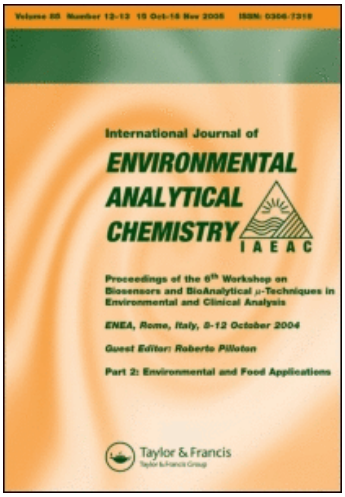

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Comparative Effects of Ionic- and Nonionic-Resin Purification Treatments on theChemistry of Dissolved Organic Matter

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COMPARATIVE EFFECTS OF IONIC- AND NONIONIC-RESIN PURIFICATION TREATMENTS ON THE CHEMISTRY OF DISSOLVED ORGANIC MATTER

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The effect of purification using ionic and non-ionic resin chromatography on the charge density and fluorescence properties of dissolved organic matter (DOM) was evaluated. Aqueous extracts of a forest soil O horizon and field corn residue were used to represent DOM from a well humified DOM source and the initial reactants for DOM formation in agricultural soils. Treatments included purification with a H^+-saturated cation exchange resin (CAT), H^+ + OH^--saturated mixed bed resin (MBR), and serial columns of XAD-8 and XAD-4 resins (XAD). In general, the alteration of DOM characteristics by purification was less for the O horizon DOM than for the corn residue DOM. The commonly used CAT purification did not alter the chemical properties measured in this study for the O horizon DOM. The CAT purification did result in a 15% loss of carbon content for corn residue DOM, but caused no difference in charge density. The benefits of MBR purification in removing both inorganic cations and anions are offset by decreased DOM recovery and charge density, especially for the corn residue extract. The use of XAD purification significantly favors a DOM fraction with a higher charge density than in the control (CTL) extract. The pragmatic need to concentrate DOM in natural solutions with low dissolved organic carbon concentration may favor the use of a XAD treatment. Careful evaluation of how purification affects the chemical characteristics of the DOM is required, especially with plant-derived materials, to ensure that the DOM used in investigation is representative of DOM in the ecosystem.

Keywords: Dissolved organic matter; carboxyl groups; fluorescence; XAD resin; sample purification

INTRODUCTION

Traditionally, organic matter has been extracted from soils using strong base extractants followed by separation into operationally-defined acid-insoluble hu-

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mic acid and acid-soluble fulvic acid fractions[1]. An alternative and increasingly common approach to studying organic matter is the use of aqueous extracts of environmental samples such as soil[2,3], sewage sludge[4], and leaf litter[5]. The organic matter in an aqueous extract of soil approximates the dissolved organic matter (DOM) in soil solution. Dissolved organic matter in soils may complex with trace metals[6], bond with organic contaminants[7] and inhibit mineral precipitation[8]. The flux of DOM also represents an important component of carbon cycling in ecosystems[9].

The use of DOM or aqueous extracts of soil horizons in complexation or precipitation studies presents several analytical challenges. Generally the organic components of an extract must be separated from the inorganic components; for some purposes the DOM must be concentrated as well as purified of inorganic contaminants. An ideal extraction and purification method allows for isolation and concentration of organic matter without altering the chemical characteristics of the sample. Despite the large number of methods proposed for isolation of soluble organic matter, few studies have examined the question of how the proposed method perturbs the chemical characteristics of natural samples.

Chromatographic approaches, which are both rapid and efficient, have been used to reduce concentrations of interfering inorganic ions in aqueous extracts of environmental samples and of aquatic humic substances. Such approaches include H^+-saturated cation exchange resin treatment[10] and XAD-8 macroporus resin followed by H^+-saturated cation exchange resin treatment[3,11]. Mixed-bed ion exchange resins have been used to remove inorganic components from xanthan solutions[12]. Cation exchange resins may be effective at removing the cationic fraction from DOM, but inorganic anions cannot be separated from DOM using this methodology. A mixed bed resin which would adsorb both inorganic cations and anions also has the potential to sorb negatively charged organic matter.

Non-ionic, macroporous sorbents such as the XAD series of resins, particularly XAD-8, have been used extensively to concentrate and purify DOM from a number of aquatic environments. The principle driving force for sorption of DOM on these resins is the "hydrophobic effect"[13]. Generally, samples are acidified before passage through XAD resins. Protonation of the organic matter reduces its charge and increases its affinity for the hydrophobic resins. XAD-8 is an acrylic ester with an average pore diameter of 25 nm and a specific surface area of 140 m^2g^{-1}, and XAD-4 is a styrene divinylbenzene resin with an average pore diameter of 5 nm and a specific surface area of 750 m^2g^{-1}[14]. XAD-4 is aromatic and hydrophobic, while XAD-8 is non-aromatic and more hydrophilic than XAD-4[14].

