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

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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 characterized chromophoric DOM (CDOM) from different Australian freshwater types (circumneutral, naturally acidic and groundwater-fed waterways), climatic regions and seasons. CDOM quality was characterized using absorbance and fluorescence spectroscopy. Excitation emission scans followed by parallel factor (PARAFAC) analysis showed that CDOM was characterized by three main components: protein-like, fulvic-like and humic-like components commonly associated with various waters globally in the Openfluor 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 and 50 $cm^2\,mg^{-1}$ and 3.9–5.7 $mg\,C^{-1}\,m^{-1}$ respectively), humic-like CDOM of high molecular weight (as indicated by $abs_{254/365}$ which ranged from 3.8 to 4.3). In contrast, circumneutral waters were dominated by fulvic-like CDOM of lower aromaticity (SAC_{340}: 7–21 $cm^2\,mg^{-1}$ and $SUVA_{254}$: 1.5–3.0 $mg\,C^{-1}\,m^{-1}$) 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 $cm^2\,mg^{-1}$ and $SUVA_{254}$: 0.58–1.1 $mg\,C^{-1}\,m^{-1}$). 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.

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

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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, 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 and Woodward, 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 (Fig. 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.

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 $TOC\text{-}V_{CSH/CSN}$, 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 MΩ/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, 2012, 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

