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# Characterization of fulvic acid fractions of dissolved organic matter during ice-out in a hyper-eutrophic, coastal pond in Antarctica

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## Abstract

Dissolved humic material (HDOM) is ubiquitous to all natural waters and its source material influences its chemical structure, reactivity, and bioavailability. While terrestrially derived HDOM reference materials distributed by the International Humic Substances Society (IHSS) have been readily available to engineering and scientific communities, a microbially derived reference HDOM was not, despite the well-characterized differences in the chemistry and reactivity of HDOM derived from terrestrial versus microbial sources. To address this gap, we collected a microbial reference fulvic acid from Pony Lake (PLFA) for distribution through the IHSS. Pony Lake is a saline coastal pond on Ross Island, Antarctica, where the landscape is devoid of terrestrial plants. Sample collection occurred over a 17-day period in the summer season at Pony Lake. During this time, the dissolved organic carbon (DOC) concentrations increased nearly two-fold, and the fulvic acid fraction (collected using the XAD-8 method) accounted for 14.6% of the DOC. During the re-concentration and desalting procedures we isolated two other chemically distinct fulvic acid fractions: (1) PLFA-2, which was high in carbohydrates and (2) PLFA-CER, which was high in nitrogen. The chemical characteristics (elemental analysis, optical characterization with UV-vis and fluorescence spectroscopy, and <sup>13</sup>C NMR spectroscopy) of the three fulvic acid fractions helped to explain their behavior during isolation.

**Keywords:** fulvic acid, Pony Lake, XAD-8, PLFA, fluorescence, NMR

 Online supplementary data available from [stacks.iop.org/ERL/8/045015/mmedia](http://stacks.iop.org/ERL/8/045015/mmedia)



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## 1. Introduction

Dissolved organic matter (DOM) is a ubiquitous and important component in aquatic ecosystems because it fuels the food web (Carpenter *et al* 2005), acts as a sunscreen for aquatic organisms by absorbing ultraviolet radiation (Minor and Stephens 2008), and is the source of harmful disinfection byproducts during drinking water treatment (Fujii *et al* 1998, Bergamaschi *et al* 1999). DOM also plays a role in the mobilization and transport of trace metals and contaminants (McKnight *et al* 1992, Haitzer *et al* 2002). The roles of DOM in aquatic processes depend on its chemical composition. The chemical composition of DOM depends on its sources, which in most natural waters include both terrestrial organic matter from degrading plant and soil material delivered from the watershed and microbial organic matter from the breakdown products of bacterial and algal matter in the water column (Thurman 1985, McKnight *et al* 2003).

Despite the fact that DOM in most aquatic ecosystems is a mixture of terrestrial and microbial derived material, studies have relied mainly on the terrestrial end-member reference sample, Suwannee River fulvic acid (SRFA), available from the International Humic Substance Society (IHSS), to study the roles of humic DOM (HDOM) in aquatic ecosystems (Malcolm *et al* 1989). Isolates like SRFA have been especially useful for experiments requiring higher DOM concentrations than present in natural waters (McKnight *et al* 2002) or for studies requiring minimal interferences from inorganic aquatic constituents (Cory *et al* 2010). For example, SRFA has been used to investigate the molecular composition of DOM (Stenson *et al* 2003), size distribution of DOM molecules (Chin *et al* 1994), and the role of DOM in photochemistry in natural waters (Vaughan and Blough 1998) and disinfection byproduct formation (Westerhoff *et al* 2004). However, DOM composition in natural waters is dynamic, exhibiting short-term fluctuation due to diurnal influence from photodegradation (Cory *et al* 2007, Spencer *et al* 2007) or from storm event driven inputs of DOM (Boyer *et al* 2000, Carstea *et al* 2009, Fellman *et al* 2009). Changing biogeochemical processes in the watershed over seasonal time scales are also reflected as a shift in DOM composition. For example, seasonal patterns in the elemental, isotopic and optical properties of DOM were interpreted as a shift in DOM source from predominantly terrestrial to more microbial from snowmelt to mid-summer in alpine lakes (Hood *et al* 2005). Given that DOM composition varies along the end-member continuum from terrestrial to microbial in aquatic ecosystems, there is pressing need for a microbial end-member reference sample available to researchers to investigate the role of microbially derived DOM in biogeochemical processes.

To fill this gap, a microbial reference material was collected from Pony Lake for the International Humic Substances Society (IHSS) during January 2006 when the rapid growth of phytoplankton produced increasing concentrations of DOC. Pony Lake was chosen as the water source for the aquatic microbial end-member fulvic acid because (1) the landscape surrounding the site is devoid of terrestrial plants, and DOM in the pond is exclusively of

