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Abstract

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Dissolved organic matter signatures vary between naturally acidic, circumneutral and groundwater-fed freshwaters in Australia

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Graphical Abstract

Highlights

- Naturally acidic waters dominated by aromatic, humic-like, high MW DOM
- Circumneutral waters dominated by less aromatic, fulvic-like DOM of lower MW
- Groundwater-fed site contained more proteinous DOM of lowest aromaticity and MW
- DOM was more autochthonous-like during summer/autumn than during winter/spring

Abstract

Dissolved organic matter (DOM) plays important roles in both abiotic and biotic processes within aquatic ecosystems, and these in turn depend on the quality of the DOM. We collected and characterised chromophoric DOM (CDOM) from different Australian freshwater types (circumneutral, naturally acidic and groundwater-fed waterways), climatic regions and seasons. CDOM quality was characterised using absorbance and fluorescence spectroscopy. Excitation emission scans followed by parallel factor (PARAFAC) analysis showed that CDOM was characterised by three main components: protein-like, fulvic-like and humic-like components commonly associated with various waters globally in the Openfluo database. Principal component analysis showed that CDOM quality varied between naturally acidic, circumneutral and groundwater-fed waters, with unique CDOM quality signatures shown for each freshwater type. CDOM quality also differed significantly within some sites between seasons. Clear differences in dominant CDOM components were shown between freshwater types. Naturally acidic waters were dominated by highly aromatic (as indicated by the specific absorbance co-efficient (SAC_{340}) and the specific UV absorbance ($SUVA_{254}$) values which ranged between $31 - 50 \text{ cm}^2 \text{ mg}^{-1}$ and $3.9 - 5.7 \text{ mg C}^{-1} \text{ m}^{-1}$ respectively), humic-like CDOM of high molecular weight (as indicated by $abs_{254/365}$ which ranged from $3.8 - 4.3$). In contrast, circumneutral waters were dominated by fulvic-like CDOM of lower aromaticity (SAC_{340} : $7 - 21 \text{ cm}^2 \text{ mg}^{-1}$ and $SUVA_{254}$: $1.5 - 3.0 \text{ mg C}^{-1} \text{ m}^{-1}$) and lower molecular weight ($abs_{254/365}$ $5.1 - 9.3$). The groundwater-fed site had a higher abundance of protein-like CDOM, which was the least aromatic (SAC_{340} : $2 - 5 \text{ cm}^2 \text{ mg}^{-1}$ and $SUVA_{254}$: $0.58 - 1.1 \text{ mg C}^{-1} \text{ m}^{-1}$). CDOM was generally less aromatic, of a lower molecular weight and more autochthonous in nature during the summer/autumn sampling compared to winter/spring. Significant relationships were shown between various CDOM quality parameters and pH. This is the first study to show that different freshwater types (circumneutral, naturally acidic and groundwater-fed) contain distinct CDOM quality signatures in Australia, a continent with unique flora and geology.

Keywords: Dissolved organic matter, Australia; humic substances; fluorescence; PARAFAC; tropical; temperate

1. Introduction

Dissolved organic matter (DOM) consists of a complex mixture of humic substances (humic and fulvic acids), carbohydrates, proteins and amino acids and is formed via the decomposition of plant and animal matter (Thomas 1997, Frimmel 1998, Zhao et al. 2016). DOM plays an important role in both abiotic and biotic processes within all aquatic ecosystems by acting as an available food source, influencing the physiology of organisms, altering light and nutrient availability, and reducing the bioavailability of contaminants such as metals (Steinberg et al. 2006, Wood et al. 2011). The concentration and quality of DOM will directly affect how DOM interacts with these abiotic and biotic processes (Schindler and Curtis 1997, Jaffé et al. 2008, Al-Reasi et al. 2013).

DOM is often characterized by either analytical and/or optical methods. Optical methods such as the use of absorbance and fluorescence spectroscopy are useful techniques in characterizing the components, source, aromaticity and approximate molecular weight of DOM within aquatic environments (Curtis and Schindler 1997, McKnight et al. 2001, Fellman et al. 2010) and are generally less expensive and time consuming than traditional analytical methods such as ^{13}C nuclear magnetic resonance. DOM characterized via these methods is often referred to as chromophoric DOM (CDOM) for short. Fluorescence excitation emission (FEEM) scans can indicate the key components of CDOM such as

humic-like, fulvic-like and protein-like compounds and their potential source - terrestrially derived (allochthonous) and/or microbially derived (autochthonous) (Coble 1996, McKnight et al. 2001, Fellman et al. 2010). Calculations based on absorbance measurements at key wavelengths such as 254, 340, and 365 nm can also be used to indicate relative aromaticity and molecular weight (Dahlén et al. 1996, Curtis and Schindler 1997, Weishaar et al. 2003).

Due to the complex nature and variety of the source materials and the chemical and biological processes involved in deriving DOM, no two waterways will contain DOM of exactly the same quality, although DOMs may be similar (Thomas 1997, Jaffé et al. 2008, Lambert et al. 2015). Similar DOMs may contain similar amounts of the dominant components; humic, fulvic and/or protein-like material, however these will slightly vary in their aromaticity, molecular weight and abundance of various functional groups (Thomas 1997, Jaffé et al. 2008, Lambert et al. 2015). DOM concentration and composition have also been linked to a number of abiotic and biotic processes such as rainfall, pH, temperature, land cover and abundance and composition of microbiota (Larson et al. 2014, Winterdahl et al. 2014, Lambert et al. 2015, Weyhenmeyer et al. 2016). Since differences in DOM concentration and quality have been linked previously with key water quality parameters such as pH, differences are likely to occur between naturally acidic and circumneutral waters. Naturally acidic waters are found world-wide and are optically darker, and generally contain higher amounts of humic substances than circumneutral waters (Thurman 1985, Holland et al. 2012, Holland et al. 2014) and thus are likely to contain DOM of a different quality than waters of neutral pH. However, comparisons with regard to DOM quality between naturally acidic waters and circumneutral waters are currently lacking and thus needed. Ground waters also contain DOM that differs to that of surface waters (Chen et al. 2010, Inamdar et al. 2012) and thus surface waters fed directly from groundwater may also contain DOM of a distinct nature. Limited information is also available on the quality of freshwater DOM isolated from the southern hemisphere compared to the information available for the northern hemisphere (McDonald et al. 2007, Petrone et al. 2011, Burrows et al. 2013, Siebers et al. 2016). DOM in Australian waters is likely to differ to other countries given that Australian flora is not found on any other continent (Shugart et al. 2011) and its soils are more weathered and do not result from glaciation like those in the northern hemisphere (Paton et al. 1995). This is the first study to optically characterize CDOM from various pristine freshwater types (naturally acidic, circumneutral and groundwater-fed) in Australia and to demonstrate that clear distinctions in CDOM quality exist among them.

