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Characterisation of Dissolved Organic Matter Fractions Released from Scottish Peatlands

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Abstract

A high concentration of dissolved organic matter is intimately related to the chemistry and ecology of water environments linked to peatlands. To understand the variations in the chemical characteristics of peat derived dissolved organic matter, those in drainage water from natural vegetation and an area containing Sitka spruce (*Picea sitchensis*), surface water (dubh lochans), and stream water associated with a peatland in central Scotland were analysed after fractionation into two fulvic acids that were desorbed from an XAD-8 column with water (FAs(H$_2$O)) and 0.1 M NaOH (FAs(NaOH)), humic acids, and dissolved non-humic substances. The elemental composition and the carbon composition as estimated by $^{13}$C cross polarisation/magic angle spinning nuclear magnetic resonance did not differ significantly between the FAs(H$_2$O) and FAs(NaOH), whilst the FAs(H$_2$O) were differentiated from the FAs(NaOH) by the greater proportion of carboxy groups with a low $pK_a$ at approximately 2. The carboxy group content and the distribution of carboxy groups with respect to the $pK_a$ of dissolved non-humic substances were similar to those of FAs(H$_2$O), suggesting their importance as a metal carrier in water systems associated with peatland.

Keywords Acidity constant, carboxy group, $^{13}$C NMR, dissolved organic matter, drainage, fulvic acids, peatland
Introduction

With a high content of organic matter and a high groundwater level, peatlands are a major source of dissolved organic carbon (DOC) in rivers\(^1\)–\(^4\). The DOC flux from peatlands in temperate regions has been reported to be 4–43 Mg C km\(^{-2}\) y\(^{-1}\)\(^5\)–\(^9\). It has also been reported that the DOC concentrations in fresh water including those in peat soil catchments are increased\(^10\), \(^11\) and that climate change\(^12\), \(^13\) and land use change\(^14\), \(^15\) are included among the driving factors for this trend. The structures of dissolved organic matter (DOM) determine their degradability and functionality, such as chelating capacity and radical-removing activity, and vary depending on their origin and their genesis and diagenesis conditions\(^16\). Humic substances, mainly fulvic acids (FAs), generally account for 20 to more than 90% of the DOM in fresh water\(^17\)–\(^19\). Complexes with DOM frequently account for more than 50% of the polyvalent metals such as Cu and Fe in natural water\(^20\)–\(^22\). It is generally considered that a large proportion of metal ions are complexed with FAs that contain abundant levels of acidic functional groups\(^23\), \(^24\), whereas the presence of dissolved non-humic substances (DNHS) that form complexes with Fe and supply it to aquatic biota has also been reported\(^25\). Changes in the concentration and chemical structure of respective DOM fractions may ultimately affect the biogeochemistry of any associated aquatic environment as well as the global C cycle\(^26\)–\(^28\). Thus, variations in the chemical structure of distinct DOM fractions need to be clearly understood.

The objective of the present study was to evaluate the chemical characteristics of different DOM fractions that are released from peatlands. For this purpose, DOM in drainage water from Scottish peat with and without spruce trees planted on it, the surface water of an undisturbed bog, and stream water in a catchment dominated by a forested peatland was fractionated into FAs, HAs and dissolved non-humic substances (DNHS). The FAs were further fractionated into two fractions that were eluted from an adsorption resin at neutral and basic solutions. Since desorption of FAs from XAD-8 with increasing pH is considered to occur due to a decrease in hydrophobicity as a result of dissociation of H\(^+\) on acidic functional groups, the two fractions were expected to...
have different distributions of acidic functional groups\textsuperscript{29, 30}. Their structural properties were
analysed by means of elemental and $^{13}$C cross polarisation/magic angle spinning (CPMAS)
nuclear magnetic resonance (NMR) analyses. The potential of these materials to form complexes
with metallic cations was estimated from the content and distribution of acidic functional groups
with respect to dissociation constants.