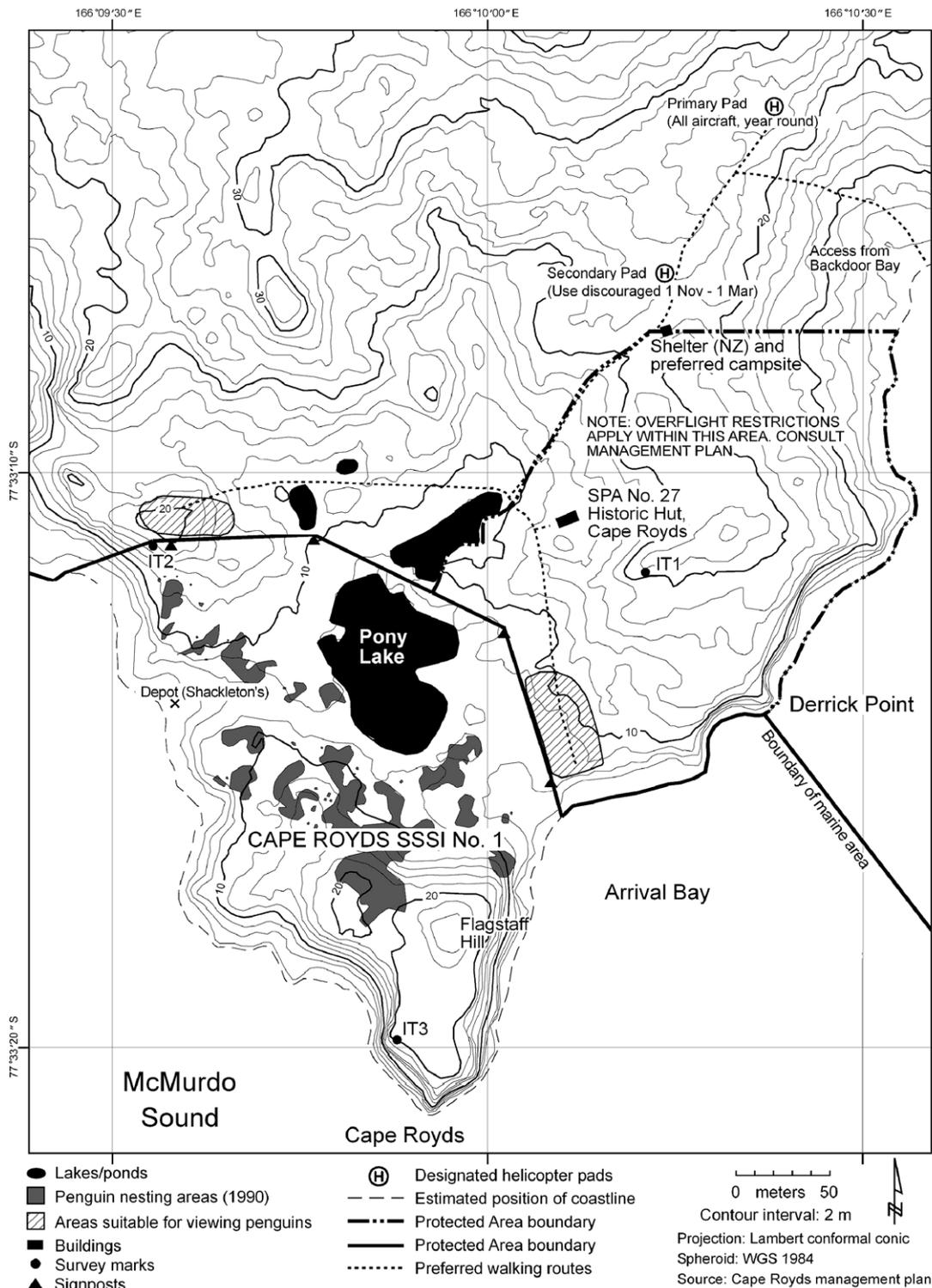
microbial origin; (2) DOC in Pony Lake is high compared to other Antarctic lakes and ponds; and (3) the pond appears to have been a stable ecosystem since the site was occupied by Shackleton's Nimrod expedition in 1907. In addition, the site is readily accessible from McMurdo Station making it possible to transport the large-volume water sample back to the Crary Laboratory for processing.

Coastal ponds like Pony Lake, which melt seasonally, are a type of cryospheric ecosystem in Antarctica that harbor life under extreme conditions (Brown *et al* 2004, Foreman *et al* 2011, Diesler *et al* 2013). These ponds are extremely productive with high concentrations of recently produced carbon in contrast to the reservoirs of older organic matter in perennially ice covered lakes, such as those in the McMurdo Dry Valleys. High concentrations of dissolved organic matter (DOM) can be found in Pony Lake and other coastal Antarctic ponds located on Cape Royds (up to 100 mg l<sup>-1</sup>; Brown *et al* 2004). In Pony Lake there are two main microbial sources of organic matter: photosynthetic algae and mixotrophic algae (capable of consuming small particles in addition to using photosynthesis as a source of carbon) (Brown *et al* 2004). The DOM pool in Pony Lake is enriched in nitrogen due to a nearby nutrient source (penguin guano) and is hypothesized to be actively cycled by the microbial community throughout the austral summer (Brown *et al* 2004). The microbial community in Pony Lake fluctuates throughout the summer season (Foreman *et al* 2011, Diesler *et al* 2013) and the period directly following ice-out may be critical for understanding the biogeochemistry of the lake and its DOM characteristics.