2. Materials and Methods

2.1 Study Sites

DOM was collected from nine sites within tropical and temperate Australia, over two seasons (winter or spring: July-September 2016) and (summer or autumn: February-May 2017) from various freshwater types (naturally acidic, circumneutral and groundwater fed (Figure 1). Within each seasonal sampling period, sampling was conducted over several months due to the logistical difficulties in travelling long distances to remote and often poorly accessible sites. Three replicate samples were collected from each site, and each sampling run. Sites S1-3 represent tropical circumneutral waters, S4 represents a tropical system fed by groundwater, S5 represents a tropical naturally acidic waterway, S6-7 are temperate circumneutral systems and S8-9 are temperate naturally acidic waterways. An important criterion for site selection was that they should have few (if any) anthropogenic inputs, which limited our choices. All finally selected sites were located within National Parks or reserves except S7, which was downstream from Budderoo National Park. All sites differed with respect to the surrounding vegetation and the amount of macrophytes present. S1 (water storage dam) and S2 (natural

billabong) were dominated by *Eucalyptus* and *Melaleuca*, with high amounts of submerged and free-floating macrophytes. S3 (rainforest stream) and S4 (Crater Lake fed via groundwater with no inflow from other waterways) were surrounded by tropical rainforest. S5 (wallum stream) was a riparian zone consisting of subtropical rainforest with surrounding wallum heathlands. S6 (lake) was surrounded by rugged Australian bush dominated by *Eucalyptus* trees. S7 (stream) flowed through temperate rainforest into pastoral land. S8 (tannin stained stream) and S9 (natural lake) were in the midst of buttongrass plains on top of peatlands.

Figure 1: Map showing the location of sampling sites, photos of sites and the colour of the DOC concentrate from each site.

2.2 Physicochemical Parameters

Physicochemical parameters were measured in the field using a Global Water WQ770 Turbidity meter and YSI Professional Plus Series Meter (YSI Inc/Xylem Inc, USA) with pH, conductivity, oxygen, and temperature probes calibrated as per manufacturer's instructions. Samples (60 ml) for determination of dissolved organic carbon (DOC) concentration were collected from the surface waters (top 10 cm) from all sites and immediately filtered in the field through 0.45 μm filters (Sartorius cellulose acetate) and placed in amber glass bottles on ice for transport to the laboratory. DOC concentrations were analysed using a Shimadzu TOC-V_{CSH/CSN}, TOC/TN analyser with autosampler as recommended in US EPA method 415.3. Potassium hydrogen phthalate was used as a standard. There were three replicate samples from each site which were analysed three times each or until the standard deviation between the three replicates was less than 10% and the mean of those three replicates was used in subsequent analyses. Ultrapure water (18.2 M Ω /cm, Milli-Q, Millipore) was used as the blank and to flush equipment between samples.

DOM concentrates were collected using a portable reverse osmosis machine (Compact L300; RO water Australia) following the recommendations in Serkiz and Perdue (1990). Concentrates were placed in 5-L bottles on ice in the dark for transport back to the laboratory. Excess salts were removed via the use of a cation exchange resin (Amberlite IR20). On arrival, concentrates were stored in a cold room in the dark until analysis.

2.3 CDOM quality

CDOM quality was characterized in concentrates using a range of absorbance and fluorescence measurements as previously described (Al-Reasi et al. 2011, Al-Reasi et al. 2012, Al-Reasi et al. 2013). There was no difference between ambient water samples and DOM concentrates in regards to abundance of dominant components and CDOM quality indices. To limit the effect that differences in pH may have on CDOM quality parameters, DOM concentrates were set to $\text{pH } 7.0 \pm 0.2$ using 0.1 M NaOH or HCl, and adjusted to a concentration of 10 mg/L C with ultrapure water (18.2 M Ω /cm). Absorbance was measured in three replicate samples using a 1-cm quartz cuvette in a Shimadzu UV-1800 Spectrophotometer. The specific absorbance coefficient at 340 nm (SAC_{340}) and the specific UV absorbance coefficient at 254 nm (SUVA_{254}) were calculated using equations 1 and 2 and gave an indication of aromaticity (Curtis and Schindler 1997, Weishaar et al. 2003). The Molecular weight was indicated by the absorbance ratio at 254 nm to 365nm ($\text{Abs}_{254/365}$) (Dahlén et al. 1996) and spectral slope by $S_{275-295}$ (Helms 2008).

Equation 1: $\text{SAC}_{340} = 2.303 \times \text{absorbance at 340 nm} / \text{DOC concentration}$

Equation 2: $\text{SUVA}_{254} = \text{absorbance at 254nm} / \text{DOC concentration}$.

Fluorescence excitation emission (FEEM) scans were performed using a 1-cm quartz fluorimeter cuvette in an HORIBA Aqualog® (HORIBA Scientific). FEEM scans along with simultaneous absorbance measurements were conducted on all samples, with excitation wavelengths in 10-nm steps between 250–450 nm, and emission wavelengths of 250–620 nm. The absorbance of a blank (ultrapure water) was automatically subtracted from each sample. Inner filter effects along with the 1st and 2nd order Rayleigh and Raman scatter were also removed using the HORIBA software. The excitation emission matrices were then analysed in MATLAB R2014b (MathWorks, Inc. © 1994–2016) to produce three-dimensional FEEMs and contour plots. The fluorescence Index (FI) was determined using equation 3 to provide an indicator of CDOM source: allochthonous and/or autochthonous (McKnight et al. 2001, Cory et al. 2010).

Equation 3: $\text{FI} = \text{emission 470} / \text{emission 520 at excitation 370 nm}$

The FEEMs were modelled using parallel factor analysis (PARAFAC) (PLS-toolbox in MATLAB: Eigenvectors Research Inc, WA, USA) (Al-Reasi et al. 2012, Johannsson et al. 2016). The model was validated following the recommendations in Murphy et al. (2013). No clear consistent patterns and peaks were visible in the residual plots, core consistency was 69%, and split-half analysis results were consistent with the model. The PARAFAC model was also compared to those in the literature using the Openfluor database (Murphy et al. 2014a).

2.4 Statistical Analyses

Two-way ANOVAs were used to assess differences in CDOM indices and components among freshwater types (temperate and tropical naturally acidic; temperate and tropical circumneutral and the tropical groundwater-fed site) over different seasons (winter/spring;

summer/autumn) (IBM SPSS 24). Principal Component Analysis (PCA) was conducted on normalised CDOM quality data to determine trends among waters using PRIMER 7 software (PRIMER 7, PRIMER-E Ltd. Lutton, UK). The PCA plot was not rotated. Similarity between sites was determined using Euclidean distance, followed by cluster analysis on the group average using the PRIMER 7 software. Differences in CDOM quality between water type, climatic regions and season were investigated using the Euclidean distance as the similarity measure for the PERMANOVA within the PRIMER 7 software package. Pair-wise comparisons were conducted using the Monte Carlo simulation. Pearson product moment correlations were used to determine significant relationships (P values of ≤ 0.05) between water quality and CDOM characteristics (SigmaPlot 13.0).

