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Changes in freshwater organic matter fluorescence intensity with freezing/thawing and dehydration/rehydration

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[1] The effects of photodegradation and biodegradation upon aquatic organic matter lability have been extensively researched in all aquatic systems because of the impact of these processes upon carbon cycling, with most studies undertaken on the dissolved organic fraction. Little research has been published into the effect of freezing/thawing and dehydration/rehydration although these are mechanisms which are often encountered in nature. In this work, 13 freshwaters from central England were analyzed for chemical water quality, total organic carbon, and organic matter fluorescence using excitation-emission-matrices (EEMs). Samples were stored unfiltered under dehydrated or frozen conditions, then rehydrated or thawed, and analyzed for fluorescence over five cycles. The effect of freezing/thawing and dehydration/rehydration upon total organic matter fluorescence was assessed through changes in fluorescence intensity of four common peaks measured on the EEM spectra. Sample spectra were found to respond in a sample specific manner after one and five cycles of analysis; although fluorescence intensity generally decreased, the magnitude of decrease was variable between fluorescence peaks and samples. Freezing/thawing and dehydration/rehydration provide useful information on the sensitivity of freshwater organic matter fluorescence to these environmental processes.

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1. Introduction

[2] In most aquatic systems the use of organic carbon through microbial respiration exceeds autochthonous (in system) production [*Cole and Caraco*, 2001]. Thus allochthonous (external) sources of carbon, which are either labile or have the potential to be processed to labile products, are also required to maintain system stability. Aquatic organic matter is commonly considered to constitute a mixture of highly labile, often protein rich materials associated with human or microbial activity and more stable, highly processed humic-like materials from the breakdown of terrestrial lignin-based materials. More recently the materials which were previously considered to be highly processed and recaltriant, e.g., terrestrial humic and fulvic acids have been found to be affected by biochemical and photochemical processes, and it is suggested that these "stable" compounds are in fact less stable than previously thought and may be an important metabolite source in fluvial and marine systems [*Battin et al.*, 2008; *Cory et al.*, 2007]. Biochemical and photochemical processes have been found to be highly influential in changing the lability and evasion potential of aquatic carbon, influencing the carbon load of the hydrological system and are considered to be highly influential in the process of carbon cycling. The effects of photodegradation and biodegradation have been studied extensively in lake [*Tranvik and Bertilsson*, 2001], fluvial [*Gao and Zepp*, 1998; *Patel-Sorrentino et al.*, 2004; *Smith and Benner*, 2005], estuarine [*Moran et al.*, 2000], and marine systems [*Skoog et al.*, 1996].

[3] Fluvial systems are of particular interest as they have the potential to expose organic matter to intense microbial or photo exposure having variable residence times and are often highly influenced by local human activity. Organic matter may be processed rapidly or may be shielded and protected from transformation by settlement or delay in quiescent zones. It is considered that only about half the carbon that enters river systems from land is transported and exported to sea [*Cole et al.*, 2007] implying that the other half of this carbon budget settles within or is degassed from river systems. Emphasis is usually placed upon the dissolved organic fraction in fluvial systems as the particulate fraction is considered to be less mobile, settling into sediments with transport occurring as a series of events [*Battin et al.*, 2008]. While photochemical processing and the effect of microbial activity are discussed in detail there is little consideration given to the effect of changes in organic matter concentration and character as a result of freezing/thawing or dehydration/rehydration in freshwaters, both of which are processes which may be experienced by organic matter in fluvial systems, depending upon latitude. Research that has been

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published on the effect of freezing and thawing in both marine [*Coble*, 1996; *Del Castillo and Coble*, 2000] and freshwater samples [*Spencer et al.*, 2007; *Fellman et al.*, 2008] relates to the dissolved fraction rather than the total organic matter. General conclusions are that marine organic matter is largely unaffected by freezing and thawing, in that changes in nutrient concentrations are so low as to be negligible [*Avanzino and Kennedy*, 1993; *Dore et al.*, 1996]. In freshwaters, however, nutrient concentrations are found to be largely affected by the process of sample freezing, leading to a decrease in concentration [*Fellman et al.*, 2008]. Furthermore, freshwater organic matter fluorescence is also seen to be affected by the freezing process [*Spencer et al.*, 2007]. No work has been carried out on the impact of dehydration and rehydration upon freshwater nutrient concentrations or fluorescence properties.

[4] In this work the impact of freezing/thawing and dehydration/rehydration upon unfiltered samples is considered in lowland rural and urban freshwater. Both processes have the potential to cause changes in concentration and character of the organic matter present, and thus affect calculation of the downstream carbon budget. We assess the impact of such processes on the total carbon content of the water, not simply the dissolved fraction, to obtain a direct indication of the effect of such environmental processes upon total organic carbon in the environment. We hypothesize that river waters are likely to demonstrate dramatic changes in fluorescence intensity due to changes in organic matter concentration as a result of the freezing/thawing and dehydration/hydration processes. We will investigate if the extent of change in fluorescence intensity is related to either the chemical water quality or the initial organic matter characteristics.

2. Materials and Methods

2.1. Sample Site Identification

[5] Water samples were collected from thirteen freshwaters in central England, between November 2006 and February 2007. The waters were chosen to include a range of urban and rural characteristics and, as logistically it was not possible to gain any sort of temporal replicate in the period of time available, it was decided to work with a larger spatial distribution of samples to capture the widest possible diversity of organic matter. The watercourses sampled can be grouped into geographically similar areas, and these groups ultimately feed into the same higher-order watercourses. The grid reference of each sample collected for this analysis is shown in Table 1.

2.2. Sample Collection and Storage

[6] Samples were collected directly into previously unused unwashed 1 L bottles. As no sampling aids were used the distance of sampling from the bank was < 1 m and sampling depth was around 10–20 cm. No field analysis of samples was undertaken other than a visual assessment of low- or high-flow status and visual or odor indicators of obvious pollution incidents.

[7] Samples were returned to the laboratory and stored in the refrigerator (4°C, dark) until analysis. One full 1 L bottle of each sample was sent to the Environment Agency for analysis in cold, dark conditions (cool box) within 6 h of sample collection. These samples were registered by the Environment Agency laboratory within 2 days and full chemical water quality analysis was undertaken. The reported parameters and abbreviated titles were Alkalinity ($CaCO_3$) (Alk), Ammoniacal Nitrogen (AmN), BOD_5, Chloride (Chl), Total Oxidized Nitrogen (as N) (TON), Nitrate (as N), Orthophosphate (as P) (Orthop), Silicate (SiO_2) (Si), Phosphate (Phos), Conductivity (Cond), pH, turbidity (Turb), Nitrate (as N). Initial water chemistry and fluorescence values are presented in Table 1.