Here we describe the collection and isolation of PLFA and two other fractions of Pony Lake HDOM with different chemical characteristics. Comparison of the chemistry of the three Pony Lake fulvic acid isolates collected during the dynamic early ice-out period of the season was based upon elemental analysis, fluorescence spectroscopy, and <sup>13</sup>C NMR spectroscopy. Information about the collection and characteristics of the Pony Lake HDOM isolates will be useful for future studies aimed at understanding how microbial HDOM differs from terrestrial HDOM and the impact of microbial HDOM on environmental processes. PLFA has already been used to study fate and transport of pharmaceuticals and herbicides (Guerard *et al* 2009a, 2009b, Hakala and Chin 2010, Jacobs *et al* 2011), the production of reactive oxidants (Page *et al* 2011), the role of DOM in metal speciation (Agrawal *et al* 2009, Berkovic *et al* 2013, Xiao *et al* 2013), and presence of harmful algal blooms in the environment (Cawley *et al* 2013). As expected based on differences in chemical composition as a function of DOM source, these studies reported differences between PLFA and other terrestrially derived HDOM samples. Here we provide information about the collection and characterization of PLFA to advance the use of microbial HDOM in studies investigating the role of DOM composition in aquatic biogeochemical processes.

## 2. Site description

Pony Lake is located on Cape Royds, Ross Island (figure 1), adjacent to Ernest Shackleton's hut from the 1907 Nimrod



**Figure 1.** Map of Pony Lake showing penguin colony, helicopter pads, and topographic features surrounding the lake (adapted from Cape Royds Site of Special Scientific Interest (SSSI) management plan).

expedition. The ecology of the pond was first studied during this expedition (West and West 1911), and the biogeochemistry and algal community in Pony Lake have been studied episodically since that time. Unlike many other Antarctic lakes that are highly oligotrophic, Pony Lake is hyper-eutrophic and may receive a supply of nitrogen and phosphorus rich guano aerosols from a nearby Adelie penguin

colony. Strong winds also transport marine aerosols and fine particulates from the surrounding landscape into Pony Lake, making it brackish (5.5 ppt; Brown *et al* 2004). Although penguin guano contributes to the fine particulates entering Pony Lake, NMR spectroscopy shows that its chemical signature is different than that of the dissolved fulvic acid in Pony Lake (Mao *et al* 2007). There are multiple sources of microbial

organic matter in Pony Lake, including photosynthetic and mixotrophic algae, and heterotrophic bacteria that are capable of surviving varying concentrations of ions and temperatures present in the lake throughout the year (Brown *et al* 2004, Foreman *et al* 2011, Dieser *et al* 2013).

Previous studies have shown that salinity, concentration of major ions, and DOC concentration in Pony Lake vary year to year and seasonally, likely due to changes in the timing of ice melt and algal bloom formation (Brown *et al* 2004) (table S1, available at [stacks.iop.org/ERL/8/045015/mmedia](http://stacks.iop.org/ERL/8/045015/mmedia)). In association with the *Chlamydomonas* blooms, reaching cell densities above 225 000 cell ml<sup>-1</sup>, DOC concentrations in past seasons have increased from 15 to 110 mg C l<sup>-1</sup> (McKnight *et al* 1994, Brown *et al* 2004). The fulvic acid fraction in Pony Lake typically accounts for 16–21% of the DOM and despite differences in the overall DOM concentration, the HDOM character is relatively constant (McKnight *et al* 1994, Brown *et al* 2004). The fulvic acid isolates from Pony Lake have low aromaticity (11.7–16.5%), a high aliphatic content (>60%), and are high in nitrogen (4.4–7.6%) (Brown *et al* 2004). Brown *et al* (2004) recorded a decrease in the nitrogen content of the fulvic acid fraction during the transition in dominant phytoplankton species from *Chlamydomonas* to the mixotroph *Cryptomonas spp.* The samples collected for the PLFA IHSS isolation were taken during a 17-day period in January 2006 while the ice cover had just started decreasing on the pond and the phytoplankton was dominated by *Chlamydomonas* (table S1). During the collection of the PLFA isolates in January 2006 the lake did not reach an ice-free or mostly ice-free state and the algal community continued to be dominated by *Chlamydomonas*.

### 3. Material and methods

#### 3.1. Pony Lake water characterization

Dissolved oxygen, pH, and conductivity were measured using YSI meters from the ice edge of Pony Lake on a daily basis. Whole water samples for dissolved oxygen measurement by the Winkler method (according to limnological methods for the McMurdo Long Term Ecological Research Program) were collected and fixed in the field prior to transport to McMurdo Station for titration with sodium thiosulfate. Water samples collected for chemical characterization were collected in the field as filtered aliquots from the large scale filtration procedure described below. Fluorescence samples were collected in combusted (4 h at 500 °C) glass vials that were crimp sealed. DOC concentrations were measured using a Shimadzu TOC-5000 analyzer at Crary Labs at McMurdo Station and on a Shimadzu TOC-V at CU-Boulder. Samples for inorganic analysis using ion chromatography, anions (Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>-2</sup>) and cations (Mg<sup>+2</sup>, Ca<sup>+2</sup>, Na<sup>+</sup>, K<sup>+</sup>), were collected in plastic bottles and cation samples were acidified prior to transport.