3. Results and Discussion

Water quality parameters for each site are shown in Table 1. Sites S5, S8 and S9 were naturally acidic (pH values < 6), while circumneutral sites had pH values ≥ 6.8 . S4 is also the only site completely fed by groundwater. DOC concentrations varied among sites and ranged from 1.4 to 14 mg/L, with no clear pattern between different climatic regions or freshwater types (Table 1).

Three main components of CDOM were determined via PARAFAC analysis (Figure 2). The three components (C1, C2 and C3) described 99.08% of the variability within the data. The PARAFAC model was compared against other published models in the Openfluor database, with $\geq 95\%$ similarity match to 31 out of 70 models. C1 has been previously reported in 21 models, C2 in 15 and C3 in 4 models (Supplementary Table 1). The components match others previously reported from a variety of different aquatic environments (streams, rivers, groundwater, wastewater, marine) around the globe (Supplementary Table 1).

Figure 2. Spectra of the three main components determined via PARAFAC analysis, used to describe the organic matter present within different Australian freshwaters. These three components, a) humic-like, b) fulvic-like and c) protein-like, explained 99.08% of the variation between samples, with a core consistency of 69%.

Table 1. Physicochemical parameters measured at each site during winter/spring and summer/autumn

Site	Freshwater Type	Season	Temp (°C)	Specific Conductance ^a (μS/cm)	DO ^b (% sat)	TDS ^c (mg/L)	pH	Turbidity (NTU)	DOC ^d (mg/L)
S1	Tropical Circumneutral	winter/spring	31	100	110	65.0	8.3	10	14
S2	Tropical Circumneutral	winter/spring	26	32.5	91.0	21.1	6.8	0.0	6.9
S3	Tropical Circumneutral	winter/spring	22	60.8	88.5	39.7	6.8	0.40	1.9
S4	Groundwater fed	winter/spring	22	49.4	78.4	17.9	7.4	0.0	1.4
S5	Tropical Naturally acidic	winter/spring	16	124	85.6	80.3	4.6	0.90	12
S6	Temperate Circumneutral	winter/spring	14	83.5	78.6	54.3	6.9	2.5	6.2
S7	Temperate Circumneutral	winter/spring	12	161	102	105	7.1	0.78	6.5
S8	Temperate Naturally acidic	winter/spring	6.2	19.1	94.5	12.4	4.9	0.0	8.9
S9	Temperate Naturally acidic	winter/spring	7.0	17.8	90.1	11.1	5.7	0.0	5.5

Table 1 (cont). Physicochemical parameters measured at each site during winter/spring and summer/autumn

S1	Tropical Circumneutral	summer/autumn	29	65.0	70.7	NA	7.8	0.0	6.0
S2	Tropical Circumneutral	summer/autumn	26	19.0	48.3	NA	6.8	5.0	4.6
S3	Tropical Circumneutral	summer/autumn	26	48.2	81.5	31.2	7.4	14	3.2
S4	Groundwater fed	summer/autumn	28	49.2	79.3	31.9	7.8	7.5	3.1
S5	Tropical Naturally acidic	summer/autumn	17	206	78.7	134	4.3	0.0	11
S6	Temperate Circumneutral	summer/autumn	26	88.1	88.2	57.3	7.6	0.0	5.4
S7	Temperate Circumneutral	summer/autumn	23	223	80.9	145	7.4	1.2	4.9
S8	Temperate Naturally acidic	summer/autumn	10	25.7	82.0	16.9	4.9	0.0	11
S9	Temperate Naturally acidic	summer/autumn	13	17.1	92.4	11.1	5.4	0.0	7.0

^a = Specific Conductance at 25°C; ^b = Dissolved Oxygen % saturation; ^c = Total Dissolved Solids ^d = Dissolved Organic Carbon.

C1 has previously been related to high molecular weight, aromatic humic material defined as peaks A and C in the literature, most abundant in wetlands and forested environments (Coble 1996, Fellman et al. 2010, Coble et al. 2014) (Supplementary Table 1). Naturally acidic waterways were shown to contain higher abundances of this component (Figure 3). C2 (similar to Peak M in the literature) is often associated with newly formed, low molecular weight CDOM, derived from biological activity (Fellman et al. 2010, Coble, Spencer et al. 2014), and is likely to represent fulvic acids (Supplementary Table 1). Fulvic acids fluoresce at shorter wavelengths and at lower emissions than humic acids (Sierra et al. 2005). Fulvic-like CDOM was the dominant component of organic carbon within circumneutral waters in these study sites (Figure 3).

Figure 3. Percent relative abundance of each of the three PARAFAC components associated with the organic matter from different Australian freshwaters; 1. Winter/Spring; 2. Summer/Autumn. NA = Naturally Acidic; C = Circumneutral and G = groundwater fed. Data expressed as mean $\pm$ SD. Upper case letters indicate a significant difference between freshwater types and * indicates a significant difference between seasons within the same freshwater type. A-D: humic like; M-P: fulvic-like; R-T: protein-like.

C3 is often associated with autochthonous proteinaceous material such as amino acids, and/or free or bound proteins (Fellman et al. 2010) (Supplementary Table 1). Therefore it is likely to reflect the proteinaceous material produced by microbes and phytoplankton within the water bodies. C3 is also often associated with wastewater inputs (Coble et al. 2014), however, this is unlikely to be the source within this study as all sites (except S7) were located within national parks free from anthropogenic inputs. The groundwater-fed site contained higher amounts of protein-like CDOM compared to the naturally acidic and circumneutral waters (Figure 3). Groundwater generally contains higher amounts of protein-like CDOM than surface waters (Chen et al. 2010, Inamdar et al. 2012).

Aromaticity, as indicated by SAC_{340} and $SUVA_{254}$ values, varied among freshwater types: naturally acidic, circumneutral and the groundwater-fed sites (Figure 4). Aromaticity was greatest for CDOM isolated from the tropical naturally acidic sites and lowest in the groundwater-fed site (Figure 4). $SUVA_{254}$ values for waterways sampled within this study were within the range $0 - 6 \text{ L mg C}^{-1} \text{ m}^{-1}$ generally reported for surface waters (Hansen et al. 2016) (Figure 4). Tropical and temperate naturally acidic waters had values towards the upper end of the range found in the literature (as high as $5.7 \text{ mg C}^{-1} \text{ m}^{-1}$), and the groundwater-fed site had values towards the lower end (as low as $0.58 \text{ mg C}^{-1} \text{ m}^{-1}$) (Table 2 and Figure 4). SAC_{340} values in this study ranged from $2 - 50 \text{ cm}^2 \text{ mg}^{-1}$, with highest values recorded for tropical naturally acidic waters and lowest for the groundwater-fed site (Figure 4). SAC_{340} values reported in the literature range between $1 - 73 \text{ cm}^2 \text{ mg}^{-1}$, with most waters reported as having values less than $40 \text{ cm}^2 \text{ mg}^{-1}$ (Al-Reasi et al. 2011, Al-Reasi et al. 2012, Johannsson et al. 2016) (Table 2 and Figure 4). Exceptions to this are the naturally acidic blackwaters of the Amazon (Duarte et al. 2016, Holland et al. 2017). CDOM from the small number of naturally acidic waters (especially in Australia and Brazil) appear to be more aromatic in nature as indicated by higher SAC_{340} and $SUVA_{254}$ values compared to other regions (Table 2).