2.3. Sample Preparation

[8] In order to be more representative of organic matter in the natural system samples were not filtered prior to analysis. Within 6 h of sample collection, 40 ml of unfiltered sample was decanted into new unwashed, sterile 50 ml HDPE bottles in duplicate for each sample which allowed a margin for volume increase during freezing. The bottles were then placed in a laboratory freezer at approximately −20°C (batch 1, 20 November 2006 to 9 January 2007 is average −19.9°C ± 0.67°C, batch 2, 11 January to 1 February 2007 is average −19.71°C ± 0.52°C).

[9] Eight ml of unfiltered sample was also decanted into sterile Petri dishes in duplicate and placed, uncovered, in an oven which had been previously sterilized by washing with 70% ethanol/IMS (Industrial Methylated Spirit). The over temperature was maintained at around 30°C (batch 1, 20 November 2006 to 9 January 2007 is average 36.58°C ± 0.46°C, batch 2, 11 January to 1 February 2007 is average 31.57°C ± 1.06°C) An ambient temperature of 30°C was chosen as it was considered that this was sufficiently environmentally relevant to produce meaningful results while also dehydrating the sample within a timescale that allowed for the experimental phase to be completed within the time available. For the same reason 8ml of sample was decanted for dehydration and rehydration analysis as this was found to dehydrate overnight under the temperature conditions. This allowed a rapid recovery of results.

[10] In addition, 40 ml of sample was decanted into unwashed, sterile 50 ml HDPE bottles and stored under refrigerated conditions as a control, against which to measure changes in fluorescence as a result of freezing and thawing and dehydration/rehydration These samples were refrigerated at around 4°C, in the dark, and were analyzed again for fluorescence only on the last day of the test.

2.4. Sample Analysis

[11] Twenty-four hours prior to analysis the frozen samples were removed from the freezer and allowed to thaw in an environmental cabinet in cycles of light and dark at approximately 11°C. Dehydrated samples were removed from the oven, rehydrated with 8 ml 18 MΩ deionized water and covered with the petri dish lid. One hour before analysis commenced all samples were taken to the fluorescence laboratory where they were stored at room temperature under laboratory lights until analysis. No other preparation was undertaken prior to fluorescence analysis. It was necessary to dilute some samples due to their turbidity or very high fluorescence intensities which made the test ''destructive,'' with a volume of sample being removed from the bulk and not returned. This is considered to be of negligible impact as each fluorescence analysis used only 400 μl, from a total

Table 1. Sample Names, Reference Numbers, Locations, Initial Chemical Characteristics, and Fluorescence Data[a]

Sample Name	Reference	Sample GR	BOD (mg/l)	Alkalinity (mg/l)	Ammoniacal Nitrogen (mg/l)	Chloride (mg/l)	TON (mg/l)	Nitrite (mg/l)	Orthophosphate (mg/l)	Silicate (mg/l)	Phosphate (mg/l)	Conductivity (μS/cm)	pH	Turbidity (FTU)	Nitrate (mg/l)	TOC (mg/l)	TC (mg/l)	TIC (mg/l)
River Tame	1	SP174914	<3.0	177	0.21	135	7.5	0.145	1.88	11.2	2.29	929	7.70	9.3	7.3	6.49	49.98	43.49
Wood Brook	2	SK033815	25.9	160	7.47	35	3.0	0.759	0.90	10.6	1.20	479	7.61	14.5	2.2	ND	ND	ND
River Rea	3	SP067840	<3.0	158	0.06	80	2.8	0.055	0.13	11.6	0.13	682	7.95	1.4	2.7	3.64	43.68	40.04
Harborne Brook North	4	SP026836	5.6	75	0.53	796	1.8	0.139	0.15	6.8	0.16	2490	7.05	10.5	1.7	12.46	30.54	18.08
Harborne Brook South	5	SP026836	<3.0	87	0.16	627	1.9	0.068	0.16	7.1	0.16	2000	7.30	6.6	1.9	9.71	31.19	21.48
Vale Lake	6	SP052847	<3.0	85	0.97	23	0.3	0.024	0.12	7.8	0.12	282	7.35	5.2	0.3	6.51	28.02	21.51
Bartley Brook	7	SK022827	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Merritt's Brook	8	SK034811	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
River Trent	9	SK254223	<2.9	111	0.28	41	7.1	0.093	0.54	9.8	0.66	512	7.68	54.3	7.0	ND	ND	ND
Repton Brook	10	SK307265	<3.0	225	<0.03	45	6.6	0.019	0.04	9.6	0.04	659	7.95	7.2	6.6	2.03	58.67	56.64
Hilton Brook	11	SK242302	<2.9	135	0.13	22	6.9	0.044	0.28	9.2	0.34	421	7.82	62.5	6.8	ND	ND	ND
River Dove	12	SK214295	<3.0	160	<0.03	31	4.1	0.019	0.17	7.5	0.16	520	8.05	1.7	4.1	3.64	44.90	41.26
Alder Brook	13	SK235276	<2.9	192	0.14	29	8.1	0.043	0.26	14.7	0.29	734	8.04	49.3	8.0	ND	ND	ND
18 MΩ Control			<3.0	<15	<0.03	<1	<0.2	<0.004	<0.02	<0.2	<0.02	<10	6.95	<1.0	<0.2	0.20	0.40	0.20

			Fluorescence Character Recorded on Day 1											
			Peak T_1			Peak T_2			Peak C			Peak A		
Sample Name	Reference	Sample GR	Excitation	Emission	Intensity	Excitation	Emission	Intensity	Excitation	Emission	Intensity	Excitation	Emission	Intensity
River Tame	1	SP174914	283	362	147	232	351	355	331	414	191	239	420	370
Wood Brook	2	SK033815	280	371	396	228	362	689	325	419	291	222	413	857
River Rea	3	SP067840	285	350	69	232	349	224	330	420	146	237	407	319
Harborne Brook North	4	SP026836	282	344	210	233	360	754	316	420	280	237	414	756
Harborne Brook South	5	SP026836	281	342	134	231	345	534	300	416	220	231	407	554
Vale Lake	6	SP052847	285	348	135	231	369	505	316	424	168	237	411	470
Bartley Brook	7	SK022827	282	344	64	230	342	149	335	421	197	231	425	417
Merritt's Brook	8	SK034811	282	344	83	233	350	197	341	419	305	236	430	493
River Trent	9	SK254223	280	344	99	233	356	252	332	422	324	238	415	560
Repton Brook	10	SK307265	280	359	49	233	360	96	321	429	107	238	417	235
Hilton Brook	11	SK242302	285	355	136	227	348	340	331	421	488	236	429	868
River Dove	12	SK214295	288	344	38	232	368	143	341	429	165	237	420	317
Alder Brook	13	SK235276	278	361	139	228	354	235	340	418	469	237	439	868
18 MΩ Control			280	354	2	231	352	13	336	417	2	227	398	6

[a]Sites sorted by decreasing urban character. ND, no data. (Fluorescence intensity corrected for Raman and dilution factors and intensity values recorded for 18 MΩ control.)