**Experimental**

**Collection of water samples**

Drainage water samples were collected from afforested (Sitka spruce; *Picea sitchensis*) and
natural vegetation plots established on a pristine deep peat (average of 5 m) within the Rumster
Forest (central Caithness in northern Scotland). Surface water was collected from a pool in the
undisturbed bog (dubh lochans) near the plots. The major vegetation on both undisturbed bog and
natural vegetation plot was a *Sphagnum* (L.) species dominated by *S. capillifolium* (Ehrh.) Hedw.
and *Trichophorum cespitosum* (L.) Hartman. Details of the site and drainage system have been
described by Anderson et al. (2000)\textsuperscript{31}). Stream water affected by drainage from the forested peat
(96% peat with a 0.5–2.0 m thickness), where the dominant tree species was Sitka spruce, was
also collected from the Kelty Water (Loch Ard Forest in central Scotland). Sample collections
were done in September, later dry summer, avoiding the influence of rain event. Insoluble
materials in the water samples were removed using throughflow centrifugation (Gyro Tester
centrifuge, De Laval, USA), and the pH and DOC concentration (total organic C analyser Model
800, OI Analytical, Texas, USA) were then determined.

**Fractionation of DOM**

The pH of the water sample was regulated at 1.5 with 6 M HCl, and the sample solution
(50 l) was passed through a column (length 50 cm; diameter 12 mm) packed with XAD-8
(Amberlite, Rohm & Haas, Philadelphia, USA). The XAD-8-adsorbed organic materials were eluted from the column with a 0.1 M NaOH solution, acidified to pH 1.5 with a 6 M HCl solution and allowed to stand overnight at 4°C. The precipitates, which were regarded as HAs, were separated by centrifugation, washed twice with a 0.1 M HCl solution and ultrapure water, respectively, and freeze-dried.

The supernatant, including FAs, was treated with XAD-8 a second time. The FAs that were adsorbed to the column were eluted with two column volumes of 0.1 M HCl, and combined with the XAD-8 non-adsorbed fraction. The remaining HCl was removed by elution with ultrapure water, and a portion of the FAs (FAs(H2O)) were then recovered by elution with ultrapure water\(^{29}\).

After the complete elution of FAs(H2O), the remaining FAs were eluted from the column with 0.1 M NaOH (FAs(NaOH)). The two sub-fractions of FAs were converted into the H\(^+\) form by treatment with a cation exchange resin (AG-MP-50-H\(^+\), Bio-Rad, Hercules, CA, USA), and the resulting solution was concentrated (<38°C) and freeze-dried.

The XAD-8-non-adsorbed fraction was passed through a column packed with Bio-Rad AG-MP-50 in the H\(^+\) form, neutralised with a NaOH solution, condensed by a rotary evaporator (<38°C), and desalted by ultrafiltration (Amicon YC-05; MW cut-off, 500; Beverly, MA, USA) at 4°C. The condensed solution was treated again with the cation exchange resin to remove Na\(^+\) and the resulting solution was freeze-dried (DNHS).

**Elemental composition and \(^{13}\)C CPMAS NMR**

After drying under a vacuum over P\(_2\)O\(_5\) at room temperature, the C, H and N contents of the DOM fractions were determined with an elemental analyser (Perkin Elmer 2400, Wellesley, USA).

\(^{13}\)C CPMAS NMR spectra were recorded at 75.6 MHz on a Chemagnetic CMX-300 spectrometer (Varian, Palo Alto, USA). Samples were packed into a cylindrical zirconia rotor (inner diameter, 7 mm). The operating conditions were as follows: spinning rate, 5 kHz; contact time, 1 ms; pulse delay time, 1 s; and number of accumulations, 2,000–12,000, and the spectra
were recorded with 50 Hz of line broadening. The spectra were divided into four regions of 0–45 (saturated alkyl C), 45–110 (alkyl C substituted by a hetero atom; represented by O-alkyl C), 110–160 (aromatic C), and 160–190 ppm (carbonyl C), and the relative area of each region in the total spectral area was determined. A correction for spinning side bands (SSBs) was conducted according to Watanabe and Fujitake (2008). The experimental error in the estimation of any of the C functional groups under these conditions was <4% total C. The $^{13}$C CPMAS NMR spectrum of the surface water HAs was not obtained because of their low yield.