Recently, serial columns of XAD-8 and XAD-4 resin have been used for simultaneous desalting and concentration of DOM fractions[14]. It was suggested that the XAD-8 column sorbs a "hydrophobic" fraction of DOM thought to contain humified organic material, aromatic carboxylic acids and phenols, and aliphatic carboxylic acids containing five to nine carbons. The subsequent XAD-4 column, due to its large specific surface area, sorbs a lower molecular weight fraction deemed "hydrophilic" and generally increases DOM recovery beyond that possible with a single XAD-8 column. Neither resin has a significant affinity for inorganic ions, so this method represents an efficient means of separating organic and inorganic solutes in natural samples. Using this system, DOM recovery rates ranging from 30 to 83% for six aquatic samples from diverse environments have been reported[14]. A similar approach with XAD-8 and XAD-4 columns in tandem recovered 85% of lake water DOM[15].

The objectives of this study were to evaluate the comparative effects of treatment with H^+-saturated cation exchange resin, mixed bed resin, and sequential XAD-8 and XAD-4 resin chromatography on charge density and fluorescence properties of DOM derived from soil and plant residue. The presence of acidic functional groups is perhaps the single most important chemical characteristic of DOM, since its involvement in metal complexation reactions, binding of pesticides, mineral weathering and stabilizing soil structure is dependent on its charge properties[16]. Functional group content of DOM probably regulates its interaction with mineral surfaces[8,17]. Charge densities of soil fulvic acids typically range between 12 to 25 $mmol_{(-)}\ g^{-1}\ C$[18].

Fluorescence spectroscopy is a sensitive technique for probing the structural chemistry of organic matter[19]. Fluorescence studies of dissolved organic matter have typically used excitation-, emission- or synchronous-scan to characterize the material. Recently, excitation-emission matrix (EEM) fluorescence spectroscopy, which uses the total fluorescence intensity profile of the sample to characterize the fluorophores, has been used in characterizing Suwannee River fulvic acid[20], organic matter isolated from seawater[21], and the Al and Cu complexes of aqueous pine litter extract[22]. Our primary objective was not to assign particular chemical structures to fluorescence peaks exhibited by our samples, but instead to use the position and intensity of peaks observed before and after resin treatment as an indicator of treatment effects.

The two sources of organic matter used in this study, plant residue and organic horizon soil represent the initial and latter stages of the organic matter decomposition process. Crop residues, like litterfall in forest ecosystems, are a source of fresh organic material input into soils. These non-humified materials are rich in water-soluble carbon that is quickly released into soils during decomposition. Organic matter extracted from an organic soil horizon represents material that

has undergone extensive humification, has a lower water-soluble C content, and typifies the end product of decomposition of organic materials[23]. These two sources represent endpoints on a continuum of soil organic matter.

EXPERIMENTAL SECTION

Aqueous Extracts

Fresh O horizon was collected in August, 1994 from a mature red pine (*Pinus resinosa* Ait.) stand described in a previous paper[10]. Triplicate forest floor samples were extracted at a 10:1 water to soil ratio in a refrigerator for 18 hours. Deionized water (DI-H_2O) with greater than 18.0 megohm-cm resistivity was used in this study. Corn (*Zea mays* L.) residue was obtained from a farm field in Iowa, dried at 60°C, ground to pass a 1-mm sieve, and stored at room temperature. The residue sample was extracted at a 40:1 water to residue ratio in a refrigerator for 18 hours. Suspensions from both extracts were centrifuged at 2500 revolutions min^{-1} for 15 min. The O horizon soil extract was passed through a perforated Buchner filter funnel to remove large solids prior to vacuum filtering through a GF/F (Whatman International; Maidstone, England) glass fiber filter. The corn residue extract was filtered sequentially through an A/E (Gelman Sciences, Ann Arbor, MI) glass filter and a 0.4 μm Nuclepore polycarbonate filter (Costar; Cambridge, MA). Each extraction was replicated in triplicate.

The filtered solutions of each replicate were divided into four subsamples for the purification treatments. One aliquot served as the unpurified control (CTL). The cation exchange resin purification treatment (CAT) was conducted by passing the solution through a H^+-saturated AG 50W-X8 cation exchange resin. The mixed bed resin purification treatment (MBR) was conducted by passing the solution through a H^+-OH^- saturated AG 501W-X8 resin. Gravity flow was used for the CAT and MBR treatments.

The XAD-8 and XAD-4 resins (20–50 mesh) were cleaned with rinses of 0.1 *M* NaOH and water, followed by sequential Soxhlet extraction with methanol, acetonitrile, and ether. After rinsing with distilled water, the resins were slurry packed into 30 cm long plastic chromatography columns with a volume of 15 mL. Prior to use, the resin columns were rinsed with 10 column volumes each of distilled water, 0.1 *M* NaOH, 0.1 *M* HCl, and distilled water. Acidified 50 mL samples (pH 2) were pumped through the XAD-8 columns at a flow rate of 2.0 mL min^{-1}, and the eluate was collected in glass. The last of the 50 mL sample

was chased through the column with 15 mL of distilled water. The XAD-8 eluant was then pumped at 2.0 mL min^{-1} through the XAD-4 column, followed by a 15 mL rinse of distilled water. Each column was then back-eluted at 2.0 mL min^{-1} with 25 mL of 0.1 *M* NaOH, followed by a 15 mL rinse of distilled water. The combined NaOH extracts from the XAD-8 and XAD-4 columns were next pumped through the H^+-form AG-50W-X8 cation exchange column at 3.0 mL min^{-1}, followed by a rinse of distilled water. The collected eluted volumes were 77 mL for the O horizon and 92 mL for the corn residue materials. All resins except the Amberlite (Rohm and Haas, W. Philadelphia, PA) XAD-8 and XAD-4 were analytical grade obtained from Bio-Rad (Melville, NY).