Figure 4. DOC quality indices for different Australian waters. NA = Naturally Acidic; C = Circumneutral and G = Groundwater fed. Data expressed as mean $\pm$ SD. Capital letters indicate significant difference between freshwater types and lower case letters indicate significant difference between seasons within the same freshwater type.

Table 2: Various absorbance indices (ranges) obtained during this study for Australian DOC compared with results obtained from other countries.

Country	SAC ₃₄₀	FI	SUVA ₂₅₄	Abs _{254/365}	S ₂₇₅₋₂₉₅
Australia ^a	2.3 - 50	1.19 - 1.76	0.58 - 5.7	3.7 - 8.3	0.01 - 0.03
Brazil ^b	30 - 73	1.10 - 1.57	0.90 - 7.4	3.4 - 4.6	0.02 - 0.04
Canada ^c	3.7 - 39	1.19 - 2.54	1.4 - 4.4	2.5 - 15	
Norway ^d		1.13 - 1.33	4.9		
USA ^e	13 - 49	1.13 - 1.75	0 - 7.5		0.01 - 0.03
Congo ^f		1.22 - 1.45	3.1 - 3.6		0.01 - 0.02
		1.65 - 1.77	0.19 - 3.1		0.01 -
Uruguay ^g					0.02
Germany ^h		1.30 - 1.90	2.0 - 3.2		0.01- 0.02

^aData collected from this study; Cawley et al 2012; ^bData estimated from tables and figures in Johannsson et al. (2016); Duarte et al. (2016); Holland et al. 2017; Brandão et al. 2016; ^cAl-Reasi et al. (2011, 2012, 2013); Glover et al (2005); Schwartz et al. (2004); Williams et al. 2010 ^dAl-Reasi et al 2011; Gjessing et al. (1998); Jaffe et al. (2008); ^eHelm et al. 2008; Yamashita et al. 2010b; Al-reasi et al. 2011; ^fSpencer et al. 2010; ^gAmaral et al. 2016; ^hGraeber et al. 2012.

FI values varied between 1.19 - 1.75 and fell within the range reported for other surface waters (Table 2). FI values were lower in naturally acidic and groundwater-fed waters compared to circumneutral waters, indicating CDOM was of a more allochthonous terrestrial nature (Figure 4). Naturally acidic waters were also characterized by CDOM of a higher molecular weight compared to circumneutral and groundwater-fed sites as indicated from the lower Abs_{254/365} ratio (average Abs_{254/365}: naturally acidic = 4.13; circumneutral = 6.19; groundwater-fed = 8.18) and the higher spectral slope S₂₇₅₋₂₉₅ (average S₂₇₅₋₂₉₅: naturally acidic = 0.0173; circumneutral = 0.0170; groundwater-fed = 0.0142)(Figure 4). Given that CDOM is mostly dominated by humic substances (humic and fulvic acids) it is not surprising that they dominate the quality of the CDOM. Humic-like CDOM significantly relates to greater aromaticity and molecular weight and to decreases in fulvic and protein-like components, and to lesser autochthonous DOM (Table 3). This is not surprising given that the humic-like components are likely to represent humic acids (Thurman et al. 1982, Thurman 1985, Perminova et al. 2003). Increased concentrations of fulvic-like CDOM were related to higher FI values and lower humic-like and protein-like CDOM (Table 3). This was expected as the FI targets the region in the EEM commonly associated with fulvic-like CDOM (Cory et al. 2010). As expected aromaticity (SAC₃₄₀ and SUVA₂₅₄) was negatively related to protein-like DOM as proteins will contain significantly less aromatic rings than the humic substances.

Table 3. Pearson Correlation coefficients relating DOC quality parameters to each other and water quality. *P* values ≤ 0.05 are highlighted in bold and suggest a significant relationship.

		pH	Abs _{254/365} (molecular weight)	FI (source)	SUVA ₂₅₄ (aromaticity)	SAC ₃₄₀ (aromaticity)	Protein- like	Humic- like	Fulvic- like
Fulvic-like	Correlation coefficient	0.366	-0.062	0.734	-0.164	-0.24	-0.375	-0.321	
	P value	0.135	0.653	<0.001	0.237	0.081	0.005	0.017	
Humic-like	Correlation coefficient	-0.962	-0.851	-0.465	0.942	0.971	-0.757		
	P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
Protein-like	Correlation coefficient	0.69	0.877	-0.046	-0.809	-0.785			
	P value	0.001	<0.001	0.744	<0.001	<0.001			
SAC ₃₄₀ (aromaticity)	Correlation coefficient	-0.935	-0.848	-0.428	0.99				
	P value	<0.001	<0.001	0.001	<0.001				
SUVA ₂₅₄ (aromaticity)	Correlation coefficient	-0.909	-0.828	-0.394					
	P value	<0.001	<0.001	0.003					
FI (source)	Correlation coefficient	0.532	-0.063						
	P value	0.023	0.653						
Abs _{254/365} (molecular weight)	Correlation coefficient	0.796							
	P value	<0.001							