#### 3.2. Sampling procedure

The large scale Pony Lake water sample was collected over 17 days in January 2006. On days when we collected water

for the PLFA fractionation we also collected samples for water quality and DOC. Ultimately 18 m<sup>3</sup> of Pony Lake water were collected in black, acid-washed and deionized water (DIW)-rinsed drums made from high density polyethylene (~55 gallons) for isolation of the IHSS reference fulvic acid sample. The water was pumped through Teflon-lined, stainless steel tubing throughout the collection and filtration process into a sequence of three filters (100 μm spiral wound cartridge pre-filter followed by Balston DH glass fiber filter of ~25 μm and AH filter of ~0.9 μm) using a 3/4 hp carbonator pump. The drums were transported via helicopter to the Crary Laboratory at McMurdo Station where the water was acidified within 48 h to pH 2 (±0.1) with HCl.

#### 3.3. Isolation methods

The isolation scheme (figure S1, available at [stacks.iop.org/ERL/8/045015/mmedia](http://stacks.iop.org/ERL/8/045015/mmedia)) is described in detail in the supplemental materials (available at [stacks.iop.org/ERL/8/045015/mmedia](http://stacks.iop.org/ERL/8/045015/mmedia)) and is based off of Thurman and Malcolm (1981). Briefly, the acidified Pony Lake water was processed in parallel through two 4 L columns packed with XAD-8 resin. The acidified lake water was applied to the columns and back eluted with 0.1 N NaOH. The eluent was acidified to pH 2 with HCl immediately and stored at 4 °C until re-concentration. The eluate from the larger columns was applied to a 2 L column and the effluent from the 2 L column was captured as PLFA-2. The 2 L column was rinsed with DIW to remove Cl<sup>-</sup> and back eluted with 0.1 N NaOH. The NaOH eluent from the 2 L column was immediately applied to a cation exchange resin column to remove the sodium and freeze-dried resulting in a proton-saturated PLFA sample. Material retained on the cation exchange resin was also eluted sequentially with acid then base to yield the PLFA-CER fraction. Finally, the PLFA-2 fraction was re-concentrated, de-salted, and freeze-dried similarly to the PLFA fraction. The PLFA-CER fraction was de-salted using a 1 kDa ultrafiltration method prior to being freeze-dried. A fourth fraction of the HDOM, the neutral fraction (HPON), was the fraction that sorbed to the XAD-8 resin, but was not eluted by 0.1 N NaOH. HPON concentration was calculated as the difference between the fraction retained on the XAD-8 resin and the fulvic acid fraction eluted from the column. HPON was not isolated and characterized along with the other fulvic acid fractions due to difficulty with purification of the sample.

#### 3.4. HDOM characterization

The freeze-dried fulvic acid samples were analyzed for elemental composition (C, H, O, N, S and P) and <sup>13</sup>C-NMR spectra. Elemental analysis was performed by Huffman Labs in Golden, CO and reported as ash-free dry mass. <sup>13</sup>C-NMR spectra of freeze-dried HDOM were collected using a cross polarization-magic angle spinning (CP-MAS) method based on Dria *et al* (2002) at the University of Colorado at Boulder NMR facility. HDOM solutions for excitation emission matrices (EEMs) and UV-vis spectra were prepared by dissolving 3–4 mg of sample in 500 ml of

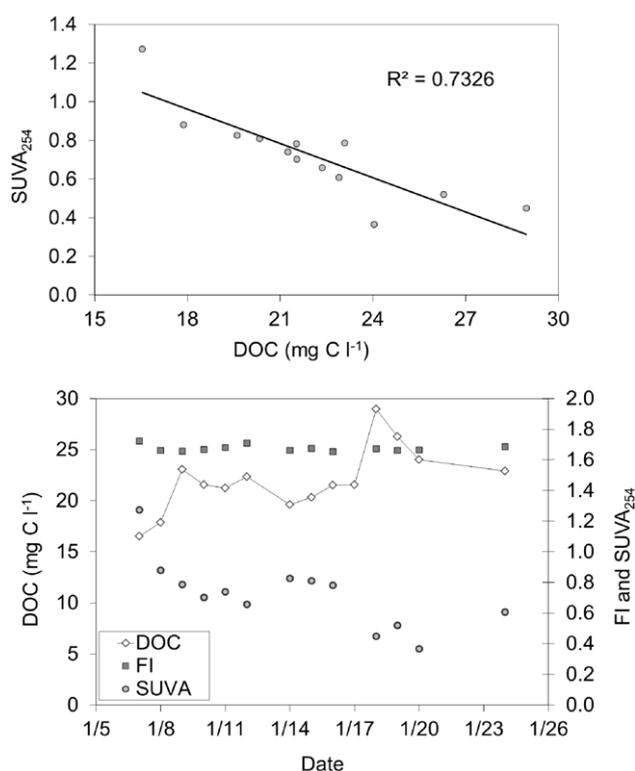
nanopure water or  $10^{-5}$  M carbonate buffer and pH adjusted to 6.5–7.0 prior to analysis. UV–vis absorbance spectra were collected on an Agilent 8453 spectrophotometer over a range of 190–1100 nm. Fluorescence EEMs were collected on a Fluoromax-2 or -3 (Horiba Scientific) over an excitation range of 240–450 nm every 10 nm and an emission range of 300–600 nm every 2 nm. An integration time of 0.25 s was used and slit widths were set at 5 nm for excitation and emission. The EEMs were corrected following Cory *et al* (2010) for instrument optics and inner-filter effects and then Raman normalized. The fluorescence index was calculated as the ratio of the emission intensities at ex370/em470 to ex370/em520 nm. The specific UV absorbance ( $SUVA_{254}$ ) was calculated by dividing the UV absorbance by DOC concentration according to Weishaar *et al* (2003). All samples were diluted to an absorbance below 0.2 if their original absorbance was above 0.2 prior to fluorescence or absorbance analyses.