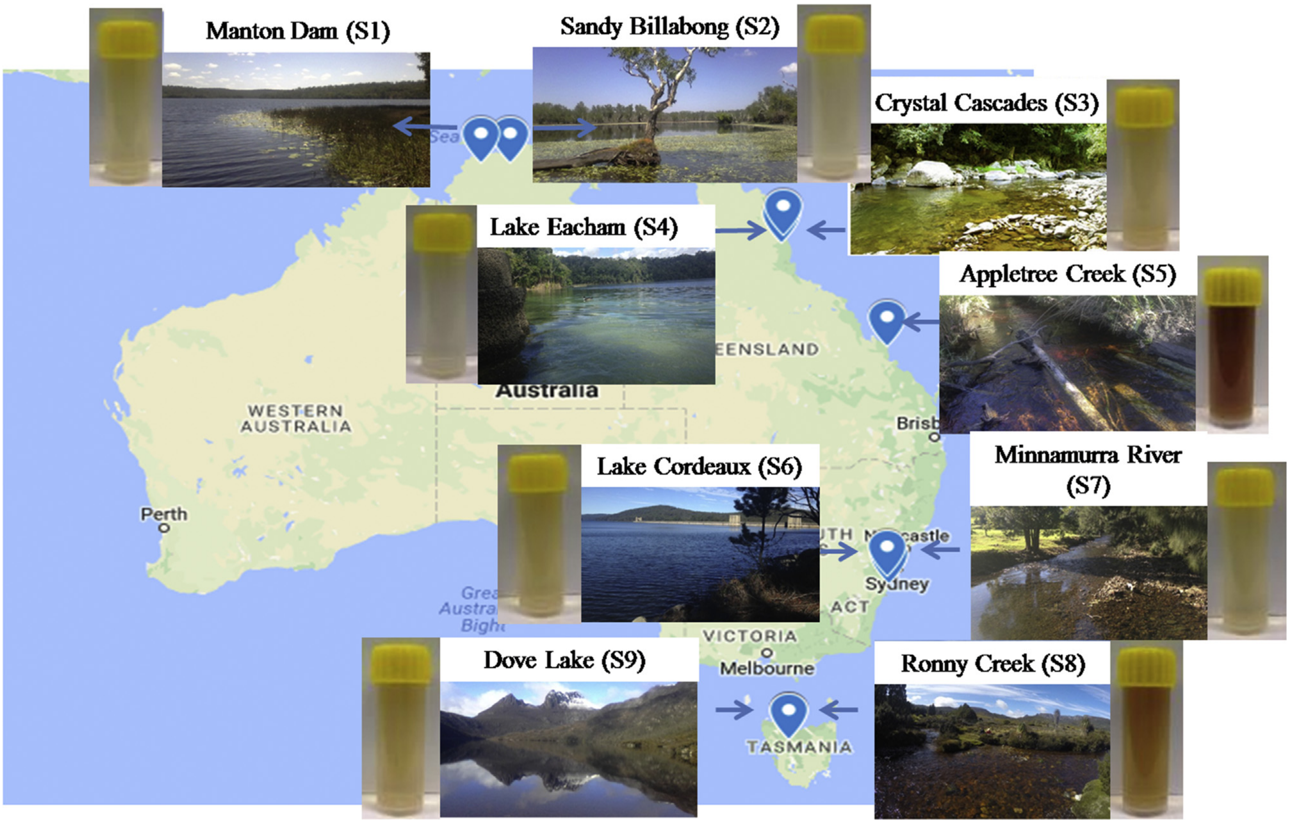

Fig. 1. Map showing the location of sampling sites, photos of sites and the colour of the DOC concentrate from each site. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

parameters, DOM concentrates were set to pH 7.0 ± 0.2 using 0.1 M NaOH or HCl, and adjusted to a concentration of 10 mg/L C with ultrapure water (18.2 MΩ/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 365 nm ($Abs_{254/365}$) (Dahlén et al., 1996) and spectral slope by $S_{275-295}$ (Helms et al., 2008).

$$SAC_{340}=2.303 \text{ x absorbance at 340 nm/DOC concentration} \quad (1)$$

$$SUVA_{254}= \text{absorbance at 254nm/DOC concentration.} \quad (2)$$

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 and 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).

$$FI = \text{emission 470/emission 520 at excitation 370 nm} \quad (3)$$

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 characterisitics

(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 (Fig. 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 ≥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).

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 (Fig. 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 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 (Fig. 3).

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

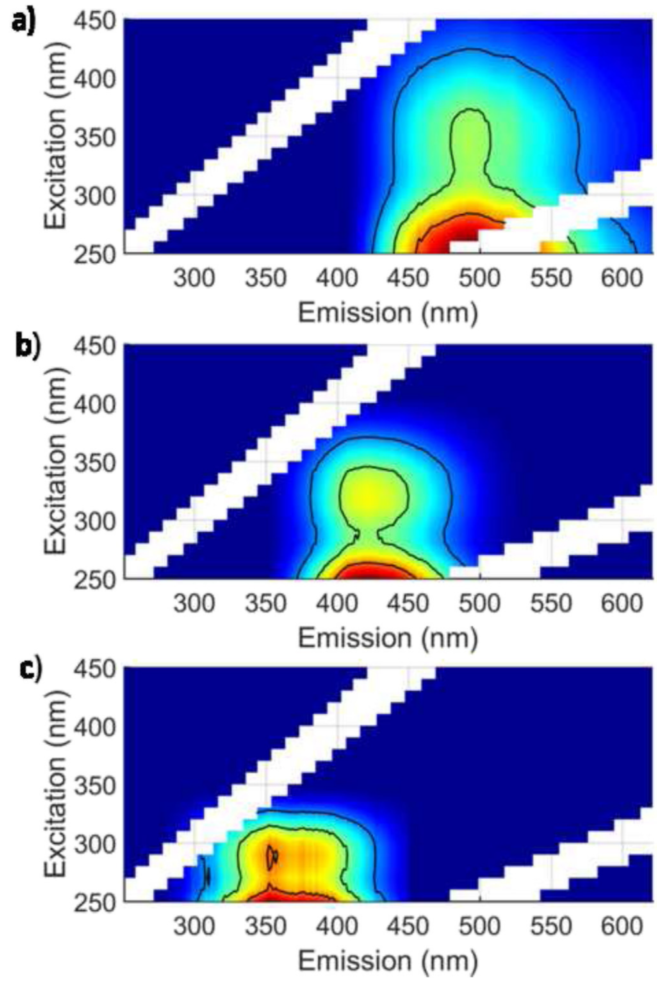

Fig. 2. Spectra of the three main components determined via PARAFAC analysis, used to describe the organic matter present within different Australian freshwaters. These three components, a) humic-like, b) fulvic-like and c) protein-like, explained 99.08% of the variation between samples, with a core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