The concentrations of total soluble carbon (C_{TS}) in extracts were measured using an infrared analyzer (O.I. Analytical, model 700, College Station, TX). Concentrations of K, Ca, Mg, Na, Al and P in extracts were determined using inductively-coupled plasma-atomic emission. Concentration of NH_4^+ and NO_3^- were determined using a Lachat QuikChem AE flow injection system (Lachat; Milwaukee, WI). Nitrate was determined by passing the sample through a cadmium-containing column; the resulting nitrite was reacted with sulfanilamide followed by N-(1-naphthyl)ethylenediamine dihydrochloride. The complex was quantified by absorbance at 520 nm (QuikChem method No. 10-107-04-1-F). Ammonia was determined by the salicylate method with detection at 660 nm (QuikChem method No. 12-107-06-2-A).

To determine DOM precipitation versus pH profiles of the CTL extracts, light scattering at 90° was used as a surrogate for precipitation. Light scattering was measured using a Hitachi F-4500 spectrofluorimeter (Danbury, CT) with both excitation (EX) and emission (EM) set at a visible wavelength of 400 nm. Both EX and EM slits were set at 2.5 nm. Fluorescence intensities averaged over 10 s were obtained in triplicate for each data point. Scattering data on duplicate 50 mL extracts were obtained as the pH was lowered from native value to ~2.0 using 5 N HCl. The 1 mL aliquot removed for each fluorescence measurement was returned to the extract after the scattering values were recorded and dilution, which was <5%, was explicitly corrected for each data point.

Acid-Base Chemistry

Potentiometric titrations of the extracts were conducted in a Plexiglas capped, glass reaction beaker thermostatted at 25.0 ± 0.1°C. The solutions were bubbled with humidified high purity N_2 to minimize CO_2 contamination. A Ross combination electrode was standardized at pH 4.01 and 10.01 using buffers held at 25.0 ± 0.1°C. Titration solutions for the corn residue extracts were adjusted to

20 m*M* C_{TS} and 10 m*M* ionic strength by diluting an appropriate volume of treatment sample and 0.50 mL of 1.00 *M* KCl to a volume of 50.0 mL with DI-H_2O. The O horizon soil extracts were prepared similarly to contain 6 m*M* C_{TS} and 10 m*M* ionic strength. Two replicates of the MBR treatment, which had 5.40 and 5.57 m*M* C_{TS} concentrations, were titrated without dilution after ionic strength adjustment. Ionic strength of 10 m*M* was selected as representative of typical soil solutions[24]. Because of the difficulty in determining equivalence points in the titration of DOM, operational beginning and end points of pH 3 and pH 7, respectively were selected for carboxyl groups[25,26]. Solution pH values were adjusted to 3.0 by the addition of HCl or NaOH. The solutions were equilibrated for 15 min prior to titration with NaOH standardized to 0.0479 ± 0.003 *M* using phenolphthalein. The NaOH was added using an Eppendorf (Brinkmann, Westbury, NY) pipet. Solutions were mixed with a magnetic stir bar to ensure mixing of the added titrant. Blank titrations consisted of 0.01 *M* KCl solution adjusted to pH 3. Titrations were corrected for the blank titrations.

Presence of titratable inorganic components in the extracts was not accounted for by the KCl blank titration. The MBR and XAD treatments were predominately free of both cations and anions, while the CAT treatment removed only the cations (Table I). The titratable inorganic components are Al and acid phos-

TABLE I Comparison of purification effects on the selected elemental concentration of aqueous O horizon soil and corn residue extracts

Sample	*Element*	*Treatment*			
		CTL	*CAT*	*MBR*	*XAD*
		μmol L^{-1}			
O horizon	K	123 ± 3	<3	<3	7 ± 3
	Ca	99 ± 9	<1	2 ± 1	2 ± 1
	Mg	38 ± 2	<2	<2	<2
	Na	185 ± 4	<4	<4	86 ± 63
	Al	24 ± 2	28 ± 4	25 ± 1	8 ± 3
	NH_4^+	65 ± 10	13 ± 1	17 ± 5	28 ± 4
	NO_3^-	<4	<4	<4	24 ± 5
	P	30 ± 3	33 ± 3	4 ± 0.4	122 ± 25
Corn Residue	K	12200 ± 120	650 ± 90	840 ± 50	<3
	Ca	1020 ± 100	40 ± 4	50 ± 10	<1
	Mg	1930 ± 30	<40	50 ± 10	<40
	Na	150 ± 30	50 ± 10	60 ± 20	20 ± 3
	Al	<0.08	<0.08	<0.08	<0.08
	NH_4^+	1650 ± 30	220 ± 10	220 ± 10	20 ± 1
	NO_3^-	300 ± 10	290 ± 10	10 ± 10	<10
	P	1750 ± 30	1630 ± 10	70 ± 10	20 ± 5

†All values are means of three replicates ± standard deviation.

phate for the pH 3 to 7 range used in our study. The Al in the O horizon extract is complexed by organic matter and was assumed not to be involved in proton dissociation reactions. The geochemical code MINTEQA2[27] was used to calculate the correction for H^+ dissociated by phosphate species as pH was raised from 3 to 7 in the titration. This correction was less than 1.4% for the O horizon extract and was ignored. The phosphate correction for the corn residue extracts accounted for 7.1, 7.8 and 3.3% of the titratable protons for the CTL, CAT, and MBR treatments, respectively.