Clear separation of naturally acidic, circumneutral and groundwater-fed sites were shown within the PCA plot, indicating that CDOM quality differs among freshwater types (Permanova, Pseudo- $F_{2,44}=127.02$, $P=0.001$) (Figure 5). Naturally acidic waters were characterized by a higher abundance of humic-like CDOM, more allochthonous in nature (as indicated by the fluorescence index), of higher aromaticity (as indicated by SAC_{340} values and $SUVA_{254}$ values) and of greater molecular weight (as indicated by $Abs_{254/365}$ values and $S_{275-295}$) than circumneutral waters and the groundwater-fed site (Figure 3, 4 and 5). The tropical naturally acidic site contained CDOM of a slightly higher molecular weight, which was more aromatic, and allochthonous in nature, with higher amounts of humic-like components than the temperate naturally acidic waters. Significant relationships between pH and a number of CDOM quality parameters were found (Table 3). Protein-like CDOM, FI and $Abs_{254/365}$ had a positive relationship with pH (Table 3), with circumneutral and groundwater-fed sites characterized by higher abundance of protein-like CDOM, of a more autochthonous nature and lower molecular weight. On the other hand humic-like CDOM, SAC_{340} , and $SUVA_{254}$ displayed a negative relationship with pH (Table 3), supporting the idea that naturally acidic waters contain a higher abundance of humic-like, allochthonous, aromatic, high molecular weight CDOM (Table 3). The significant negative relationship between pH, humic-like CDOM, and aromaticity ($SUVA_{254}$ and SAC_{340} values) is also not surprising given that the natural acidity within these waters is caused by the input of large amounts of humic acids (Collier et al. 1990, Holland et al. 2012, Holland et al. 2014). Humic acids leach from the surrounding peat and vegetation into waters of low buffering capacity, thus decreasing pH naturally within these systems (Collier et al. 1990, Holland et al. 2012, Holland et al. 2014). The low pH and high CDOM stain the water brown in colour, decreasing light penetration, limiting primary production and decomposition of the DOM (Schoenberg et al. 1990, Thomas 1997, Steinberg et al. 2006). As a result, this limits the amount of autochthonous DOM produced and allows for the persistence of the more aromatic, humic-like CDOM of a higher molecular weight within these waters.

Figure 5: Principal Component Analysis (PCA) plot showing the separation of sites with regard to DOC quality. Clusters indicates similarity between samples based on Euclidean distance. Up-right triangles = tropical naturally acidic; upside down triangle = temperate naturally acidic; square = temperate circumneutral; diamond = tropical circumneutral; circle= groundwater fed. Closed symbols indicate winter/spring and open symbols indicate summer/autumn.

CDOM quality at each site varied significantly between seasons (Permanova, Pseudo- $F_{1,44} = 12.58$, $P = 0.001$) with a significant interaction between freshwater type and season (Permanova, Pseudo- $F_{2,44} = 3.38$, $P = 0.007$) (Figure 5). CDOM quality was similar between seasons within the circumneutral waters, but significantly different within the naturally acidic and groundwater-fed sites. Significant differences in percent relative abundance of humic and fulvic components were shown between seasons within the tropical naturally acidic waters, and within groundwater-fed waters. CDOM also appeared to be of a more autochthonous nature within the tropical and temperate naturally acidic and groundwater-fed sites as indicated by higher FI values during the summer/autumn sampling. CDOM was significantly less aromatic (as indicated by SAC_{340} , $SUVA_{254}$) at all sites except the temperate circumneutral waters during summer/autumn (Figure 4). Molecular weights (as indicated by $Abs_{254/365}$ ratio and $S_{275-295}$) were generally not significantly different during the winter/spring sampling relative to the summer/autumn campaign (average $Abs_{254/365}$: winter/spring = 5.8; summer/ autumn 5.4; average $S_{275-295}$: winter/spring = 0.016; summer/ autumn 0.017) (Figure 4). An exception to this was the significant differences in $S_{275-295}$ within the groundwater-fed water. Seasonal variation in a number of DOM quality indices have been reported previously from a number of different studies (Jaffe et al. 2008, Wen et al. 2016, Awad et al. 2017, Holland et al. 2017). Phytoplankton and periphyton growth is known to increase at warmer temperatures (Hennemann and Petrucio 2010, Schabhüttl et al. 2013). The significant difference in aromaticity and abundance of humic-like and fulvic-like components, respectively, suggests that higher amounts of autochthonous DOM produced during the summer/autumn season may have led to differences in CDOM shown in this study. Photodegradation may also play a role (Winterdahl et al. 2014).

In this study, a greater difference in CDOM quality was associated with the type of freshwater (naturally acidic, circumneutral or fed via groundwater), than with the influences of climate and seasons. Our conclusions are based on nine sites (5 circumneutral, 3 naturally acidic and 1 groundwater fed) over two seasonal sampling runs. While the number of sites was limited by logistic constraints (see Section 2.1), they do represent a good cross-section of water types with, great variation in quality of CDOM between different freshwater types as indicated in the PCA (Figure 5), and the significant relationships between CDOM quality and pH (Table 3). Further research is needed to determine whether these distinct signatures (dominant component, aromaticity and MW) shown for naturally acidic, circumneutral and groundwater-fed systems extend to other freshwaters within Australia and around the globe.

4. Conclusions

- CDOM quality varies among freshwater types (naturally acidic, circumneutral and groundwater-fed).
- Naturally acidic waters are dominated by humic-like DOM which is highly aromatic and of a greater molecular weight compared to DOC from circumneutral waters which is dominated by fulvic-like DOM and is less aromatic. Groundwater-fed sites contained more protein-like DOM of the lowest aromaticity and molecular weight.
- CDOM quality varies between seasons with a trend towards less aromatic, lower molecular weight and more autochthonous CDOM during the summer/autumn sampling compared to winter/spring.