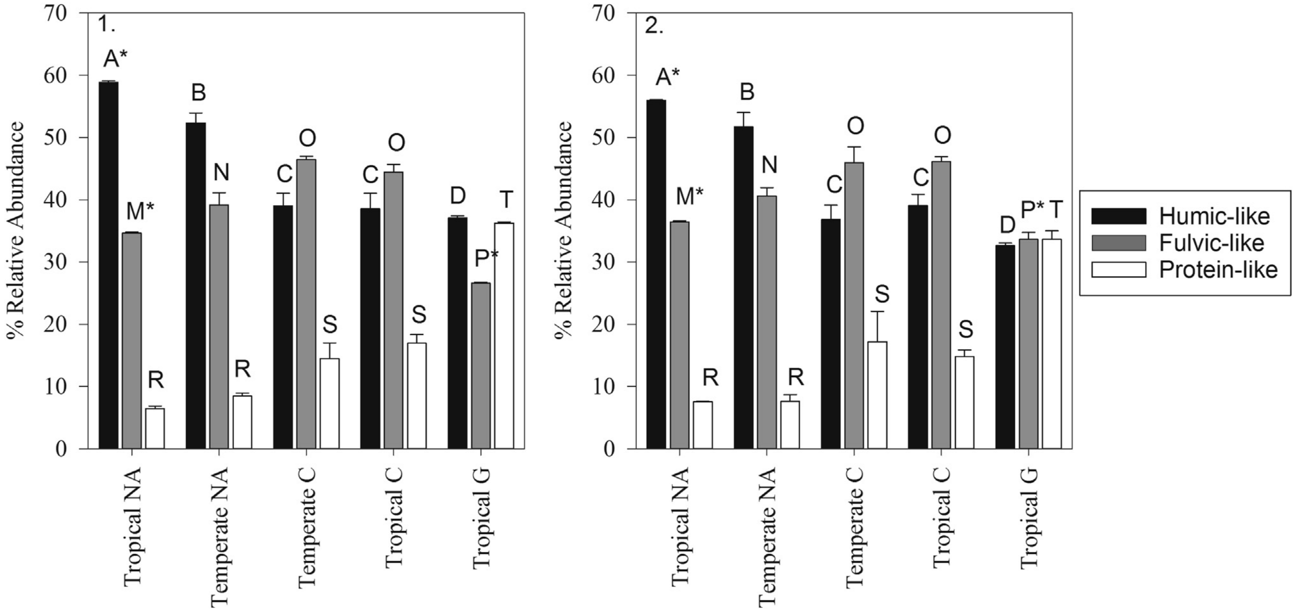

Fig. 3. Percent relative abundance of each of the three PARAFAC components associated with the organic matter from different Australian freshwaters; 1. Winter/Spring; 2. Summer/Autumn. NA = Naturally Acidic; C = Circumneutral and G = groundwater fed. Data expressed as mean ± SD. Upper case letters indicate 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.

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 (Fig. 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 (Fig. 4). Aromaticity was greatest for CDOM isolated from the tropical naturally acidic sites and lowest in the groundwater-fed site (Fig. 4). $SUVA_{254}$ values for waterways sampled within this study were within the range 0–6 $L\,mg\,C^{-1}\,m^{-1}$ generally reported for surface waters (Hansen et al., 2016) (Fig. 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 $mg\,C^{-1}\,m^{-1}$), and the groundwater-fed site had values towards the lower end (as low as 0.58 $mg\,C^{-1}\,m^{-1}$) (Table 2 and Fig. 4). SAC_{340} values in this study ranged from 2 to 50 $cm^2\,mg^{-1}$, with highest values recorded for tropical naturally acidic waters and lowest for the groundwater-fed site (Fig. 4). SAC_{340} values reported in the literature range between 1 and 73 $cm^2\,mg^{-1}$, with most waters reported as having values less than 40 $cm^2\,mg^{-1}$ (Al-Reasi et al., 2011, 2012; Johannsson et al., 2016) (Table 2 and Fig. 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).