Dissociation properties of acidic functional groups

An accurately weighed DOM sample (3 mg) was completely dissolved in 10 ml of 1.0 M NaNO$_3$ in a water-jacketed cell. A ROSS semimicro multiple electrode (9103BN, ThermoOrion, Beverly, USA) connected to a pH meter (720Aplus, ThermoOrion) was positioned in the sample solution under a N$_2$ atmosphere at 25°C for 30 min to maintain a constant electric potential. Temperature-regulated water (25°C) was circulated in the jacket (Asahi, Hiroshima, Japan) to maintain a constant temperature inside the cell. Pure N$_2$ (99.99995%) was bubbled through the solution throughout the measurement, taking care not to allow water to evaporate. While 0.05 or 0.10 ml of a (0.01 M NaOH + 1.0 M NaNO$_3$) mixture solution was dropped using a burette (APB-410-20B, Kyoto Electronics Manufacturing, Kyoto, Japan), the electric potential was measured at intervals of 10 s. When the variation in the average of the previous 6 time measurements was <0.10 mV, the average value was recorded and the titration was continued. The NaOH solution was prepared with decarbonised water under a N$_2$ atmosphere in an anoxic chamber (EAN-140, Espec, Osaka, Japan). The standard electric potential of the cell, $E^\prime$, and the accurate concentration of NaOH solution ([NaOH]) were obtained from a Gran plot. The Gran plot was recorded just before and after the titration of each sample following the same procedure as was used in the sample measurement using a mixture of 1 ml of (0.01 M HNO$_3$ + 1.0 M NaNO$_3$) solution and 9 ml of 1.0 M NaNO$_3$ solution.

The apparent acid dissociation constant ($pK_{app}$) of humic substances generally increases
with the degree of dissociation ($\alpha$) due to the nature of polyelectrolytes, even under conditions of high ion strength, in which the $pK_{app}$–$\alpha$ plots show the essential acid dissociation equilibrium without electrostatic effects\textsuperscript{34, 35}). Thus, the true $pK_a$ values, ($pK_a$)$_i$, for carboxy groups and the relative abundances in total carboxy groups, $f_i$, can be estimated based on a discrete site model\textsuperscript{36}) (Ephraim et al., 1986). In the present study, the carboxy groups in FAs and DNHS were divided into 4 groups according to $pK_a$, and the most probable values of ($pK_a$)$_i$ and $f_i$ for them were then estimated.

**Results**

**Yields of four DOM fractions**

Table 1 shows the pH, DOC concentration, and the yields of the four DOM fractions from the four water samples. Because a part of the DNHS became insoluble during the condensation procedure, the recovery of DOC from the Rumster surface water was low (58%). The surface water was characterized by low levels of DOC (13.8 mg l$^{-1}$), HAs (0.4 mg l$^{-1}$), and FAs(H$_2$O) (1.1 mg l$^{-1}$). In the spruce plot drainage, the concentration of HAs was half that in the spruce plot drainage, whereas the sum of the yield of FAs(H$_2$O) and FAs(NaOH) was 1.4–1.7 times as large as that from the sphagnum plot drainage (Table 1). The stream water was characterised by a larger proportion of FAs(H$_2$O) (28%) and a smaller proportion of DNHS (20%) as total DOM.