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6. References

- Al-Reasi, H.A., Smith, D.S. and Wood, C.M. (2012) Evaluating the ameliorative effect of natural dissolved organic matter (DOM) quality on copper toxicity to *Daphnia magna*: Improving the BLM. *Ecotoxicology* 21(2), 524-537.
- Al-Reasi, H.A., Wood, C.M. and Smith, D.S. (2011) Physicochemical and spectroscopic properties of natural organic matter (NOM) from various sources and implications for ameliorative effects on metal toxicity to aquatic biota. *Aquatic Toxicology* 103(3-4), 179-190.
- Al-Reasi, H.A., Wood, C.M. and Smith, D.S. (2013) Characterization of freshwater natural dissolved organic matter (DOM): Mechanistic explanations for protective effects against metal toxicity and direct effects on organisms. *Environment International* 59, 201-207.
- Amaral, V., Graeber, D., Calliari, D. and Alonso, C. (2016) Strong linkages between DOM optical properties and main clades of aquatic bacteria. *Limnology and Oceanography* 61(3), 906-918.
- Awad, J., van Leeuwen, J., Chow, C.W.K., Smernik, R.J., Anderson, S.J. and Cox, J.W. (2017) Seasonal variation in the nature of DOM in a river and drinking water reservoir of a closed catchment. *Environmental Pollution* 220, 788-796.
- Brandão, L.P.M., Staehr, P.A. and Bezerra-Neto, J.F. (2016) Seasonal changes in optical properties of two contrasting tropical freshwater systems. *Journal of Limnology* 75(3), 508-519.
- Burrows, R.M., Fellman, J.B., Magierowski, R.H. and Barmuta, L.A. (2013) Allochthonous dissolved organic matter controls bacterial carbon production in old-growth and clearfelled headwater streams. *Freshwater Science* 32(3), 821-836.
- Catalá, T.S., Reche, I., Fuentes-Lema, A., Romera-Castillo, C., Nieto-Cid, M., Ortega-Retuerta, E., Calvo, E., Álvarez, M., Marrasé, C., Stedmon, C.A. and Álvarez-Salgado, X.A. (2015) Turnover time of fluorescent dissolved organic matter in the dark global ocean. *Nature Communications* 6, 5986.
- Cawley, K.M., Ding, Y., Fourqurean, J. and Jaffé, R. (2012) Characterising the sources and fate of dissolved organic matter in Shark Bay, Australia: a preliminary study using optical properties and stable carbon isotopes. *Marine and Freshwater Research* 63(11), 1098-1107.
- Chen, M., Price, R.M., Yamashita, Y. and Jaffé, R. (2010) Comparative study of dissolved organic matter from groundwater and surface water in the Florida coastal Everglades using multi-dimensional spectrofluorometry combined with multivariate statistics. *Applied Geochemistry* 25(6), 872-880.
- Coble, P., Spencer, R., Baker, A. and Reynolds, D. (2014) *Aquatic Organic Matter Fluorescence*. Coble, P., Lead, J., Baker, A., Reynolds, D. and Spencer, R. (eds), Cambridge University Press, New York.
- Coble, P.G. (1996) Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chemistry* 51(4), 325-346.
- Collier, K.J., Ball, O.J., Graesser, A.K., Main, M.R. and Winterbourn, M.J. (1990) Do organic and anthropogenic acidity have similar effects on aquatic fauna? *Oikos* 59(1), 33-38.
- Cory, R.M., Miller, M.P., McKnight, D.M., Guerard, J.J. and Miller, P.L. (2010) Effect of instrument-specific response on the analysis of fulvic acid fluorescence spectra. *Limnology and Oceanography: Methods* 8(2), 67-78.
- Curtis, P.J. and Schindler, D.W. (1997) Hydrologic control of dissolved organic matter in low-order Precambrian Shield lakes. *Biogeochemistry* 36(1), 125-138.
- Dahlén, J., Bertilsson, S. and Pettersson, C. (1996) Effects of UV-A irradiation on dissolved organic matter in humic surface waters. *Environment International* 22(5), 501-506.
- Duarte, R.M., Smith, D.S., Val, A.L. and Wood, C.M. (2016) Dissolved organic carbon from the upper Rio Negro protects zebrafish (*Danio rerio*) against ionoregulatory disturbances caused by low pH exposure. *Scientific Reports* 6, 20377.

- Fellman, J.B., Hood, E. and Spencer, R.G.M. (2010) Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. *Limnology and Oceanography* 55(6), 2452-2462.
- Frimmel, F.H. (1998) Characterization of natural organic matter as major constituents in aquatic systems. *Journal of Contaminant Hydrology* 35(1-3), 201-216.
- Garcia, R.D., Reissig, M., Queimaliños, C.P., Garcia, P.E. and Dieguez, M.C. (2015) Climate-driven terrestrial inputs in ultraoligotrophic mountain streams of Andean Patagonia revealed through chromophoric and fluorescent dissolved organic matter. *Science of The Total Environment* 521-522(Supplement C), 280-292.
- Graeber, D., Gelbrecht, J., Pusch, M.T., Anlanger, C. and von Schiller, D. (2012) Agriculture has changed the amount and composition of dissolved organic matter in Central European headwater streams. *Science of The Total Environment* 438(Supplement C), 435-446.
- Hansen, A.M., Kraus, T.E.C., Pellerin, B.A., Fleck, J.A., Downing, B.D. and Bergamaschi, B.A. (2016) Optical properties of dissolved organic matter (DOM): Effects of biological and photolytic degradation. *Limnology and Oceanography* 61(3), 1015-1032.
- Helms, J.R., Stubbins, A., Ritchie, J.D., Minor, E.c., Kieber, D.J. and Mopper, K. (2008) Absorption spectral slopes and slope ratios as indicators of molecular weight, source and photobleaching of chromophoric dissolved organic matter, *Limnology and Oceanography* 53(3), 955-969.
- Hennemann, M.C. and Petrucio, M.M. (2010) Seasonal phytoplankton response to increased temperature and phosphorus inputs in a freshwater coastal lagoon, Southern Brazil: a microcosm bioassay. *Acta Limnologica Brasiliensia* 22, 295-305.
- Holland, A., Duivenvoorden, L.J. and Kinnear, S.H.W. (2012) Naturally acidic waterways: Conceptual food webs for better management and understanding of ecological functioning. *Aquatic Conservation: Marine and Freshwater Ecosystems* 22(6), 836-847.
- Holland, A., Duivenvoorden, L.J. and Kinnear, S.H.W. (2014) Effect of key water quality variables on macroinvertebrate and fish communities within naturally acidic wallum streams. *Marine and Freshwater Research* 66(1), 50-59.
- Holland, A., Wood, C.M., Smith, D.S., Correia, T.G. and Val, A.L. (2017) Nickel toxicity to cardinal tetra (*Paracheirodon axelrodi*) differs seasonally and among the black, white and clear river waters of the Amazon basin. *Water Research* 123, 21-29.
- Inamdar, S., Finger, N., Singh, S., Mitchell, M., Levia, D., Bais, H., Scott, D. and McHale, P. (2012) Dissolved organic matter (DOM) concentration and quality in a forested mid-Atlantic watershed, USA. *Biogeochemistry* 108(1), 55-76.
- Jaffé, R., McKnight, D., Maie, N., Cory, R., McDowell, W.H. and Campbell, J.L. (2008) Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties. *Journal of Geophysical Research: Biogeosciences* 113(G4), 2156-2202.
- Johannsson, O.E., Smith, D.S., Sadauskas-Henrique, H., Cimprich, G., Wood, C.M. and Val, A.L. (2016) Photo-oxidation processes, properties of DOC, reactive oxygen species (ROS), and their potential impacts on native biota and carbon cycling in the Rio Negro (Amazonia, Brazil). *Hydrobiologia*, 1-23.
- Lambert, T., Darchambeau, F., Bouillon, S., Alhou, B., Mbega, J.D., Teodoru, C.R., Nyoni, F.C., Massicotte, P. and Borges, A.V. (2015) Landscape control on the spatial and temporal variability of chromophoric dissolved organic matter and dissolved organic carbon in large African rivers. *Ecosystems* 18(7), 1224-1239.
- Lambert, T., Teodoru, C.R., Nyoni, F.C., Bouillon, S., Darchambeau, F., Massicotte, P. and Borges, A.V. (2016) Along-stream transport and transformation of dissolved organic matter in a large tropical river. *Biogeosciences* 13, 2727-2274.
- Larson, J.H., Frost, P.C., Xenopoulos, M.A., Williams, C.J., Morales-Williams, A.M., Vallazza, J.M., Nelson, J.C. and Richardson, W.B. (2014) Relationships between land cover and dissolved organic matter change along the river to lake transition. *Ecosystems* 17(8), 1413-1425.