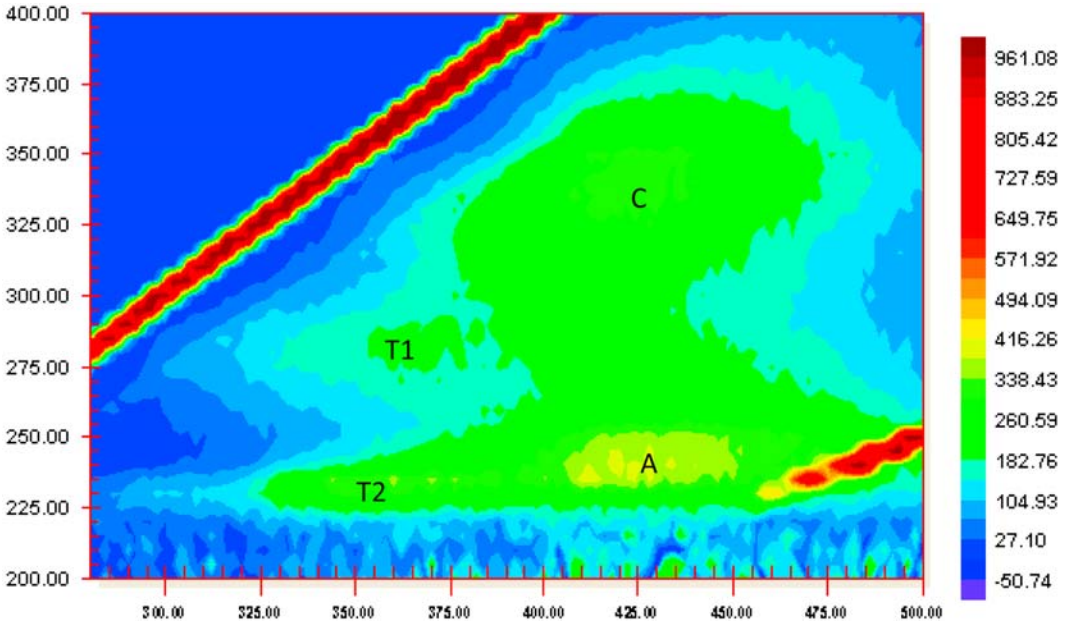

Figure 1. An example of a common freshwater EEM.

volume of 40 ml (1%). However, dehydrated/rehydrated sample volumes were reduced by 5% on each analysis as 400 μl was removed from 8 ml.

[12] Following fluorescence analysis the thawed samples were returned to the freezer for another cycle of freezing and the rehydrated samples returned, uncovered, to the oven. This cycle was repeated five times with fluorescence analysis being undertaken after each cycle.

2.5. Fluorescence Analysis

[13] An EEM was created for each sample using a Varian Cary Eclipse fluorescence spectrometer. The Cary Eclipse uses a xenon light source which flashes at up to 80 flashes per second at 2–3 μs intervals, a single Czerny-Turner monochromator which splits the excitation and emitted light into constituent colors, a range of adjustable filters and two photomultiplier tube detectors.

[14] Excitation and emission were scanned simultaneously at wavelengths from 200 to 400 nm and 280–500 nm at 5 nm and 2 nm intervals, respectively, with a 5 nm band pass at 9600 nm/min scan rate and at 20°C (regulated by a Peltier temperature controller). The position (excitation and emission wavelength pair) and intensity in arbitrary fluorescence units (AFU) of points of fluorescence maxima were manually determined on the EEM using the Cary Eclipse software and were recorded. Correction for instrument-specific wavelength bias is commonly applied in work of this nature; however, such corrections have not been applied to this data as, for the Cary-Eclipse spectrophotometer, the corrections cannot be accurately applied at wavelengths shorter than 220 nm. An attempt to apply these correction factors would, therefore, exclude correction of much of the peak A and T_2 data which are of interest in these waters. However, if corrections were to be applied they would be, in the region of peak T_1 = x1.84 +/− 0.21 and peak C = x1.37 +/− 0.05. In this work the same spectrophotometer was used throughout. Fluorescence analysis was undertaken after each freeze/thaw and dehydration/rehydration event.

[15] The Raman value of water (vibrational effect of excitation of the H-O-H molecules) at excitation wavelength 348 nm, derived daily from a manufacturer supplied sealed water cell, was used as an internal standard to test for instrument drift. Fluorescence intensity results are normalized for this value (average 24.859 units). A quartz microcuvette was used in which 400 μl of sample was analyzed at a path length of 1 cm.

[16] Data for four common fluorescence peaks is presented in this work; tryptophan-like (or protein-like) and humic/fulvic-like. Tryptophan-like fluorescence demonstrates two peak positions in the region of $\lambda_{ex/em}$ 280/350 nm and $\lambda_{ex/em}$ 215–220/340 nm which will be referred to as T_1 and T_2, respectively. Humic-like material is represented by two distinct fluorophores, commonly referred to in literature as humic-like and fulvic-like and which are referred to in this work as peaks C and A at peak regions $\lambda_{ex/em}$ 380/420–480 nm and 260/380–460 nm, respectively [*Coble*, 1996]. An example freshwater EEM is shown in Figure 1.

[17] Changes in fluorescence intensity are presented in this paper as percentage change in peak intensity from the initial value. Fluorescence intensity values have been corrected for changes in fluorescence intensity observed in 18 MΩ distilled water which was prepared and stored under the same environmental conditions. In this instance the fluorescence intensity in AFU for each peak recorded in the 18 MΩ distilled water was subtracted from the measured sample fluorescence intensity for each equivalent peak and change in sample fluorescence intensity as a percentage was calculated on the subsequent corrected fluorescence intensity. The purpose of this correction was to account for any contaminants which may enter the sample from the storage container or air. On average peak T_1 was corrected by −4 AFU, T_2 by −43 AFU, peak C by −3 AFU and peak A by −21 AFU, negligible values (within instrument variability) except peak T_2. Where no corresponding 18 MΩ distilled water was stored (batch 2 of frozen samples, Alder Brook on) an average correction

Table 2. Minimum, Maximum, and Mean Recorded Values of One Standard Deviation From the Mean Fluorescence Intensity of Each of the Four Common Fluorescence Peaks for 424 Triplicate Samples[a]

Standard Deviation	T_1		T_2		C		A	
	a.u.	Percent	a.u.	Percent	a.u.	Percent	a.u.	Percent
Minimum	0	0	0	0	0	0	1	0
Maximum	23	**27**	40	**18**	12	**8**	95	**17**
Mean	4	6	9	5	3	3	10	3

[a]Values in bold are the maximum value of one standard deviation reported across the 424 samples as a percentage of the mean intensity of that specific group of triplicate samples.

factor for each peak for the 18 MΩ distilled water data available was used.