Fluorescence

A Hitachi F-4500 spectrofluorimeter was used to obtain the fluorescence spectra. All solutions were adjusted to 3 m*M* C_{TS} and pH 4.5. Fluorescence EEMs of both materials were obtained by varying the EX wavelength from 250 to 400 nm and the EM wavelength from 300 to 550 nm in 3 nm increments. Contour plotting was chosen to graphically present the data where x- and y-axes represent emission and excitation wavelengths, respectively and iso-fluorescence intensity contours are mapped on the xy-plane. Two-dimensional contour plots are advantageous over isometric three-dimensional projection since very intense Rayleigh scattering lines (typically 10 to 20 times the fluorophore intensities) in the foreground can hide less intense peaks in the background[28]. Contours were calculated using Systat for Windows v. 5.03 with distance weighted least squares smoothing. Instrumental parameters were: EX and EM slits, 5 nm; response time, 8 s; and scan speed, 12,000 nm min^{-1}.

RESULTS AND DISCUSSION

Inorganic Chemistry

The control corn residue extracts contained greater inorganic ion content than the control O horizon soil extract (Table I). The CAT and MBR treatments were effective in reducing the basic cation (the sum of K, Ca, Mg and Na) contents in both O horizon (98% removal) and corn residue extracts (94% removal). However, the CAT and MBR treatments removed only 67% and 60% of the Na, respectively, from the corn residue extracts. Neither CAT nor MBR treatment reduced the Al concentration of the O horizon extract, implying that the Al is completely complexed and is not electrostatically attracted to the purifying resins (Table I). The extract Al concentration was reduced by 67% during XAD

adsorption, indicating that the Al is predominately complexed by hydrophilic material not retained by XAD resins. This supports previous work[29] which has shown that charged organic matter with oxygen-containing functional groups form strong complexes with Al^{3+}. The CAT and MBR treatments reduced the NH_4^+ concentration by 74 to 87% which suggests that the remainder is associated with the DOM (Table I). The concentration of NO_3^- and P in the corn residue extract was reduced by greater than 97% by both the MBR and XAD resins indicating that these anions were in a form that reacted with the MBR and passed through the XAD resin columns (Table I). The O horizon soil extract behaved differently, with enrichment of NO_3^- and P content in the XAD treatment. The hydrophobic fraction of the O horizon soil DOM must contain a relatively higher proportion of N and P than the DOM as a whole. Dissolved organic matter presumably does not contain free NO_3^-, but rather the NO_3^- is being converted from the organic-N form to free NO_3^- during the analytical determination of NO_3^- in the flow injection analysis. The high Na content of the XAD treatment is probably due to incomplete protonation of the DOM after elution of DOM from the XAD resins using NaOH.

Organic Matter Chemistry

High DOM recovery rates in the purification steps are desirable, not only for the greater yield obtained, but also for decreasing the chance of unwanted fractionation of DOM. For the O horizon extract, the loss of carbon content was not statistically significant for the CAT treatment (95% recovery), but the loss of carbon content was significant for the MBR treatment (71% recovery) (Table II). The DOM recovery was significantly lower for the corn residue DOM with 85 and 35% recovery for the CAT and MBR treatments, respectively (Table II). The

TABLE II Treatment effects on the dissolved organic carbon (DOC) mass balance in aqueous extracts of O horizon soil and corn residue and the calculated DOC recovery for the purification treatments

		Treatment			
Sample	*Parameter*	*CTL*	*CAT*	*MBR*	*XAD*
O horizon	DOC†, mmol C	0.399 ± 0.31‡	0.379 ± 0.015	0.284 ± 0.017*	0.219 ± 0.007*§
	%DOC recovery	–	95.2 ± 4.3	71.1 ± 1.3	54.8 ± 2.3
Corn residue	DOC†, mmol C	8.05 ± 0.09	6.85 ± 0.12*	2.80 ± 0.25*	2.44 ± 0.06*
	%DOC recovery	–	84.9 ± 2.2	34.7 ± 3.4	30.2 ± 0.9

†Asterisks indicate significant differences at $P < 0.05$ using a t-test between the CTL and purification treatment DOC values for each material. Carbon content based on 50 mL of extract for both sample types.
‡All values are means of three replicates ± standard deviation.
§DOC concentration of combined elutant from XAD-8 and XAD-4 columns.

XAD treatments followed the same trend with 55% recovery for the O horizon extract versus 30% for the corn residue extract (Table II), suggesting that the O horizon extract DOM was more hydrophobic than the corn residue extract. The large loss of carbon content in the corn residue by the MBR treatment is probably due to high proportion of DOM which carries a negative charge at pH 3.55 and thus is retained on the anion exchange resin.