## 4. Results and discussion

### 4.1. Daily and seasonal lake chemistry

DOC increased from  $16.5 \text{ mg C l}^{-1}$  on the initial sampling date, 5 January, up to a maximum value of  $29.0 \text{ mg C l}^{-1}$  on 18 January (figure 2). However, DOC values were ~3–4 times lower compared to previous years (table S1). Brown *et al* (2004) reported a seasonal trend in DOC concentrations in Pony Lake, which correlated with progressive melt of the ice cover, mixing of the water column and the rise and fall of different algal blooms. During the Austral summer 05–06, Pony Lake ice melted relatively late and dense algal blooms were not observed (Dieser *et al* 2013) providing a plausible explanation for the lower DOC concentrations. Unlike DOC, the concentrations of cations ( $Mg^{+2}$ ,  $Ca^{+2}$ ,  $Na^+$ ,  $K^+$ ) in the lake were only slightly lower than those measured in previous years, indicating that there may have also been some dilution due to freshly melted ice or limited mixing within the lake (table S1).

The FI of the whole water varied from 1.65 to 1.72, characteristic of a microbially derived source, and did not show a consistent increasing or decreasing trend over the collection period (figure 2). Specific UV absorbance ( $SUVA_{254}$ ) showed a consistent decrease with increasing DOC concentration ( $R^2 = 0.7326$ ) indicating a decrease in the aromaticity of the DOM over time likely due to increasing contributions of aliphatic DOM from microbial production (figure 2). In addition to the production of aliphatic DOM by micro-organisms, photo-exposure could also contribute to the decrease in  $SUVA_{254}$  values in Pony Lake (Cory *et al* 2007). Nitrate values remained below  $40 \text{ mg l}^{-1}$  (a threshold likely to interfere with  $SUVA_{254}$  values, Weishaar *et al* 2003) on all sampling days and on average contributed 0.5% of the absorbance at 254 nm. No other relationships were found between the concentrations of dissolved ions, DOC, or FI values during the sampling period.



**Figure 2.** Daily DOC concentration and characteristics for Pony Lake water during sample collection.

### 4.2. Recovery of HDOM from Pony Lake

The percentage of the total DOC retained on the XAD-8 columns varied from 23 to 34% during the isolation procedure, and the percentage retained did not vary with influent DOC concentration (figure S2, available at [stacks.iop.org/ERL/8/045015/mmedia](http://stacks.iop.org/ERL/8/045015/mmedia)). The fulvic acid fraction of Pony Lake (the amount of carbon that sorbed to the XAD-8 resin and was eluted in base; % fulvic acid) averaged 16% and varied only from 14 to 17%. The percentage of fulvic acid was also not correlated with influent water DOC concentration. In the re-concentration step, the three sub-fractions (PLFA, PLFA-2, and PLFA-CER) accounted for 14.6%, 0.8%, and 0.5% of the DOC in Pony Lake, respectively. Lastly, the HPON accounted for 13% of the Pony Lake DOC. The 2006 PLFA and the fulvic acid samples collected in 1992 and December 1997 had similar percentages of fulvic acid: 14.6%, 13.0%, and 17.5%, respectively (table 1).

### 4.3. Characteristics of the fulvic acid fractions

Among the three fulvic acids, PLFA had the highest per cent carbon, oxygen, and phosphorus compared to either PLFA-2 or PLFA-CER (table 1). PLFA-CER had the highest nitrogen content at 9.6% (table 1). C:N and Al/Ar ratios have been used to compare fulvic acids from a range of terrestrially and microbially dominated environments (McKnight *et al* 1994, 1997). The three HDOM samples collected during the early ice-out in January 2006 had C:N ratios that were much lower than terrestrially influenced aquatic systems and

**Table 1.** DOC of Pony Lake and chemical composition of six historical and the three new Pony Lake fulvic acid isolates.