**Elemental composition and $^{13}$C CPMAS NMR spectra of DOM fractions**

In the elemental composition of the four DOM fractions (Table 2), the C content was higher ($P < 0.005$; $n = 4$) in the order: HAs (52.0–52.1%), the two FA fractions (49.1–51.8%), and DNHS (41.7–45.5%). The H/C ratio was highest in the DNHS samples (0.96–1.27), followed by the HAs (0.91–1.21) and the two FA fractions (0.78–0.99) ($P < 0.05$), while the N/C ratio was highest ($P < 0.05$) in the HAs (0.026–0.052). There was no significant difference between the FAs(H$_2$O) and FAs(NaOH).
The $^{13}$C CPMAS NMR spectra of the four DOM fractions are shown in Fig. 1. Major signals were observed at 31–40 (straight or branched alkyl C), 71–75 (secondary alcohol C–O), 102–109 (acetal), 128–136 (aromatic C–H/C–C), 146–156 (aromatic C–O/C–N), and 172–177 (carboxy and amide C=O) ppm. Signals at 17–19 ppm, assignable to terminal methyl groups, were well resolved in the spectra of DNHS. More resolved signals at 56–57 ppm (methoxy C) in combination with signals at 153–156 ppm in the spectra of HAs suggest that lignin-derived structures were contributors to this fraction. The spectra of FAs(H$_2$O) and FAs(NaOH) were generally similar.

Table 3 presents the C composition of the four DOM fractions, as estimated from the $^{13}$C CPMAS NMR spectra. The C composition of the FAs(H$_2$O) and FAs(NaOH) samples were: 16–22% alkyl C, 21–29% $\text{O}$-alkyl C, 36–42% aromatic C, and 15–20% carbonyl C, suggesting aromatic rings as the major skeletal structures. The aromatic C % in the FAs(NaOH) was higher than that for the HAs and DNHS and the aromatic C % in the FAs(H$_2$O) samples was higher than that of the DNHS samples for all four samples ($P < 0.05$). The carbonyl C % in the two FAs was also higher than that in HAs and DNHS ($P < 0.05$).

The $\text{O}$-alkyl C % was higher in the DNHS than in the FAs and HAs ($P < 0.05$). The $\text{O}$-alkyl C % in the three HAs was similar, 32–34%. However, the differences in the intensities for secondary alcohol C and methoxy C signals indicate that the HAs from the drainage and stream water were structurally different. The $\text{O}$-alkyl C % in the three Rumster DNHS reached 47–58%, indicating that polysaccharides, in association with the intense peak at 71–73 ppm and the 102–103 ppm signals of secondary alcohol C and acetal C, respectively (Fig. 1), were major components. The stream water DNHS was distinct, in that it had a smaller content of $\text{O}$-alkyl C (29%) and higher contents of aromatic C (31%) and carbonyl C (18%) compared to the other DNHS.

Acid dissociation properties of the DOM fractions

Figure 2 shows $pK_a$–$\alpha$ plots of the three DOM fractions. The total carboxy group content
and the estimated $pK_a$ value and relative abundance of the four carboxy groups are presented in Table 4. The HAs could not be titrated due to the fact that they underwent flocculation at approximately pH 4. There were also difficulties in titrating the surface water DNHS, but the reason for this could not be ascertained. The carboxy group content in the FAs(H$_2$O) samples, 9.4–10.8 mmol g C$^{-1}$, was higher ($P < 0.05$) than that for the FAs(NaOH), 8.1–9.2 mmol g C$^{-1}$. The DNHS contained comparable amounts of carboxy groups, 8.8–10.6 mmol g C$^{-1}$, which tended to be higher in the stream water.

The slope of the $pK_a$–$\alpha$ plot varied among the three DOM fractions (Fig. 2). The $pK_a$–$\alpha$ plots for the FAs(NaOH) samples were similar among the four water samples, compared with FAs(H$_2$O) and DNHS. The $pK_a$ values estimated for the three fractions were: ($pK_a)_1$, 1.94–2.09; ($pK_a)_2$, 3.42–3.60; ($pK_a)_3$, 4.38–4.85; and ($pK_a)_4$, 5.63–6.22 (Table 4). The relative abundance of the carboxy groups with ($pK_a)_1$ was higher ($P < 0.005$) in the FAs(H$_2$O) (39–45%) and DNHS (39–55%) than in the FAs(NaOH) (16–27%) samples, while the order was opposite for carboxy groups with ($pK_a)_3$ ($P < 0.005$) and ($pK_a)_4$ ($P < 0.05$). The FAs(H$_2$O) and DNHS from the spruce plot drainage contained a larger proportion of carboxy groups with ($pK_a)_4$ compared to the same fractions from the other water samples.