Decreasing pH reduces the solubility of DOM and this mechanism is used to operationally define the traditional humic versus fulvic acid fractions. The pH value of the O horizon extract was 5.0 and was reduced by 1.5 to 2 pH units by the purification treatments. The corn residue extract pH was 6.0 and purification reduced the pH by 2.5 to 3.5 units. Light scattering increased steadily for the corn residue DOM in the pH 5 to 3 region indicating significant DOM loss to precipitation in this pH range (Figure 1). The precipitation loss of DOM was not concentration dependent since the scattering measurements at 159, 80 and 20 m*M* C_{TS} all show the same pattern of scattering increasing at around pH 5,

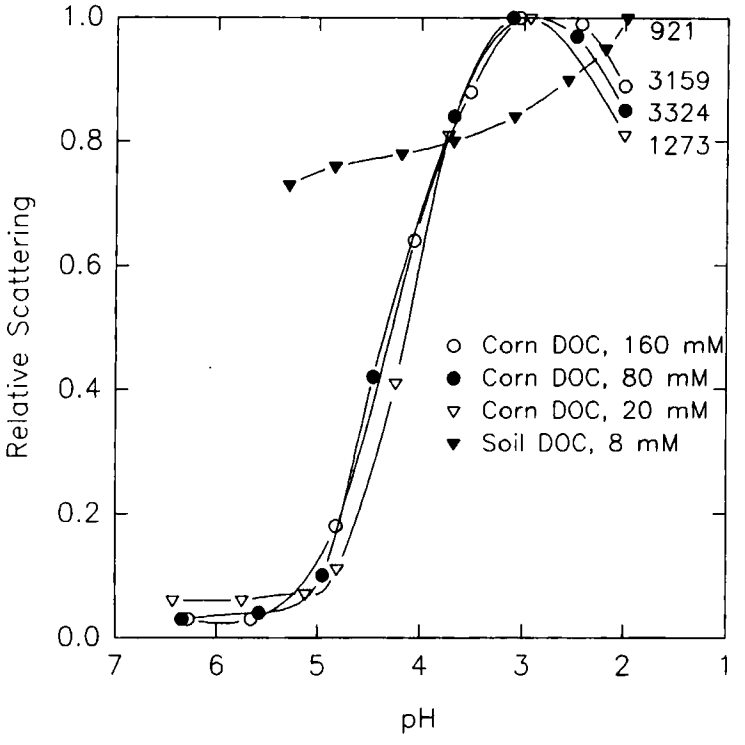

FIGURE 1 Relative scattered light intensity of the control corn residue and O horizon extract as a function of pH measured at $\lambda_{EX} = \lambda_{EM} = 450$ nm. The values adjacent to the symbols at pH 2 are the maximum observed scattering in arbitrary units. The scattering of DI-H_2O was 18 arbitrary units.

reaching a maximum at pH 3 and declining slightly as pH was lowered to pH 2 (Figure 1). The decrease in light scattering as pH decreased below 3 may reflect formation of larger-sized aggregates as the DOM is fully protonated and electrostatic repulsion is decreased. A leveling off of light scattering with increasing Cu^{2+}-induced precipitation of a soil fulvic acid has been attributed to the lower light scattering efficiency of larger particles[30]. Light scattering by the O horizon soil DOM increased only slightly as pH decreased from 5.3 to 2 indicating much less precipitation loss for the soil DOM (Figure 1). This evidence suggests that precipitation of DOM is the cause of statistically significant loss of carbon in the CAT treatment for the corn residue extract.

The charge densities ($mmol_{(-)}\ g^{-1}$ C) of the DOM are shown in Table III. There was no significant effect of CAT treatment on charge density for either the O horizon or the corn residue extract (Table III). The MBR treatment reduced the charge density of corn residue by 74% suggesting removal of a fraction with a high negative charge density. For both sources, the fraction of DOM recovered after XAD treatment had a significantly greater charge density than the respective unpurified control extracts (Table III). The 12.4 $mmol_{(-)}\ g^{-1}$ C for the XAD treatment of corn residue was greater than the 5.5 $mmol_{(-)}\ g^{-1}$ C charge density reported for wheat straw fulvic acid[31]. The difference may be due to plant species effects as well as the use of XAD-8 and XAD-4 in this work versus XAD-8 alone in the work of Grossl and Inskeep[31]. The XAD-4 resin isolates the more hydrophilic fraction of DOM which likely contains a greater negative charge density than the more hydrophobic fraction. Measurement of acidic functional groups in water-soluble soil organic matter has been limited to a sample isolated from a mineral soil and was reported to be 14.9 $mmol_{(-)}\ g^{-1}$ C for titration up to pH 10[7]. The lower value of the CTL O horizon extract in our study probably reflects sample differences as well as the pH 7 endpoint we used.

TABLE III Negative charge density of the dissolved organic matter in the control and purified extracts

	Treatment†			
Sample	*CTL*	*CAT*	*MBR*	*XAD*
	$mmol_{(-)}\ g^{-1}$ C			
O horizon	6.07 ± 0.44‡	6.17 ± 0.33	5.45 ± 0.67	9.34 ± 0.85*
Corn residue	6.20 ± 0.21	6.11 ± 0.22	1.59 ± 0.20*	12.4 ± 0.53*

†Asterisks indicate significant differences at $P < 0.05$ using a t-test between the CTL and purification treatment carboxyl group contents for each material.
‡All values are means of three replicates ± standard deviation.