2.6. Total Organic Carbon

[18] Undiluted samples were analyzed prior to experimentation for both total carbon and inorganic carbon, and the total organic carbon (TOC) then calculated by difference using a Shimadzu TOC-Vcpn analyzer. Total carbon was analyzed by combustion of the sample at 680°C with a platinized alumina catalyst and the resulting CO_2 production measured. Total inorganic carbon was analyzed by phosphoric acid digestion combined with CO_2 determination by IR detection. From these analyses results the total organic carbon was calculated by total carbon – total inorganic carbon (TOC = TC − TIC). The instrument was calibrated prior to each analysis using a dilution series of total carbon and inorganic carbon 1 molar standards (Reagecon) and for each analysis the mean of three measurements was used.

3. Results

[19] The values of one standard deviation from the mean of 424 triplicate samples from unpublished work is presented to assess whether the change in fluorescence intensity observed may be reportable change in intensity or simply analytical uncertainty. A summary of the standard deviation around the mean as a value and as a percentage of the mean intensity are shown in Table 2. The minimum, maximum, and mean standard deviation values of fluorescence intensity calculated for each fluorescence peak across the 424 samples, subjected to different storage conditions, are quoted. The maximum standard deviation value recorded across the 424 samples for each peak is considered to be representative of the maximum possible variability across a triplicate analysis. Therefore any change in fluorescence up to this value may be attributable to analytical uncertainty. Any change in observed fluorescence intensity in excess of the maximum standard deviation should be considered an actual change in fluorescence properties.

Table 3. Percent Change in Fluorescence Intensity Between Initial Fluorescence and on the Final Day of Analysis in Refrigerated Control Samples[a]

Sample Name	Sample Reference	Percent Change Fluorescence Intensity			
		T_1(%)	T_2(%)	C(%)	A(%)
River Tame	1	−19	11	**15**	**28**
Wood's Brook	2	**−81**	**−64**	**−18**	**−52**
River Rea	3	**−36**	**−28**	0	6
Harborne Brook North	4	**−41**	**−42**	**−15**	−16
Harborne Brook South	5	−20	−7	4	14
Vale Lake	6	−14	**−28**	**12**	9
Bartley Brook	7	12	**−29**	2	8
Merritt's Brook	8	−6	**−44**	**−18**	−2
River Trent	9	**−34**	**−42**	**−14**	−4
Repton Brook	10	**−32**	**−62**	7	−3
Hilton Brook	11	**−59**	**−69**	**−22**	−25
River Dove	12	−5	**−49**	−2	5
Alder Brook	13	**−62**	**−61**	**−20**	−16
Mean fluorescence change (%)		−31	−40	−5	0
Standard deviation		26	23	13	15

[a]Values in bold indicate changes outside the bounds of maximum analytical uncertainty.

Table 4. Percent Change Intensity Values for Corrected Data After One Cycle Freeze/Thaw Which Fall Outside the Bounds of Maximum Analytical Uncertainty[a]

Sample Name	Sample Reference	Percent Change Fluorescence Intensity			
		T_1(%)	T_2(%)	C(%)	A(%)
River Tame	1	−12	**−26**	**−8**	−6
Wood Brook	2	−12	−12	**−10**	−7
River Rea	3	19	**−28**	**−11**	−6
Harborne Brook North	4	11	−11	1	−2
Harborne Brook South	5	−5	−17	−7	−7
Vale Lake	6	−23	**−35**	**−20**	**−30**
Bartley Brook	7	19	−17	**10**	9
Merritt's Brook	8	−11	−14	**−8**	8
River Trent	9	−14	**−29**	−2	13
Repton Brook	10	2	**−86**	−4	−11
Hilton Brook	11	22	**−51**	**−13**	0
River Dove	12	2	**−78**	**−13**	−7
Alder Brook	13	**−37**	**−37**	−7	12
Mean fluorescence change (%)		−3	−34	−7	−3
Standard deviation		18	24	7	12

[a]Values in bold.

3.1. Stability of Refrigerated Control Samples

[20] A subsample of each water sample was stored under refrigerated conditions for the duration of the freezing/thawing and dehydration/rehydration cycles. These subsamples exhibited fluorescence intensity change over the period

Table 5. Percent Change Intensity Values for Corrected Data After One Cycle Dehydration/Rehydration Which Fall Outside the Bounds of Maximum Analytical Uncertainty[a]

Sample Name	Sample Reference	Percent Change Fluorescence Intensity			
		T_1(%)	T_2(%)	C(%)	A(%)
River Tame	1	**−30**	−17	**−15**	−12
Wood Brook	2	**−57**	**−55**	**−28**	**−33**
River Rea	3	−23	**−19**	**−19**	**−20**
Harborne Brook North	4	−19	**−29**	**−29**	**−30**
Harborne Brook South	5	12	−12	**−17**	−14
Vale Lake	6		**−22**	**−14**	**−21**
Bartley Brook	7	**−27**	**−55**	2	1
Merritt's Brook	8	**−42**	**−44**	**−29**	−14
River Trent	9	**−42**	**−53**	**−21**	−8
Repton Brook	10	**29**	**−52**	**−16**	**−26**
Hilton Brook	11	**−60**	**−59**	**−17**	6
River Dove	12	−8	**−67**	**−22**	**−21**
Alder Brook	13	**−40**	**−30**	**−15**	−8
Mean fluorescence change (%)		−26	−40	−18	−15
Standard deviation		26	19	8	11

[a]Values in bold.