Pony Lake fulvic acid	DOC	% FA	SUVA <sub>254</sub>	FI
1992	95	13–22	—	1.7
1994	110	—	—	1.7
12/4/1997	92	7.7	—	—
12/30/1997	32	2.7	—	—
12/2004	—	—	2.0	1.48
1/2005	—	—	2.3	1.44
PLFA-2	17–30	0.8	2.6	1.41
PLFA-CER	17–31	0.5	3.2	1.20
<b>PLFA</b>	17–32	<b>14.6</b>	<b>2.7</b>	<b>1.42</b>

	C	H	O	N	S	P	Ash
	Ash free, dry mass						Ash
1992	48.9	6.2	40.4	4.4	—	—	10.1
1994	—	—	—	—	—	—	—
12/4/1997	47.3	5.6	36.3	7.6	3.1	—	5.7
12/30/1997	49.9	6.1	35.8	4.9	3.2	—	7.5
12/2004	47.0	5.8	35.6	6.2	2.3	—	4.8
1/2005	49.0	5.9	34.4	6.2	3.2	—	2.1
PLFA-2	48.9	5.6	36.2	6.3	2.8	0.11	1.1
PLFA-CER	49.4	6.1	32.5	9.6	2.3	0.05	7.1
<b>PLFA</b>	52.8	5.4	31.6	6.6	3.1	0.55	1.3

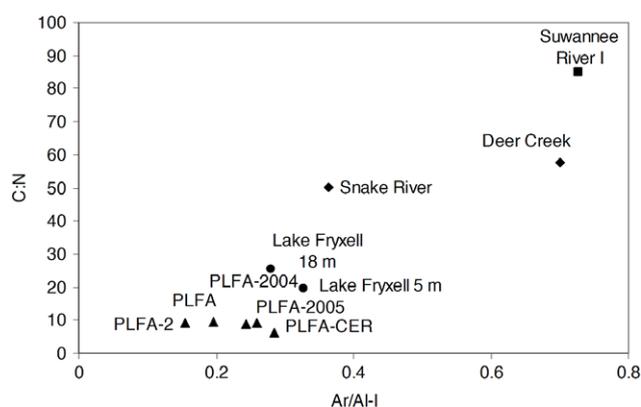
Sample	Aliphatic I 0–60 ppm	Aliphatic II 60–90 ppm	Acetal 90–110 ppm	Aromatic 110–165 ppm	Carboxyl 165–190 ppm	Carbonyl 190–220 ppm
<b>PLFA</b>	<b>63.6</b>	<b>11.8</b>	<b>0.2</b>	<b>9.8</b>	<b>14.2</b>	<b>0.3</b>
PLFA-2	62.5	11.4	0.1	9.7	16.4	0.1
PLFA-CER	58.3	8.9	0.0	16.6	16.4	0.1

	0–60 ppm	60–90 ppm	90–110 ppm	110–160 ppm	160–190 ppm	190–220 ppm
Pony Lake 1/2005	49.4	14.7	3.1	12.8	17.1	2.9
Pony Lake 12/2004	48.5	17.2	3.8	11.8	16.5	3.0
1992	42.0	16.6	6.6	16.5	16.7	2.2
1994	—	—	—	—	—	—
12/4/1997	49.5	16.1	3.8	13.5	15.9	1.1
12/30/1997	53.3	16.3	3.8	11.7	13.8	1.1

also HDOM from other Antarctic lakes (figure 3). Lower C:N is consistent with a precursor HDOM source comprised solely of algal primary production coupled with abundant sources of nitrogen provided by lake sediments or aerosols from the penguin colony.

The <sup>13</sup>C NMR spectra of the three Pony Lake fulvic acids show a characteristic broad peak in the aliphatic I region (0–60 ppm) and smaller peaks in the aliphatic II region (60–90 ppm), aromatic region (110–165 ppm), and carboxyl region (160–190 ppm) indicating a relatively high input of microbial carbohydrate and aliphatic carbon relative to aromatic carbon (figure 4). No clear carbonyl or acetal signal was present for any of the fractions (figure 4). All three fractions had the largest percentage of carbon in the aliphatic I/paraffinic region (0–60 ppm) where degradation products of lipids and biopolymers appear in <sup>13</sup>C-NMR spectra (Dria *et al* 2002). PLFA-CER had the lowest percentage of aliphatic carbon and highest percentage of sp<sup>2</sup>-hybridized (aromatic) carbon of the three fulvic acids. PLFA-CER also had a peak between 45 and 60 ppm, which corresponds to methoxyl- and amino-substituted carbon that was not present in either of the spectra for the other fulvic acids (figure 4). Given the high N content of PLFA-CER, it is likely that this peak represents amino and amide groups from the degradation



**Figure 3.** C:N and Ar/Al-I ratios for Pony Lake fulvic acids and a geographically diverse set of samples from the literature for comparison. Lake Fryxell data from Aiken *et al* (1996). Snake River data from McKnight *et al* (2002). Suwannee River data from the IHSS website. Deer Creek data from McKnight *et al* (1992).

of algal and bacterial proteins in the lake. The aromatic to aliphatic (Ar/Al) ratio of carbon determined from <sup>13</sup>C NMR for the three Pony Lake HDOM fractions varied from 0.16 for PLFA-2 to 0.28 for the PLFA-CER fraction