- McDonald, S., Pringle, J.M., Bishop, A.G., Prenzler, P.D. and Robards, K. (2007) Isolation and seasonal effects on characteristics of fulvic acid isolated from an Australian floodplain river and billabong. *Journal of Chromatography A* 1153(1-2), 203-213.
- McKnight, D.M., Boyer, E.W., Westerhoff, P.K., Doran, P.T., Kulbe, T. and Andersen, D.T. (2001) Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography* 46(1), 38-48.
- Murphy, K., Stedmon, C., Wenig, P., and Bro, R. (2014a). OpenFluor—A spectral database of auto-fluorescence by organic compounds in the environment. *Analytical Methods*, 6, 658-661.
- Murphy, K., Bro, R. and Stedmon, C. (2014b) *Aquatic Organic Matter Fluorescence*: Cambridge Environmental Chemistry Series, Cambridge University Press, Cambridge.
- Murphy, K.R., Hambly, A., Singh, S., Henderson, R.K., Baker, A., Stuetz, R. and Khan, S.J. (2011) Organic matter fluorescence in municipal water recycling schemes: toward a unified PARAFAC model. *Environmental Science & Technology* 45(7), 2909-2916.
- Murphy, K.R., Ruiz, G.M., Dunsmuir, W.T.M. and Waite, T.D. (2006) Optimized parameters for fluorescence-based verification of ballast water exchange by ships. *Environmental Science & Technology* 40(7), 2357-2362.
- Murphy, K.R., Stedmon, C.A., Waite, T.D. and Ruiz, G.M. (2008) Distinguishing between terrestrial and autochthonous organic matter sources in marine environments using fluorescence spectroscopy. *Marine Chemistry* 108(1-2), 40-58.
- Murphy, K.R., Stedmon, C.A., Graeber, D. and Bro, R. (2013) Fluorescence spectroscopy and multi-way techniques. PARAFAC. *Analytical Methods* 5(23), 6557-6566.
- Perminova, I.V., Frimmel, F.H., Kudryavtsev, A.V., Kulikova, N.A., Abbt-Braun, G., Hesse, S. and Petrosyan, V.S. (2003) Molecular weight characteristics of humic substances from different environments as determined by size exclusion chromatography and their statistical evaluation. *Environmental Science & Technology* 37(11), 2477-2485.
- Paton, T., Humphreys, G., and Mitchell, P. (1995) *Soils a New Global View*, CRC Press, New York
- Petrone, K.C., Fellman, J.B., Hood, E., Donn, M.J. and Grierson, P.F. (2011) The origin and function of dissolved organic matter in agro-urban coastal streams. *Journal of Geophysical Research: Biogeosciences* 116(1), 1-13.
- Osburn, C.L., Handsel, L.T., Peierls, B.L. and Paerl, H.W. (2016) Predicting sources of dissolved organic nitrogen to an estuary from an agro-urban coastal watershed. *Environmental Science & Technology* 50(16), 8473-8484.
- Osburn, C.L. and Stedmon, C.A. (2011) Linking the chemical and optical properties of dissolved organic matter in the Baltic–North Sea transition zone to differentiate three allochthonous inputs. *Marine Chemistry* 126(1), 281-294.
- Schabhöttl, S., Hingsamer, P., Weigelhofer, G., Hein, T., Weigert, A. and Striebel, M. (2013) Temperature and species richness effects in phytoplankton communities. *Oecologia* 171(2), 527-536.
- Schindler, D.W. and Curtis, P.J. (1997) The role of DOC in protecting freshwaters subjected to climatic warming and acidification from UV exposure. *Biogeochemistry* 36(1), 1-8.
- Schoenberg, S.A., Benner, R., Armstrong, A., Sobecky, P. and Hodson, R.E. (1990) Effects of acid stress on aerobic decomposition of algal and aquatic macrophyte detritus: direct comparison in a radiocarbon assay. *Applied and Environmental Microbiology* 56(1), 237-244.
- Serkiz, S.M. and Perdue, E.M. (1990) Isolation of dissolved organic matter from the Suwannee River using reverse osmosis. *Water Research* 24(7), 911-916.
- Siebers, A.R., Pettit, N.E., Skrzypek, G., Fellman, J.B., Dogramaci, S. and Grierson, P.F. (2016) Alluvial ground water influences dissolved organic matter biogeochemistry of pools within intermittent dryland streams. *Freshwater Biology* 61(8), 1228-1241.
- Sierra, M.M.D., Giovanela, M., Parlanti, E. and Soriano-Sierra, E.J. (2005) Fluorescence fingerprint of fulvic and humic acids from varied origins as viewed by single-scan and excitation/emission matrix techniques. *Chemosphere* 58(6), 715-733.

- Shugart, H., and Woodward, F. (2011) *Global change and the terrestrial biosphere*, John Wiley and Sons, West Sussex, UK.
- Søndergaard, M., Stedmon, C.A. and Borch, N.H. (2003) Fate of terrigenous dissolved organic matter (DOM) in estuaries: Aggregation and bioavailability. *Ophelia* 57(3), 161-176.
- Spencer, R.G.M., Hernes, P.J., Ruf, R., Baker, A., Dyda, R.Y., Stubbins, A. and Six, J. (2010) Temporal controls on dissolved organic matter and lignin biogeochemistry in a pristine tropical river, Democratic Republic of Congo. *Journal of Geophysical Research: Biogeosciences* 115(G3), 1-12.
- Stedmon, C.A. and Markager, S. (2005) Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. *Limnology and Oceanography* 50(2), 686-697.
- Stedmon, C.A., Markager, S. and Bro, R. (2003) Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Marine Chemistry* 82(3-4), 239-254.
- Stedmon, C.A., Markager, S., Tranvik, L., Kronberg, L., Slätis, T. and Martinsen, W. (2007a) Photochemical production of ammonium and transformation of dissolved organic matter in the Baltic Sea. *Marine Chemistry* 104(3-4), 227-240.
- Stedmon, C.A., Seredyńska-Sobecka, B., Boe-Hansen, R., Le Tallec, N., Waul, C.K. and Arvin, E. (2011) A potential approach for monitoring drinking water quality from groundwater systems using organic matter fluorescence as an early warning for contamination events. *Water Research* 45(18), 6030-6038.
- Stedmon, C.A., Thomas, D.N., Granskog, M., Kaartokallio, H., Papadimitriou, S. and Kuosa, H. (2007b) Characteristics of dissolved organic matter in baltic coastal sea ice: allochthonous or autochthonous origins? *Environmental Science & Technology* 41(21), 7273-7279.
- Steinberg, C.E.W., Kamara, S., Prokhotskaya, V.Y., ManusadZianas, L., Karasyova, T.A., Timofeyev, M.A., Jie, Z., Paul, A., Meinelt, T., Farjalla, V.F., Matsuo, A.Y.O., Kent Burnison, B. and Menzel, R. (2006) Dissolved humic substances – ecological driving forces from the individual to the ecosystem level? *Freshwater Biology* 51(7), 1189-1210.
- Thomas, J.D. (1997) The role of dissolved organic matter, particularly free amino acids and humic substances, in freshwater ecosystems. *Freshwater Biology* 38(1), 1-36.
- Thurman, E.M. (1985) *Organic geochemistry of Natural Waters*, Springer Netherlands.
- Thurman, E.M., Wershaw, R.L., Malcolm, R.L. and Pinckney, D.J. (1982) Molecular size of aquatic humic substances. *Organic Geochemistry* 4(1), 27-35.
- Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R. and Mopper, K. (2003) Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science & Technology* 37(20), 4702-4708.
- Wen, Z.D., Song, K.S., Zhao, Y., Du, J. and Ma, J.H. (2016) Influence of environmental factors on spectral characteristics of chromophoric dissolved organic matter (CDOM) in Inner Mongolia Plateau, China. *Hydrol. Earth Syst. Sci.* 20(2), 787-801.
- Weyhenmeyer, G.A., Müller, R.A., Norman, M. and Tranvik, L.J. (2016) Sensitivity of freshwaters to browning in response to future climate change. *Climatic Change* 134(1), 225-239.
- Williams, C.J., Frost, P.C. and Xenopoulos, M.A. (2013) Beyond best management practices: pelagic biogeochemical dynamics in urban stormwater ponds. *Ecological Applications* 23(6), 1384-1395.
- Williams, C.J., Yamashita, Y., Wilson, H.F., Jaffé, R. and Xenopoulos, M.A. (2010) Unraveling the role of land use and microbial activity in shaping dissolved organic matter characteristics in stream ecosystems. *Limnology and Oceanography* 55(3), 1159-1171.
- Winterdahl, M., Erlandsson, M., Futter, M.N., Weyhenmeyer, G.A. and Bishop, K. (2014) Intra-annual variability of organic carbon concentrations in running waters: Drivers along a climatic gradient. *Global Biogeochemical Cycles* 28(4), 451-464.
- Wood, C.M., Al-Reasi, H.A. and Smith, D.S. (2011) The two faces of DOC. *Aquatic Toxicology* 105(3-4 SUPPL.), 3-8.