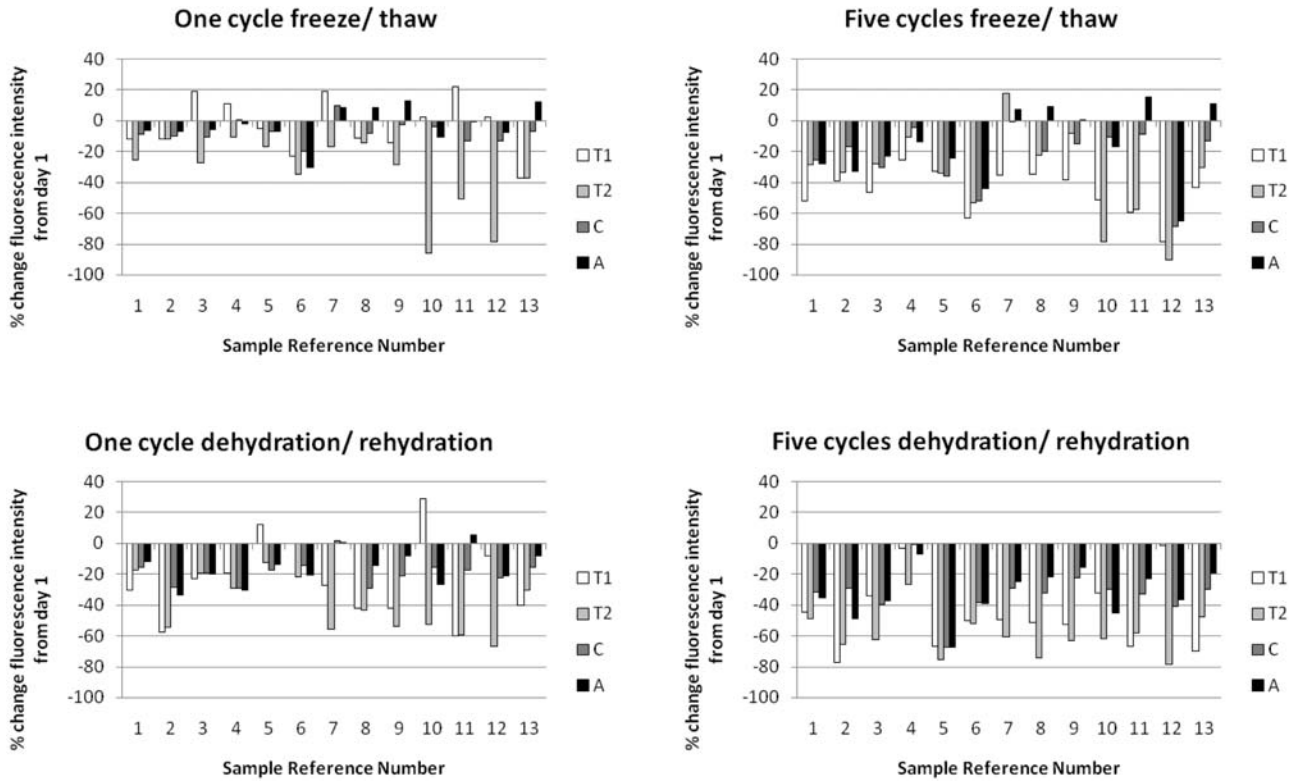

Figure 2. Percentage change in fluorescence intensity after one cycle and five cycles of freezing/thawing and dehydration/rehydration. Fluorescence intensity data corrected for 18 MΩ deionized water sample stored under same conditions.

of analysis. Table 3 shows the percentage change in intensity in refrigerated samples from Day 1. In particular, decreases in fluorescence intensity occur in peaks T_2 and C which are greater than analytical uncertainty. It is clear that even under these storage conditions the samples are not stable and are subject to oxidation/microbial activity which affect the fluorescence properties of the sample. Fluorescence intensity changes in the frozen or dehydrated samples in excess of that observed in the control sample may indicate that the processes of freezing/thawing and dehydration/rehydration are more influential than oxidation/microbial activity.

3.2. One Cycle Freeze/Thaw and Dehydration/Rehydration

[21] It is clearly shown in Table 4 that for one cycle of freeze/thaw almost all percentage changes in observed fluorescence intensity in peaks T_1 ($-3 \pm 18\%$) and A ($-3 \pm 12\%$) fall within the that of calculated analytical uncertainty. Changes in peaks T_2 ($-34 \pm 24\%$) and C ($-7 \pm 7\%$) are often outside that which can be explained by analytical uncertainty.

[22] Table 5 shows the percent change in fluorescence from that measured initially for each peak after one cycle of dehydration/rehydration. Similar to one cycle of freeze/thaw, the vast majority of changes in peaks T_2 ($-40 \pm 19\%$) and C ($-18 \pm 8\%$) fall outside that expected due to analytical uncertainty, but in this instance a large proportion of sites exhibit significant changes in peaks T_1 ($-26 \pm 26\%$), and some sites also show decreases in peak A ($-15 \pm 11\%$).

[23] Results presented in Tables 4 and 5 suggest that while freezing may be a convenient method of filtered fresh water sample storage, it is not without detrimental effect to the fluorophores present in total water samples. Substantial decreases in peak T_2 and C intensities, outside the range attributable to analytical uncertainty, may be observed over a single episode of freezing and thawing even when frozen for a relatively short space of time. Dehydration and rehydration of samples appears to be even more disruptive of sample fluorescence, as a greater proportion of samples exhibit fluorescence intensities which fall outside the bounds of analytical uncertainty. These findings have some relevance for sample stability, suggesting that dehydration more effectively disrupts the structure of the organic matter present, with more fluorescence peaks exhibiting fluorescence intensity decreases after dehydration than freezing. For both freezing and dehydration, both peaks C and T_2 are the most affected, with most samples exhibiting a significant decrease in fluorescence intensity. The general reduction in fluorescence intensity with freezing and dehydration suggests evasion of organic carbon as CO_2 as the mechanism, the precise cause is unknown but may include the mechanical breakdown of organic molecules, cell lysis/bursting, changes in colloid-organic matter interactions, etc. As it is the total organic matter fraction investigated in this work, it is not possible to determine whether this loss of organic carbon is occurring in one specific fraction (dissolved, colloidal, or particulate) or whether it occurs at the same rate and to the same degree across the fractions.