**Discussion**

In the present study, after fractionation of DOM into three fractions of HAs, FAs, and DNHS, the FAs were further fractionated into 2 sub-fractions with expecting that the content and/or $pK_a$ distribution of acidic functional groups differed between them. Although their elemental composition (Table 2) and aromaticity (Table 3) were similar to each other, the carboxy group content as estimated by the titration method was higher in the FAs(H$_2$O) than in the FAs(NaOH) (Table 4). The difference in carbonyl C % between the two fractions as estimated by the $^{13}$C CPMAS NMR method was not conclusive because of small differences in each water sample (2–3%; Table 3) compared to the confidential value of 4% in this method$^{33}$. However, the
carbonyl C% in the FAs(H2O) tended to be larger than that in FAs(NaOH) constantly, which may agree to the trend in the titration results. The titration results also indicated that the FAs(H2O) samples were more abundant in carboxy groups with \((pK_a)_1\) (approximately 2) compared to the FAs(NaOH) samples (Table 4). Leenheer et al. (1995) attributed such low \(pK_a\) values to the presence of strong electron-withdrawing functional groups that are in close proximity to carboxy groups and intramolecular hydrogen bonding effects. The common characteristics of the FAs(NaOH) was the high relative abundance of carboxy groups with \(pK_a\) values higher than 4, \((pK_a)_3\), and 5, \((pK_a)_4\), respectively. A similar \(pK_a\) to \((pK_a)_4\) was reported for polyprotic acids \((n > 3)\) that have a strong chelate-forming ability. Benzoic acids with more than three carboxy groups have been detected as structural moieties of soil FAs and aquatic DOM by means of thermochemolysis.

A higher N content and N/C ratio for the HAs compared to the other DOM fractions suggest a higher content of proteinaceous materials (Table 2), as has also been observed for another peatland in the UK. This and polysaccharides among \(O\)-alkyl C components likely contribute to the lower aromaticity in the HAs compared to the two FAs (Table 3; \(P < 0.05\)). Similar relationships have been observed for soils where the HAs have a lighter colour, e.g., Entisols. A smaller carbonyl C content (Table 3) could result in the different solubilities of HAs in the peatland associated waters compared to the FAs. Although it was not possible to use the titration method in the present study to examine HAs, the findings suggest that there are cases where HAs could influence the translocation of various elements in hydrological processes because of their high concentration or high carbonyl C content as was observed for the spruce drainage and stream water.

DNHS were characterised by a high H/C and \(O\)-alkyl C, suggesting a high polysaccharide content. However, the ratio of \(O\)-alkyl C %/alkyl C % varied from 0.10 for surface water to 0.76 for stream water, via 0.42 for sphagnum drainage water and 0.60 for spruce plot drainage water. This variation might reflect the degree of decomposition of plant derived DNHS with replacement by microbe-derived DNHS, although proteinaceous materials were distributed more into HAs. In
addition to the compositional differences, the order in the proportion of DOC present as DNHS: surface water (including DNHS lost during the preparation procedures), drainage water, and stream water, suggest the lability of DNHS in peatland ecosystems. The carboxy group content in the DNHS samples was similar to that in FAs (Table 4). The same trend was reported for the surface and river waters in a peatland in Massachusetts, USA. These observations suggest that DNHS should not be ignored as a potential carrier of metals.