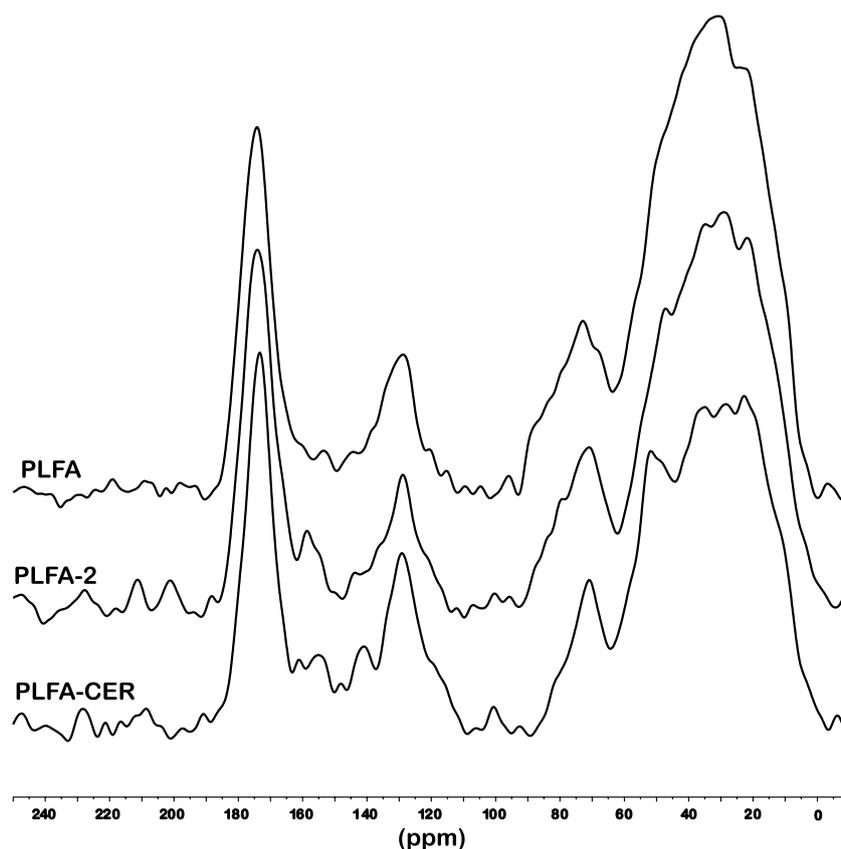


Figure 4.  $^{13}\text{C}$  NMR spectra of the three Pony Lake fulvic acid DOM isolates.

(figure 3). The Ar/Al ratios were all relatively low compared to terrestrial sites and even some other Antarctic lakes and this comparison reinforces that there are differences in the chemical characteristics of these fractions that may reflect their role in the seasonal cycling of organic matter within Pony Lake.

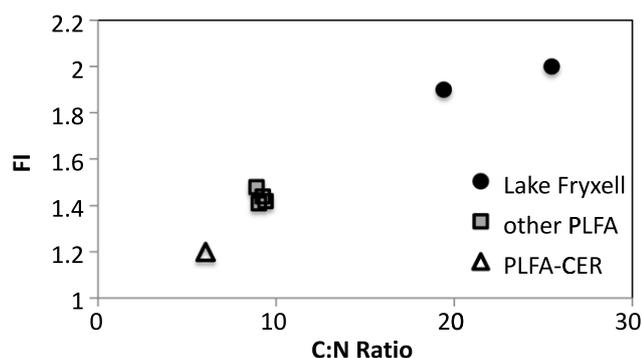
PLFA had a  $\text{SUVA}_{254}$  value of 2.7 (table 1). Similarly, PLFA-2 had a  $\text{SUVA}_{254}$  value of 2.6. PLFA-CER, however, had a much higher  $\text{SUVA}_{254}$  value of 3.2 indicative of greater aromaticity (Weishaar *et al* 2003). Compared to terrestrially derived HDOM, e.g. Suwannee River fulvic acid, the Pony Lake fulvic acids had low aromaticity and  $\text{SUVA}_{254}$  (Weishaar *et al* 2003) and higher nitrogen content (McKnight *et al* 1994, 2001) reflecting their microbial origin. The PLFA fractions have the same relationship of increasing  $\text{SUVA}_{254}$  with increasing percentage aromatic carbon determined by  $^{13}\text{C}$  NMR presented in Weishaar *et al* (2003). However, the equation presented in Weishaar *et al* (2003) to predict aromaticity from  $\text{SUVA}_{254}$  ( $\% \text{ aromaticity} = 6.52\text{SUVA}_{254} + 3.64$ ) would over-predict the aromaticity of all three of the PLFA fractions. This high absorbance relative to aromatic carbon content may have ecological significance as a UV light attenuator for algae within the lake.

The fluorescence index (FI) values for PLFA and PLFA-2 exhibited similar values (1.42 and 1.41, respectively) though PLFA-CER had a much lower value (1.20). It is noteworthy that the FI for PLFA-CER was very low for HDOM isolated from exclusively microbial precursor organic matter. Indeed,

it is similar to the FI for fulvic acid from the Suwannee River, which is dominated by terrestrially derived organic matter. Some of the abundant N in the Pony Lake fulvic acid was in the form of aromatic structures, in addition to amino groups, based on  $^{15}\text{N}$ -NMR spectroscopy (Mao *et al* 2007, Fang *et al* 2011) and aromatic moieties likely contribute to the fluorescence characterized by the FI, at longer excitation and emission wavelengths than protein-like molecules would. The EEM of PLFA-CER showed a red shifted spectrum relative to PLFA or PLFA-2, consistent with aromatic compounds (figure S3, available at [stacks.iop.org/ERL/8/045015/mmedia](http://stacks.iop.org/ERL/8/045015/mmedia)). A positive relationship between C:N ratio and FI for Pony Lake and Lake Fryxell samples also suggests that for these microbial systems higher aromatic nitrogen content may result in a lower FI (figure 5).