- Yamashita, Y., Boyer, J.N. and Jaffé, R. (2013) Evaluating the distribution of terrestrial dissolved organic matter in a complex coastal ecosystem using fluorescence spectroscopy. *Continental Shelf Research* 66(Supplement C), 136-144.
- Yamashita, Y., Maie, N., Briceño, H. and Jaffé, R. (2010a) Optical characterization of dissolved organic matter in tropical rivers of the Guayana Shield, Venezuela. *Journal of Geophysical Research: Biogeosciences* 115(G1), 1-15.
- Yamashita, Y., Panton, A., Mahaffey, C. and Jaffé, R. (2011) Assessing the spatial and temporal variability of dissolved organic matter in Liverpool Bay using excitation–emission matrix fluorescence and parallel factor analysis. *Ocean Dynamics* 61(5), 569-579.
- Yamashita, Y., Scinto, L.J., Maie, N. and Jaffé, R. (2010b) Dissolved organic matter characteristics across a subtropical wetland's landscape: application of optical properties in the assessment of environmental dynamics. *Ecosystems* 13(7), 1006-1019
- Zhao, Y., Song, K., Li, S., Ma, J. and Wen, Z. (2016) Characterization of CDOM from urban waters in Northern-Northeastern China using excitation-emission matrix fluorescence and parallel factor analysis. *Environmental Science and Pollution Research* 23(15), 15381-15394.

Supplementary Information

Supplementary Table 1: The PARAFAC component matches with other freshwater components in the Openfluor database and their associated details.

Component(s)	Openfluor model	Openfluor component(s)	TCC*	Country or Ocean	Source	Details	Reference
C1	FluorMod	C4	0.95	USA	river, stream, estuary	Aromatic of terrestrial origin	Stedmon et al. 2007
C1	CS-Flocc	C4	0.97	Denmark	river, stream, lake	Humic-like	Søndergaard et al. 2003
C1	HF2005	C2	0.97	Denmark	river, stream, lake, wetland, estuary, freshwater, seawater, groundwater, wastewater	UVA humic fractions; represents terrestrially derived fulvic acid	Stedmon and Markager 2005
C1	ZambeziRiver Basin	C2	0.96	Africa	rivers	terrestrial humic-like; common in freshwaters; high-molecular-weight and aromatic compounds	Lambert et al. 2016
C1	GreatLakesRegion	C1	0.97	Canada and USA	stream, stormwater ponds, urban ponds, lakes	Humic-like	Williams et al. 2013
C1	Horsens5	C2	0.98	Denmark	stream, river, estuary, marine	Humic-like material possibly formed during photo-degradation of terrestrial organic matter	Murphy et al 2014
C1	SharkBay	C2	0.95	Australia	surface water; marine; groundwater	Humic like	Cawley et al. 2012
C1; C2 (ModelG & H)	ModelC; ModelG; ModelH	C1; C2 (ModelG & H)	0.95	Australia	stream, reservoir, treated drinking water; coagulated water	C1 and C2: terrestrial humic-like	Shutova et al. 2014
C1; C2	TropicalRivers	C2; C3	0.97	Venezuela	stream, river	C2: terrestrial humic-like component; C3: similar to humic-like peak M, linked to increased microbial production	Yamashita et al. 2010a

C1; C2; C3	Graeber2012	C2; C1; C5	0.98	Europe	stream	C2: terrestrial humic-like, present in all environments; C1: terrestrially humic material present wide range of environments; C5: Protein, tryptophane-like, microbial-produced	Graeber et al. 2012
C2; C3	DrinkingWater2011	C1; C4	0.95	Denmark	drinking water, groundwater	C1: humic-like C-peak, found in almost all natural waters; C4: protein-like similar to amino acid tryptophan	Stedmon et al 2011
C2	Andean_Mountain_Streams	C1	0.99	Argentina	streams	Terrestrial humic-like compounds	Garcia et al. 2015
C2	CWT	C1	0.99	USA	streams	Microbial humic-like fluorophore, similar to peak M	Yamashita et al. 2013
C2	FCE	C3	0.98	USA	wetland, estuary, mangrove	Terrestrial fulvic-like	Yamashita et al. 2010b
C3	Stedmon2003	C5	0.96	Atlantic, Baltic Sea, Denmark	river, stream, lake, wetland, estuary, freshwater, groundwater, wastewater, marine	Component similar to peak N and T. Believed to represent proteinous labile autochthonous material	Stedmon et al 2003
C3	Onstreams	C5	0.95	Canada	stream	Tryptophan-like, recently produced	Williams et al. 2010
C1	Recycle_PC	C1	0.96	Australia	recycled water, wastewater, treated water, greywater	Terrestrial humic-like	Murphy et al. 2011

*TCC = Tucker congruence coefficient