FI values varied between 1.19 and 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 (Fig. 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 $S_{275\text{-}295}$ (average $S_{275\text{-}295}$: naturally acidic = 0.0173; circumneutral = 0.0170; groundwater-fed = 0.0142) (Fig. 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 (SAC_{340} and $SUVA_{254}$) was negatively related to protein-like DOM as proteins will contain significantly less aromatic rings than the humic substances.

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) (Fig. 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 $S_{275\text{-}295}$) than circumneutral waters and the groundwater-fed site (Figs. 3–5). 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

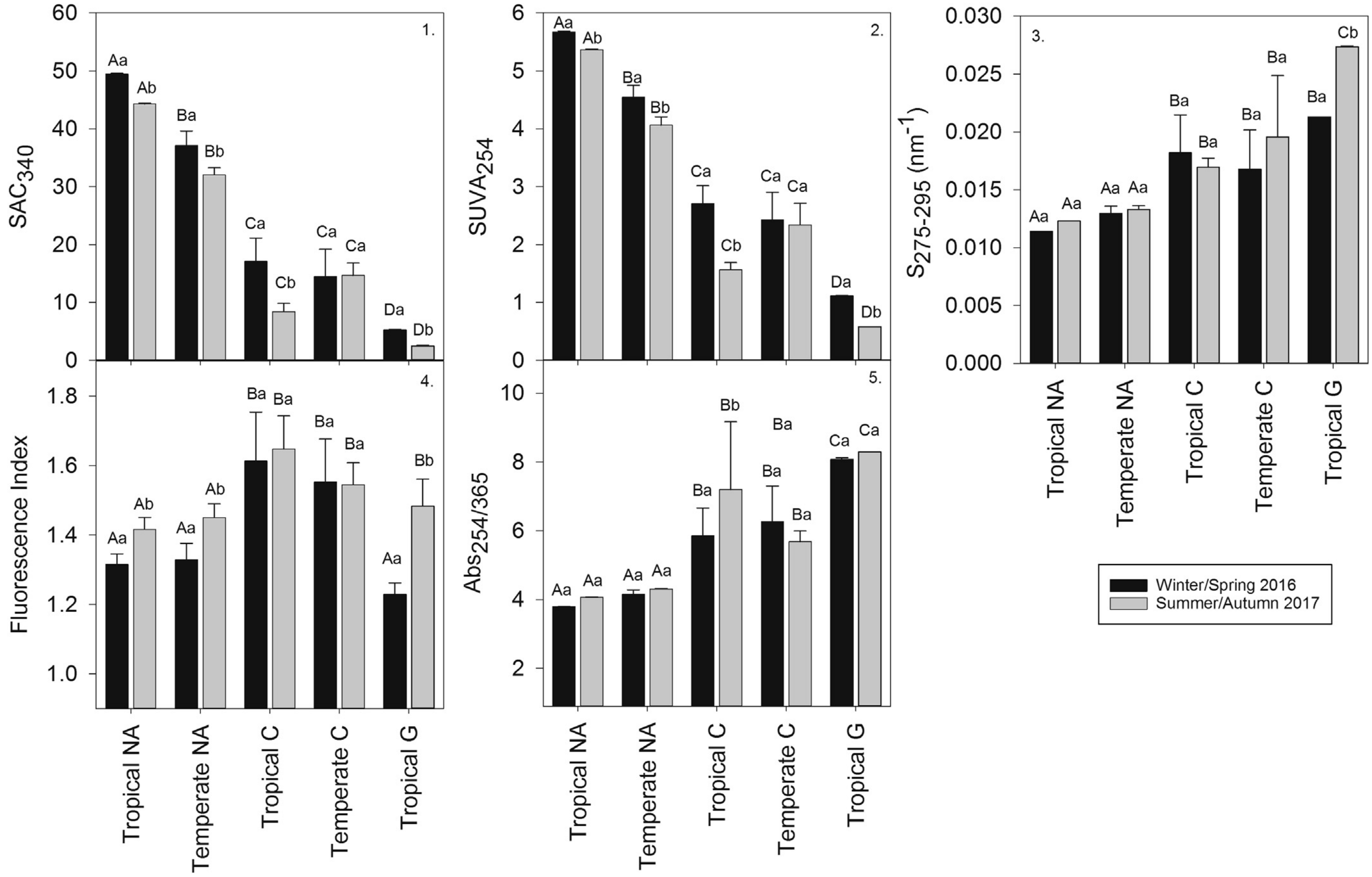

Fig. 4. DOC quality indices for different Australian waters. NA = Naturally Acidic; C = Circumneutral and G = Groundwater fed. Data expressed as mean ± SD. Capital letters indicate significant difference between freshwater types and lower case letters indicate significant difference between seasons within 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	$SUVA_{254}$	$Abs_{254/365}$	$S_{275\text{-}295}$
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
Uruguay[g]		1.65–1.77	0.19–3.1		0.01–0.02
Germany[h]		1.30–1.90	2.0–3.2		0.01–0.02

[a] Data collected from this study; Cawley et al., 2012.
[b] Data estimated from tables and figures in Johannsson et al. (2016); Duarte et al. (2016); Holland et al., 2017; Brandão et al., 2016.
[c] Al-Reasi et al. (2011, 2012, 2013); Glover et al. (2005); Schwartz et al. (2004); Williams et al., 2010.
[d] Al-Reasi et al., 2011; Gjessing et al. (1999); Jaffé et al. (2008).