#### 4.4. Comparison to other fulvic acid isolates

The major fulvic acid fraction (PLFA), which is distributed as an IHSS reference material, resembles other fulvic acids (low % fulvic acid and low aromaticity) isolated from microbially dominated aquatic systems and previously isolated samples from Pony Lake (McKnight *et al* 1994, Aiken *et al* 1996, Brown *et al* 2004). However, the FI of PLFA reported here and measured by others is not as high as the FI from of the whole water (1.65–1.72) (Miller *et al* 2010). The XAD isolation method may have segregated the most aromatic and hydrophobic fraction of the Pony Lake DOM pool



**Figure 5.** Plot of the C:N ratio versus FI to illustrate the positive relationship between FI and C:N ratio for the microbial samples.

from the microbial fluorophores responsible for the higher FI recorded in Pony Lake whole water. A similar trend of higher FI values in the whole water than in fulvic acid isolates has been reported for alpine and temperate lakes with seasonal microbial HDOM inputs (Bade *et al* 2007, Miller and McKnight 2010).

The FI for PLFA was also lower than for previous fulvic acid isolates from Pony Lake (table 1). The microbial activity taking place during the winter freeze-up and within the ice (Foreman *et al* 2011) could result in alteration of the DOM pool leading to different spectral characteristics from those we observed during summer melt conditions. Because the sample was collected only about a month after ice-out started (Dieser *et al* 2013) and before there was a large increase in DOC concentration due to algal DOM production there could be a residual fluorescence signal from the highly concentrated and reduced winter lake ice conditions.

A fraction corresponding to PLFA-2 has not been isolated from previous samples of Pony Lake water or other Antarctic lakes. No re-concentration step was performed in the 1992 or 1997 fulvic acid isolations and HDOM with characteristics similar to PLFA-2 was not separated due to changes in the fractionation protocol. However, there is evidence for the PLFA-2 fraction present in the  $^{13}\text{C}$ -NMR spectra from 1992 and early December 1997. Both spectra of the fulvic acids isolated in 1992 and 1997 showed an amide peak at  $\sim 160$  ppm that is present in PLFA-2, but not in PLFA. The  $^{13}\text{C}$ -NMR spectra from the early and late December 1997 Pony Lake fulvic acid samples provide evidence that the fraction corresponding to PLFA-2 may have been consumed by microbial or mixotrophic activity during the transition to an ice-free lake because the peak at  $\sim 160$  ppm disappeared when the dominant algal species transitioned to *Cryptomonas sp.* (Brown *et al* 2004). Thus, PLFA-2 may represent a microbially labile fulvic acid fraction with a slightly smaller XAD-8 capacity factor ( $< 100$ ) than the rest of the PLFA and therefore did not sorb to the resin during the re-concentration procedure.

The presence of a distinct peak at  $\sim 50$  ppm in the PLFA-CER  $^{13}\text{C}$  NMR spectra is likely from molecules containing carbons bonded to amide groups. This organo-amine peak detected in the PLFA-CER fraction was not present in any of the 1992 or 1997 Pony Lake fulvic acid  $^{13}\text{C}$  NMR spectra,

likely because this fraction is typically discarded following cleanup with the cation exchange resin. During the desalting procedure, the fulvic acid eluent has a high pH ( $\sim 13$ ) when applied to the cation exchange resin and thus the amine groups detected by  $^{13}\text{C}$  NMR in the PLFA-CER fraction are primarily present in neutral form. When these groups contact the cation exchange resin they will not undergo cation exchange, but the amines may be selectively retained through hydrogen bonding with any residual protons attached to the ion-exchange resin.

## 5. Summary and conclusions

In this study the biology and chemistry of Pony Lake, Ross Island, Antarctica follow a consistent annual trend from samples collected in 1908, 1992, 1997, and 2006. Historically, there are two main algal blooms during the austral summer in Pony Lake: an initial chlorophyte bloom followed by a cryptophyte bloom during ice-free conditions in the lake (Brown *et al* 2004). The samples in this study were collected during the chlorophyte bloom in January 2006, within a month of the start of ice-out while the lake was predominantly ice covered. The chemical characterization shows that the IHSS PLFA reference material is similar to previous Pony Lake fulvic acid samples in that it contains high N and low aromaticity, which are characteristics of a microbial end-member. However, it had a somewhat lower FI indicating that seasonal algal blooms may increase the FI through production of high FI HDOM and/or through abiotic processing, e.g. photochemistry, to form HDOM with a higher FI as the season and extent of open water increases. The PLFA-2 fraction has a unique  $^{13}\text{C}$  NMR spectrum compared to the other PLFA fractions and likely has a lower XAD capacity factor than the PLFA fraction. The PLFA-CER fraction has high nitrogen and aromatic carbon and may contain some unique aromatic nitrogen compounds. This was the first time the PLFA-2 and PLFA-CER fractions have been collected and characterized from Pony Lake.

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