phenolic proxy may provide a useful estimate of DOC concentration.[63] These methods could be preferable to freezing or acidifying water samples, which have been shown to affect both DOC quantity and quality.[19,33–36]

4.3.   Changes in DOC and absorbance following freeze/thaw

For most samples, only small (<5%) changes in DOC concentration were observed after samples had been frozen and thawed. Such changes are in the range of typical precision for DOC analysis.[58–60] The exception was one sample of overland flow from the Afon Ddu catchment, for which DOC concentration increased by 37%, along with large increases in absorbance. A second sample taken nearby which had almost identical EC and pH showed a loss of 6.8%. It is therefore unknown what caused the substantial increase in one sample. Fellman et al.[35] noted a strong correlation between percentage loss of DOC during freeze/thaw and original DOC concentration, but no such trend was evident in our data. Our data are similar to that presented by Spencer et al.,[36] who found no consistent change in post-freeze/thaw DOC concentration, with increases and decreases (up to ~10%) for different samples.

Freeze/thaw had a strong and inconsistent effect on the absorbance properties of the samples. At 250 and 254 nm there was an even split with half of the samples showing absorbance increases, and half decreases. It has been observed before that freeze/thaw can have contrasting results on the direction of absorbance changes.[36] As for DOC, the median change at 250 and 254 nm was small (<5%), although several samples changed by ~50%. As wavelength increased, fewer samples displayed absorbance decreases, and percentage change increased. At 665 nm, the median increase in absorbance was 100%. These changes had strong significant effects on SUVA and E ratios. For most samples, SUVA decreased after freeze/thaw and this has been noted before.[35] However, SUVA increased in seven samples, sometimes by as much as 50%. The E ratios for some samples changed very little, but increases and decreases of 30–60 % were observed for others. Taken together, these results suggest that freezing might be a useful preservation method for peatland samples when only a measurement of bulk DOC is required, but that it will lead to erroneous results if UV-vis measurements are also needed.

5.   Conclusions

Our data show that there is a significant loss of DOC concentration and absorbance in peatland water samples stored in the dark at 4 °C, over periods from 138 to 1082 days, and this loss appears to be predictable as a function of storage time. DOC quality had some effect on losses, but this effect was small when compared to the influence of storage time. It is known that photodegradation of peatland DOC can occur extremely rapidly, but our results additionally show that biological processes operate slowly and steadily in dark conditions. These results have important practical implications; water samples can be stored in dark and cold conditions for considerable time and still be used for DOC analysis, by correcting for storage-related losses. Consider an example where samples have to be stored for six months before analysis: the samples would be analysed after storage (six months) and then again on a monthly basis (e.g. for six more months) storage. This would allow a half-life to be calculated, and the original DOC concentration before storage could be estimated.

Results from the freeze/thaw experiment suggest that, for some samples, freezing will not lead to losses of DOC. However, there remains the caveat that DOC concentration may be heavily changed in a minority of samples, and such a change will be difficult to predict using water chemistry determinands such as pH or EC. Freezing is not recommended as a method of preserving samples for UV-vis analysis, as changes in absorbance will be inconsistent (increases and decreases in absorbance) and potentially large.

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