[e] Helms et al. 2008; Yamashita et al., 2010; Al-Reasi et al., 2011.
[f] Spencer et al., 2010.
[g] Amaral et al., 2016.
[h] Graeber et al., 2012.

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, 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, 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.

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-$F_{2,44} = 3.38$, $P = 0.007$) (Fig. 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 (Fig. 4). Molecular weights (as indicated by $Abs_{254/365}$ ratio and $S_{275\text{-}295}$) 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 $S_{275\text{-}295}$: winter/spring = 0.016; summer/autumn 0.017) (Fig. 4). An exception to this was the significant differences in $S_{275\text{-}295}$ within the groundwater-fed water. Seasonal variation in a number of DOM quality indices have been reported previously from a number of different studies

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**							

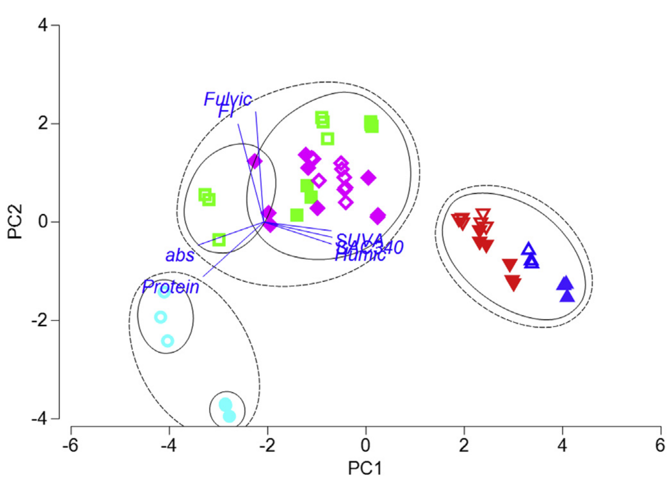

Fig. 5. Principal Component Analysis (PCA) plot showing the separation of sites with regard to DOC quality. Clusters indicates similarity between samples based on Euclidean distance. Up-right triangles = tropical naturally acidic; upside down triangle = temperate naturally acidic; square = temperate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

(Jaffé 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 (Fig. 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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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.watres.2018.02.043.

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