Fluorescence Spectra

The EEM spectra for the unpurified, control extract of O horizon soil extract revealed 2 fluorophores with differing excitation maximas, but the same emission maxima (Figure 2). The spectra from all treatments of the O horizon extracts featured similar shaped contours indicating that the same fluorophores were present; however there were differences in fluorophore intensities indicating that treatment alters the distribution of fluorophores present in DOM (Table IV). The intense fluorophore (designated A) in Figure 2 had an EX maxima in the 328 to 344 nm range and an EM maxima in the 444 to 453 nm range. The less intense fluorophore (designated B) had an EX maxima in the 253 to 262 nm range and a EM maxima range identical to fluorophore A. Emission maximas in the 435 to 460 nm range are characteristic of fulvic material extracted from a variety of terrestrial sources[9]. A single fluorophore may appear to be multiple fluorophores with the same EM maxima, but differing EX maxima. If this occurs, however, the fluorescence intensity ratio between the apparent multiple

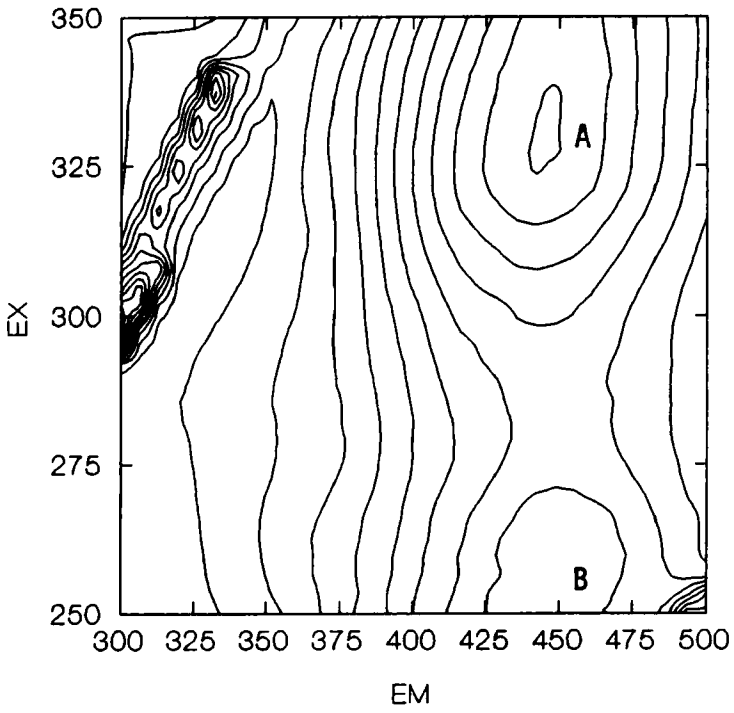

FIGURE 2 Fluorescence excitation-emission matrix of the control O horizon soil extract adjusted to pH 4.5 and 3 m*M* DOC.

TABLE IV Effect of purification treatment on the location and relative fluorescence intensities of the fluorophores isolated from O horizon soil derived dissolved organic matter. All extracts adjusted to 3 m*M* DOC and pH 4.5

Treatment	*Parameter*	*Fluorophore A*	*Fluorophore B*
CTL	Location	262 EX/447 EM	344 EX/447 EM
	Intensity	298†	358
CAT	Location	259 EX/444 EM	331 EX/444 EM
	Intensity	291	350
MBR	Location	253 EX/453 EM	340 EX/453 EM
	Intensity	192	218
XAD	Location	256 EX/447 EM	328 EX/447 EM
	Intensity	296	448

†Fluorescence intensity in arbitrary units.

fluorophores will remain constant for any treatment[21]. In our study the ratio of fluorophore A: fluorophore B intensity ranged from 1.14 to 1.51 for the different treatments suggesting the presence of two different fluorophores in the O horizon extracts.

The EEM spectrum for the unpurified, control corn residue extract is shown in Figure 3. As for the O horizon DOM spectra, treatment did not alter the general shape of the fluorescence contours. The intense fluorophore (designated C) in Figure 3 had an EX maxima in the 271–277 nm range and an EM maxima in the 336–345 nm range across all purification treatments (Table V). Peaks in this region have been attributed to protein-type fluorescence in near-surface seawater samples[32]. Tryptophan residues account for about 90% of total protein fluorescence and typically absorb at 280 nm and emit from 320 to 350 nm[33]. Aqueous crop residue extracts are likely to contain proteinaceous material. The lower intensity fluorophore (designated D) had an EX maxima in the 313–316 nm range and an EM maxima in the 435–444 nm range across purification treatments. Fluorophore D is similar to the 322 nm EX and 442 nm EM peak used for Al titration for a leaf litter extract[34]. The EX and EM maxima ranges of fluorophore D are characteristic of fulvic-type material[19]. While DOM extracted from plant material extracts does not contain "true" fulvic material since it has not undergone degradation and subsequent synthesis, classification of this fluorophore as fulvic-like is further supported by the increase in its fluorescence intensity after XAD treatment which is used to concentrate fulvic acids.