Brown et al. (1999) reported that no distinct differences were found in their Cu(II)-binding potential (at pH 6) for the sub-fractions of FAs that were eluted from an XAD column at various pH values, whereas Fujii et al. (2014) showed that the chemical characteristics of humic substances have a significant influence on Fe(III) complexation capacity with increasing from pH 6 to pH 8. In the field conditions of a peatland ecosystem (pH 3.9–4.1 in Rumster; Table 1), some of the carboxy groups in the DOM are not dissociated, and the amount of dissociated carboxy groups can directly affect the complexation capacity of the DOM. For the drainages from the sphagnum and spruce plots, the total concentration of carboxylate ions ([COOH]\text{dis}; μmol l\(^{-1}\)) at the pH of each water sample (3.9 and 4.0) was calculated using equations 1 and 2:

\[
\alpha_i = \frac{1}{1 + 10^{pK_{a,i} - \text{pH}}} \quad (1),
\]

\[
[\text{COOH}]\text{dis} = \Sigma(\alpha_i f_i) \times [\text{COOH}]\text{total} \quad (i = 1–4) \quad (2).
\]

Where [COOH]\text{total} indicates the calculated concentration of total carboxy groups contained in each DOM fraction in each water sample (μmol l\(^{-1}\)) using data in Tables 1 and 4. The estimated values of [COOH]\text{dis} in the FAs(H\(_2\)O), FAs(NaOH), and DNHS samples for the sphagnum plot drainage, 39.2, 30.7, and 51.5 μmol l\(^{-1}\), respectively, were smaller than the corresponding values for the spruce plot drainage, 52.4, 51.2, and 65.5 μmol l\(^{-1}\), respectively. However, these variations were dependent on the concentrations of the DOM fractions, because the amount of dissociated carboxy groups per unit concentration of each DOM fraction was similar between the two plots: FAs(H\(_2\)O), 6.9–7.1 mmol g C\(^{-1}\); FAs(NaOH), 4.2–4.3 mmol g C\(^{-1}\); and DNHS 6.1–6.5 mmol g C\(^{-1}\). Thus, to evaluate the influence of afforestation on the amount of active carboxy groups in the drainage, the temporal variations in the concentrations of respective DOM fractions are important.
In the temperate region, the quality of DOM in peatland may be affected by seasonal variations in temperature, precipitation, and the supply of the source materials, such as litter fall, as well as an intensive rain event. However, such information has mostly been obtained for bulk DOM and seasonal variations in the quality of each DOM fraction, in particular acidic property of FAs, is scarce. Although the isolation and fractionation of DOM samples are time-consuming procedures, the accumulation of data on their chemical characteristic is required, which may also be necessary for the development of a more convenient method to evaluate their functionality.

Conclusions

FAs, a major DOM fraction in aquatic environments, could be evaluated as a combination of two sub-fractions varying distributions of carboxy groups with a low and a high $pK_a$. Since their chemical characteristics were similar among the surface, drainage, and stream waters, regardless of whether or not spruce planting was involved, the functions of FAs, including the transport of metallic ions, depends primarily on their concentrations. The DNHS showed a potential for forming a complex that was comparable with FAs and would be expected to be even higher than FAs(NaOH) under native pH conditions in a peatland system. The present results also point to a need to elucidate the solubility of peatland-derived HAs, both during their diffusion and after their complexation with polyvalent metals.

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Fig. 1. $^{13}$C CPMAS NMR spectra of fulvic acids desorbed from XAD-8 successively with H$_2$O (FAs(H$_2$O); a) and 0.1 M NaOH (FAs(NaOH); b), humic acids (HAs; c), and dissolved non-humic substances (DNHS; d).

SSB, spinning side band.

Fig. 2. $pK_a$–$\alpha$ plots for three dissolved organic matter fractions. FAs(H$_2$O) and FAs(NaOH), fulvic acids desorbed from XAD-8 successively with H$_2$O and 0.1 M NaOH, respectively. DNHS, dissolved non-humic substances.

Open circles, Sphagnum-Surface water; open diamonds, Sphagnum-Drainage water; closed diamonds, Spruce-Drainage water; and closed squares, Spruce-Stream water.