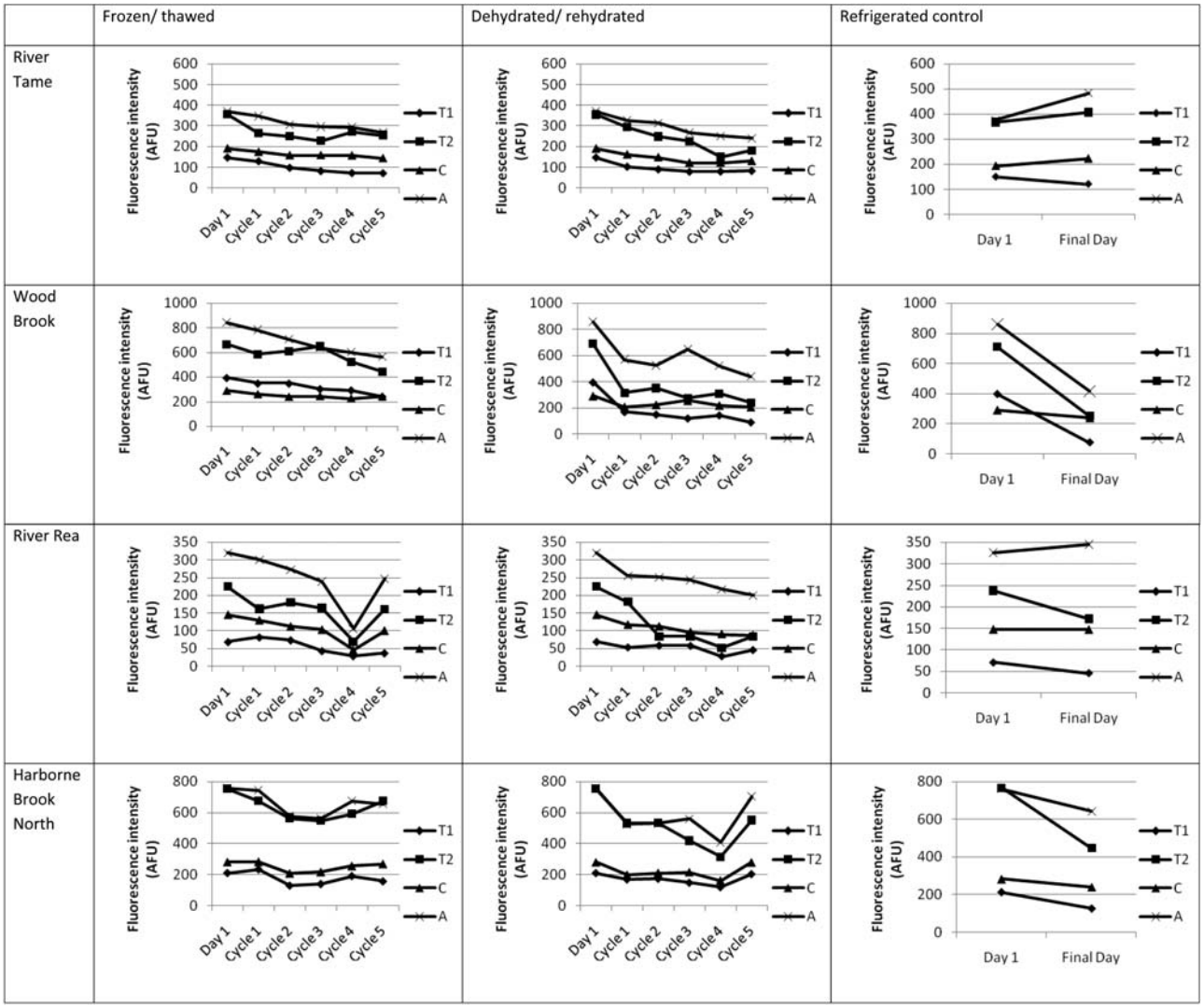

Figure 3. Graphs showing direction of fluorescence intensity change on a cycle-by-cycle basis.

3.3. Five Cycles Freeze/Thaw and Dehydration/Rehydration

[24] Percent change in fluorescence intensity values are presented for samples after the same number of cycles of freezing/thawing and dehydration/rehydration. Freeze/thaw results after five cycles are presented in Table 6, and dehydration/rehydration in Table 7, and results comparing one and five cycles are presented in Figure 2 and for all five cycles in Figure 3.

[25] Generally it can be observed that after five cycles of freezing/thawing and dehydration/rehydration the direction of fluorescence change becomes more consistent than after one cycle, with a decrease in fluorescence intensity for all peaks being common. The direction and amount of change in fluorescence is illustrated graphically for each peak over one and five cycles of freezing/thawing and dehydration/rehydration in Figure 2. In comparison with changes in fluorescence after one cycle of freeze/thaw or dehydration/rehydration it can be seen that there is less obvious peak specific pattern to responses, and in general most observed changes are greater than analytical uncertainty. Only one sample does not exhibit significant decreases in fluorescence after five cycles (Harborne Brook North); however, inspection of Figure 3 shows that this sample initially undergoes a fluorescence decrease, followed by an increase in fluorescence during later cycles.

4. Discussion

[26] Chemical water quality results (Table 1), including total and inorganic carbon results, all fall within those typical of British freshwaters. Two urban sites, Wood Brook and Harborne Brook North, can be seen to be highly influenced by organic pollution with high BOD_5 values. The urban Vale Lake has high ammonia values with no elevated BOD_5 value, suggesting an autochthonous source of ammonia, rather than an allochthonous source associated with sewage pollution. As all samples fall within that expected for British freshwaters, they have been ordered in Tables 1–7 and Figures 2 and 3 by decreasing urban/increasing rural land cover. Those samples at the more rural end of the spectrum may be seen to be more highly turbid, with higher total oxidized nitrogen and nitrate concentrations, typically of larger rivers and an agricultural source of nitrate pollution. Urban rivers had rela-