Purification effects on the fluorescence intensities of the fluorophores were different for the two sources of DOM. The CAT treatment did not alter the fluorescence intensities of fluorophores A and B (Table IV). The MBR treatment reduced the intensities of fluorophores A and B by about a third, while the XAD treatment did not alter fluorophore A intensity and increased fluorophore B in-

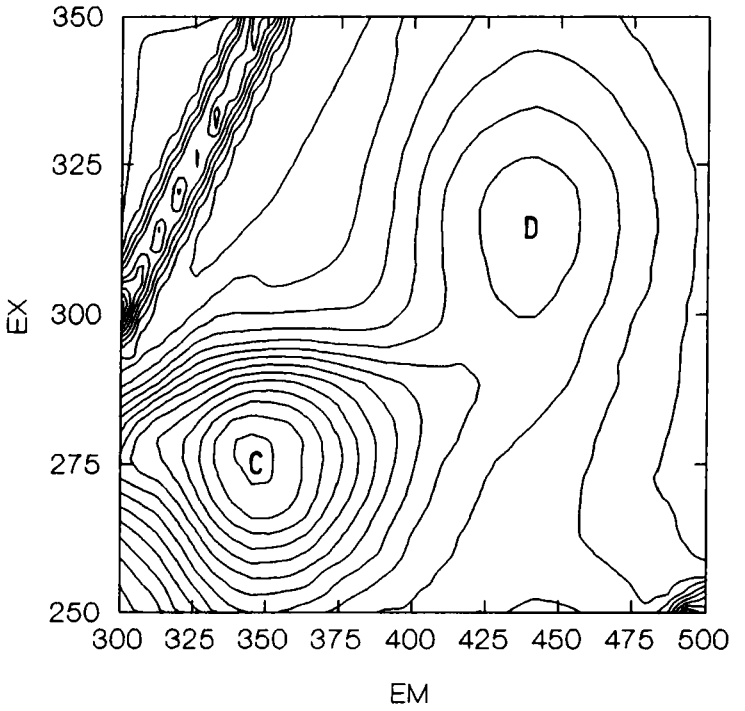

FIGURE 3 Fluorescence excitation-emission matrix of the control corn residue extract adjusted to pH 4.5 and 3 m*M* DOC.

tensity by about 25% (Table IV). There were greater treatment effects on fluorescence intensities for the corn residue DOM. Fluorophore C intensity for the CAT and MBR was about 53% of the CTL and the XAD fluorophore C intensity was about 72% of CTL (Table V). Reduction in fluorescence intensity of fluo-

TABLE V Effect of purification treatment on the location and relative fluorescence intensities of the fluorophores isolated from field corn residue derived dissolved organic matter. All extracts adjusted to 3 m*M* DOC and pH 4.5

Treatment	*Parameter*	*Fluorophore C*	*Fluorophore D*
CTL	Location	274 EX/345 EM	313 EX/440 EM
	Intensity	233†	109
CAT	Location	274 EX/336 EM	313 EX/444 EM
	Intensity	123	117
MBR	Location	271 EX/342 EM	313 EX/441 EM
	Intensity	122	171
XAD	Location	277 EX/342 EM	316 EX/435 EM
	Intensity	168	226

†Fluorescence intensity in arbitrary units.

rophore C could be result of retention of positively charge proteinaceous materials by the resin, selective precipitation of proteinaceous materials by the low pH conditions induced by the treatment or low pH-induced denaturation of the proteins[33].

To further investigate the effect of pH, the DOM retained by filtration with the 0.4 μm polycarbonate filter after acidification of the corn residue extract to pH 2 was rinsed and re-dissolved at pH 7.5. The precipitated DOM corresponded to 12.4% of the total carbon mass balance. The fluorescence EEM spectrum was collected after readjustment to pH 4.5 and 3 m*M* C_{TS} to provide comparability with the other spectra collected in this study (Figure 4). The intensities of fluorophore C and D of the re-dissolved precipitate were about equal (fluorophore C = 63, fluorophore D = 67 arbitrary units) indicating that both protein- and fulvic-type materials are precipitating at the low pH values. The intensities of the fluorophores were 27 and 61% of the CTL for fluorophore C and D, respectively. This indicates that the precipitating fraction of the DOM contains more non-fluorescent carbon than the CTL DOM.

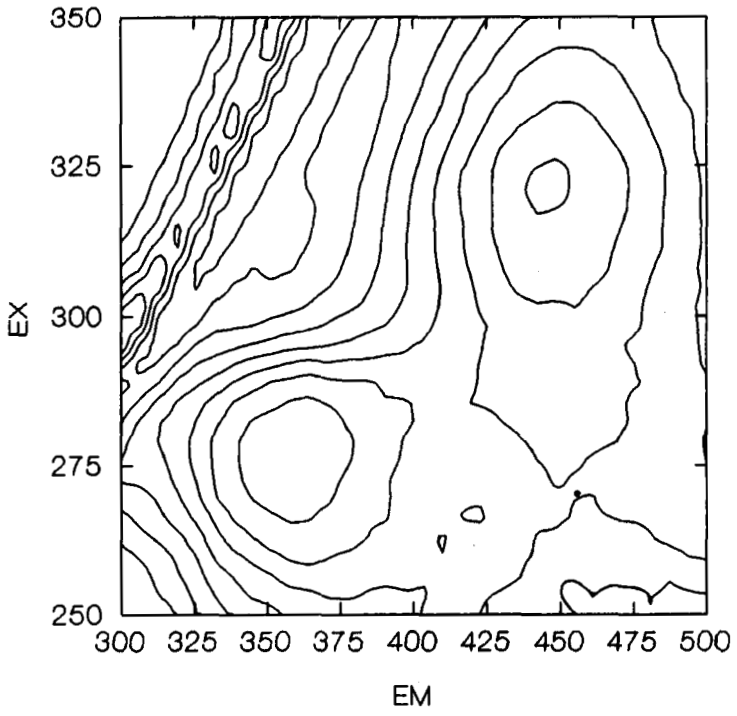

FIGURE 4 Fluorescence excitation-emission matrix of the re-dissolved corn residue precipitate adjusted to pH 4.5 and 3 m*M* DOC.

The fulvic-type fluorophore intensity of the corn residue extract was about 30% of the intensity of the O horizon extract (Tables IV and V) which was expected since plant residue extracts have not undergone humification. There was a high linear correlation, r = 0.84, between charge density and fluorophore B intensity for all the O horizon extracts (treated and untreated). Correlation coefficient between charge density of the corn residue DOM and fluorophore D was also high, r = 0.80, suggesting that the fulvic-type fluorophore is contributing much of the charge density in the corn residue DOM.

Table VI contains the location of fluorophores identified using EEM fluorescence spectroscopy for DOM samples isolated from seawater, river water, plant tissue and soils. In our study, fluorescence excitation was not scanned under 250 nm due to fluorescing contaminants introduced from the filtration of the samples. The O horizon DOM matches the spectra of 375 m deep seawater DOM[21], but does not share any fluorophore characteristics reported for a soil fulvic acid[35]. The soil fulvic acid used[35] was obtained using traditional base extraction, thus dissimilarity to the soil-derived DOM spectra of this study may reflect the different extraction method used as well as differing soil sources. An EEM for an aqueous extract of O horizon material obtained from beneath a ponderosa pine (*Pinus ponderosa* Douglas ex P. Lawson and C. Laws) plantation (their sample designated L3, Oa horizon) has been reported[22]. Their 310 nm EX/450 nm EM peak is similar to fluorophore B in this study, but no fluorophore in the 390 nm EX/500 nm EM peak was found in our DOM sample (data not shown). The linear ridge fluorophore reported for the O horizon extract[24] has been reinterpreted as being caused by Raman scatter from the water solvent (G. Sposito, personal communication). The O horizon extract[22] and our extract of pine O horizon material were conducted under similar conditions which suggests

TABLE VI Comparison of fluorophore location in samples of dissolved organic matter isolated from various sources

Sample	*Fluorophores*	*Reference*
O horizon soil	253–262/444–453†, 328–344/444–453	This study
Corn residue	271–277/336–345, 313–316/435–444	This study
1 m seawater	260/450, 285/350–360	21
375 m seawater	260/445, 345/445	21
Surface seawater	220/320, 270/320	32
Deep seawater	230/430, 310/430	32
River water	230/430	32
Suwannee fulvic acid	230/420, 300/415	20
Soil fulvic acid	350/490, 390/509, 455/521	36
Pine litter	310/450, 390/500	22

†Fluorophore location expressed EX nm/EM nm.

that distinct fluorophores signatures may be obtained from forest floor samples formed from litter from different plant species. Further work with multiple plant species under uniform extraction protocol is needed to determine the ability of EEM to differentiate DOM derived from different plant species. Evidence that synchronous-scan fluorescence signatures of forest floor extracts are strongly influenced by taxonomic differences in the forest stand has been previously reported[10]. The distinct fluorophore signature of the plant residue-derived DOM (fluorophore C) suggests that EEM fluorescence can be used to distinguish between plant residue DOM and native soil DOM. Applications of this finding could include using fluorophore C to monitor the release of DOM from plant residues in a field environment.

CONCLUSION

Soil-derived DOM was affected less by the purification treatments examined than was plant-derived DOM. Treatment of the O horizon extract by H^+-saturated cation exchange resin did not alter carbon content, charge density or the fluorophore properties of the DOM. Mixed-bed resin purification did reduce the carbon content of the extract and fluorophore intensities, but did not significantly alter charge density of the DOM. The use of serial XAD-8 and XAD-4 resins for purification had the lowest DOM recovery rate of all the treatments for the O horizon extract. This treatment increased the charge density of the DOM by a factor of 1.5 indicating preferential retention of a DOM fraction with high charge density. All purification treatments caused a significant DOM loss for an aqueous extract of field corn crop residue. Charge density of the corn residue extract was not affected by the CAT treatment, but MBR treatment reduced it by 37% and XAD treatment quadrupled the charge density. Fluorescence data indicated that purification altered the distribution of the protein-type and fulvic-type fluorophores resulting in enrichment of the fulvic-type fraction DOM.

The data presented here show that the common practice of using H^+-saturated cation exchange resin to remove inorganic cations and to protonate ***soil-derived*** DOM does not significantly alter the chemical characteristics of the DOM investigated in this study. The use of XAD purification to concentrate DOM from soil extracts results in a DOM fraction with a higher charge density, but does not greatly alter the fluorescence spectra. Our data also suggest purification of ***plant-derived*** DOM by both ionic- and nonionic-resins does alter the resultant DOM. Careful consideration is warranted to evaluate the benefits of reduction of inor-

ganic components of plant extracts to the risks of working with DOM than has been altered by the purification step.

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