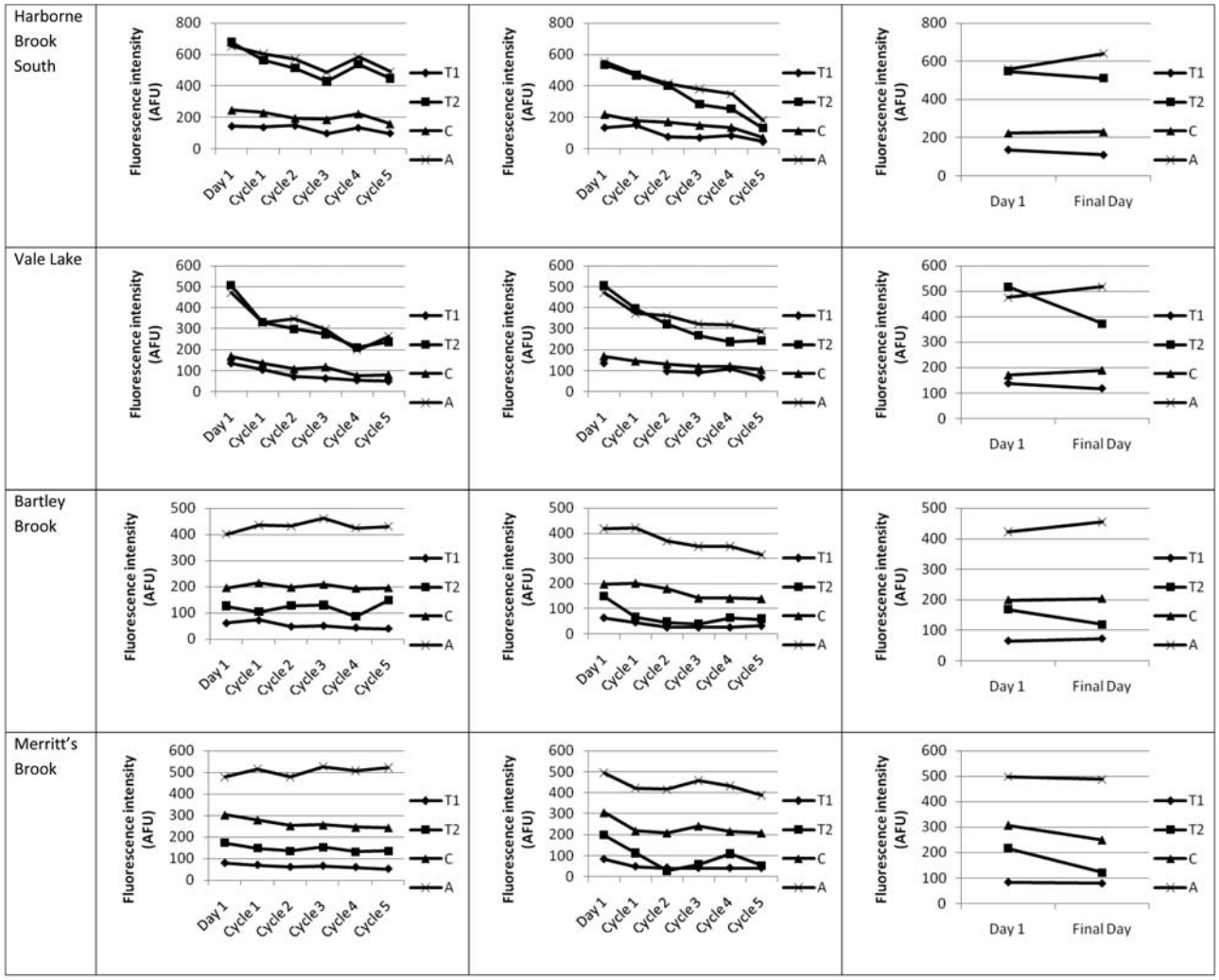

Figure 3. (continued)

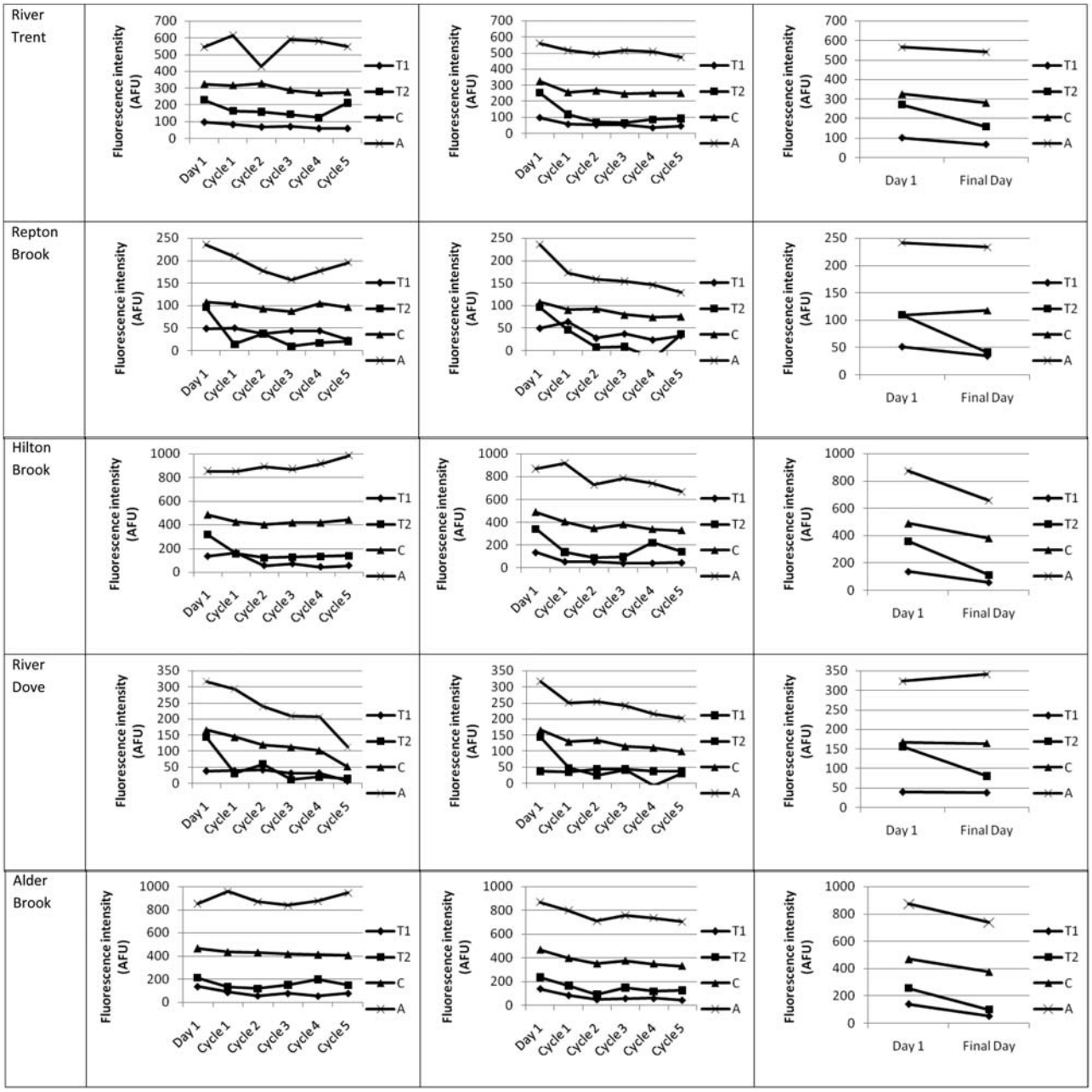

Figure 3. (continued)

Table 6. Percent Change in Fluorescence Intensity Between Initial Fluorescence and After Five Cycles of Freezing/Thawing[a]

Sample Name	Sample Reference	Percent Change Fluorescence Intensity			
		T_1	T_2	C	A
River Tame	1	**−52**	**−28**	**−25**	**−28**
Wood's Brook	2	**−39**	**−33**	**−17**	**−33**
River Rea	3	**−46**	**−28**	**−31**	**−23**
Harborne Brook North	4	−25	−11	−4	−14
Harborne Brook South	5	**−33**	**−34**	**−36**	**−24**
Vale Lake	6	**−63**	**−53**	**−52**	**−44**
Bartley Brook	7	**−35**	**18**	0	7
Merritt's Brook	8	**−34**	**−22**	**−20**	9
River Trent	9	**−39**	−8	**−15**	0
Repton Brook	10	**−52**	**−78**	**−10**	**−17**
Hilton Brook	11	**−59**	**−57**	**−9**	15
River Dove	12	**−78**	**−90**	**−69**	**−65**
Alder Brook	13	**−43**	**−30**	**−13**	11
Mean fluorescence change (%)		−46	−35	−23	−16
Standard deviation		15	29	20	24

[a]Values in bold indicate changes outside the bounds of maximum analytical uncertainty.

tively high chlorine and total organic carbon, indicative of urban runoff and sewerage contamination.

[27] After one cycle of freezing, peaks T_2 and C tend to show changes in fluorescence intensity which fall outside the bounds of analytical uncertainty. This may suggest that these peaks are intrinsically more unstable than the T_1 and A peaks, and suggests that different organic matter fractions contribute to each of the four fluorescence peaks. Few patterns between the change in fluorescence intensity after freezing and chemical water quality or initial fluorescence properties are observed, although samples which are classified as more "rural," e.g., Repton Brook, Alder Brook, Hilton Brook, and River Trent, show large changes in T_2 fluorescence intensity. No relationships are observed with parameters that might reflect greater organic matter lability (high initial peak T intensity or high BOD). After five cycles of freezing the River Dove demonstrates the greatest degree of fluorescence change for all peaks. One sample, Harborne Brook North, demonstrates an increase in fluorescence intensity during later freeze/thaw cycles, suggesting the presence of several organic matter fractions with different sensitivities to freezing.

[28] After one cycle of dehydration/rehydration most samples generally demonstrate fluorescence decreases greater than analytical uncertainty, particularly in peaks T_2 and C which are greater than those observed in frozen samples. Peak T_1 also decreases significantly. However, as in samples subjected to one cycle of freezing and thawing peaks T_1 and T_2 are seen to change independently of each other. This may suggest separate fluorophores are responsible for fluorescence of these peaks and a greater stability of the T_1 peak. Peak A intensity also decreases with dehydration more than with freezing; this is slightly more apparent for the urban sample sites. One cycle of dehydration therefore effects a greater decrease in fluorescence intensity of all fluorescence peaks that one cycle of freezing. After five cycles of dehydration all fluorescence peaks in all sample sites except the Harborne Brook North exhibit a significant decrease in fluorescence. Harborne Brook North initially decreases in fluorescence, but after five cycles increases in fluorescence for all fluorescence peaks.

[29] There appears to be no simple relationship between the initial sample characteristics and the manner in which the organic matter responds to episodes of freezing/thawing or dehydration/rehydration. There is no relationship with BOD or peak T intensity, suggesting that the processes determining organic matter degradation with freezing and dehydration are different to those determining microbial degradation. What may be summarized is that there is a common decrease in fluorescence intensity of all peaks in all samples after freezing/thawing and dehydration/rehydration, ubiquitous across all samples, regardless of catchment. Peak C intensity correlates with TOC as demonstrated by *Ferrari et al.* [1996] and *Bieroza et al.* [2009]; therefore a decrease in peak C intensity of 23% with five freezing cycles and 33% with five dehydration cycles suggests a decrease in TOC of 1−3 mg/l can be inferred. Therefore these processes are important in our understanding of carbon cycling in the environment, although they are currently largely unstudied.

5. Conclusions

[30] 1. There is a general trend for fluorescence peaks in all samples to demonstrate a decrease in fluorescence intensity after freezing/thawing and dehydration/rehydration. This may suggest a decrease in TOC during these processes which is highly important in our understanding of carbon budgets.

[31] 2. In general, fluorescence intensity continued to decrease with repeated cycles of freezing and dehydration, although for one urban site, fluorescence increased with repeated cycles.

[32] 3. T_1 and T_2 follow independent behavior in response to freeze/thaw and dehydration/rehydration. This may indicate that peaks T_1 and T_2 comprise more than one fluorophore which respond differently to freezing and dehydration.

[33] 4. Dehydration and rehydration appears to be more destructive to fluorescent organic carbon than freezing and thawing, although both are highly destructive and lead to losses of fluorescence after both one and five cycles.

[34] 5. It is likely that the observed decrease in fluorescence intensity also indicates a loss of TOC from samples through the freezing and dehydration processes. Thus it may

Table 7. Percent Change in Fluorescence Intensity Between Initial Fluorescence and After Five Cycles of Dehydration/Rehydration[a]

Sample Name	Sample Reference	Percent Change Fluorescence Intensity			
		T_1	T_2	C	A
River Tame	1	**−44**	**−49**	**−32**	**−35**
Wood's Brook	2	**−77**	**−65**	**−29**	**−49**
River Rea	3	**−34**	**−63**	**−40**	**−37**
Harborne Brook North	4	−3	**−27**	0	−7
Harborne Brook South	5	**−67**	**−75**	**−67**	**−67**
Vale Lake	6	**−50**	**−52**	**−38**	**−39**
Bartley Brook	7	**−49**	**−60**	**−29**	**−25**
Merritt's Brook	8	**−51**	**−74**	**−32**	**−22**
River Trent	9	**−53**	**−63**	**−22**	−16
Repton Brook	10	**−32**	**−62**	**−30**	**−45**
Hilton Brook	11	**−67**	**−58**	**−33**	**−23**
River Dove	12	−1	**−78**	**−41**	**−36**
Alder Brook	13	**−70**	**−47**	**−30**	**−19**
Mean fluorescence change (%)		−46	−59	−33	−32
Standard deviation		23	13	14	15

[a]Values in bold indicate changes outside the bounds of maximum analytical uncertainty.

be speculated that a reduction in the number and frequency of freezing events in winter in midlatitudes, which may occur as a result of global warming, may be another factor leading to an overall increase in TOC concentrations and color in midlatitude rivers. Conversely, in high latitudes, defrosting and freezing due to permafrost melting might be increasing, leading to a converse effect of TOC in rivers. At lower latitudes, dehydration events may occur more frequently as a result of global warming, which could contribute to a decrease in TOC concentrations in rivers in this region.

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