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Note

Investigating sorption-driven dissolved organic matter fractionation by multidimensional fluorescence spectroscopy and PARAFAC

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Abstract

Soil organic matter is involved in many ecosystem processes, such as nutrient supply, metal solubilization, and carbon sequestration. This study examined the ability of multidimensional fluorescence spectroscopy and parallel factor analysis (PARAFAC) to provide detailed chemical information on the preferential sorption of higher-molecular-weight components of natural organic matter onto mineral surfaces. Dissolved organic matter (DOM) from soil organic horizons and tree leaf tissues was obtained using water extracts. The suite of fluorescence spectra was modeled with PARAFAC and it was revealed that the DOM extracts contained five fluorescing components: tryptophan-like (peak location at excitation <255 nm:emission 342 nm), tyrosine-like (276 nm:312 nm), and three humic-substance-like components (<255 nm:456 nm, 309 nm:426 nm, <255 nm:401 nm). In general, adsorption onto goethite and gibbsite increased with increasing DOM molecular weight and humification. PARAFAC analysis of the pre- and post-sorption DOM indicated that the ordering of sorption extent was humic-like components (average 91% sorption) > tryptophan-like components (52% sorption) > tyrosine-like components (29% sorption). This differential sorption of the modeled DOM components in both the soil organic horizon and leaf tissue extracts led to the fractionation of DOM. The results of this study demonstrate that multidimensional fluorescence spectroscopy combined with PARAFAC can quantitatively describe the chemical fractionation process due to the interaction of DOM with mineral surfaces.
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1. Introduction

Soil organic matter in forested ecosystems is important for its involvement in processes such as nutrient bioavailability and metal solubilization. The preferential sorption onto metal oxyhydroxides of the higher-molecular-weight components of humic substances, natural organic matter, and dissolved organic matter (DOM) from plant biomass has been well established [1–4]. Sorption of DOM onto mineral surfaces is an important factor in the sequestration of carbon in soils. Sorption involves interaction between surface metal groups and acidic functional moieties of DOM, suggesting that sorption is sensitive to both the chemical structure of the sorbing DOM ligands and the surface properties of the minerals [5]. Adsorption of organic matter onto metal oxyhydroxides that have a preponderance of surface hydroxyl groups proceeds predominantly via ligand exchange reactions involving both carboxyl and hydroxyl groups of the organic matter and surface hydroxyl groups of the metal oxyhydroxides [6,7].

Fluorescence spectroscopy has been used to investigate the chemical character of natural organic matter. Multidimensional fluorescence spectroscopy with parallel factor analysis (PARAFAC) has been shown to decompose the spectra into *chemically meaningful* spectral components [8–10]. Studies have shown that 3–13 components can be identified in DOM from diverse aquatic environments [11–13]. DOM fractionation is ideally suited for study by PARAFAC because the model describes components with scores and loading vectors that are estimates of concentrations of the chemical analytes and their excitation and emission spectra. The objective of this

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work was to chemically describe the preferential DOM sorption process onto goethite and gibbsite minerals, which are frequently present in soils.

2. Experimental

2.1. DOM sources and extraction

DOM extracted from forest O-horizon soils and tree leaves was used in the study. Organic horizons of hardwood and softwood stands were collected from the reference Bear Brook Watershed in Maine. Details about the sampling site can be found in Fernandez et al. [14]. Tree leaf tissues from a hard wood species, red oak (*Quercus rubra*), and two softwood species, white pine (*Pinus strobus*) and blue spruce (*Picea pungens*), were sampled from about 2–3 m above the forest floor at the University of Maine Research Forest in Stillwater, Maine. Plant samples were air-dried at 40 °C prior to being ground to pass a 1-mm sieve. The organic soil horizons and plant tissues were extracted at a 10:1 or 40:1 deionized-water:sample ratio, respectively. The samples were extracted for 16 h at 4 °C and the suspension was vacuum-filtered through a glass fiber filter. The eluent was passed through an H^+-saturated cation exchange resin to remove free metals present in the eluent.

2.2. Sorption to minerals

Goethite and gibbsite minerals used in the study were synthesized using standard methods [15,16]. Sorption was determined by reacting 0.250 g of goethite and 0.125 g of gibbsite with 25 ml of extract solution containing 50 mg total soluble carbon (C_{TS}) L^{-1} in 50-ml centrifuge tubes. The pH of the extracts was initially adjusted to 5.5 with NaOH or HCl. Three replicates of each DOM–mineral combination were placed on an orbital shaker and shaken at 120 revolutions min^{-1} for 24 h at 4 °C. The suspensions were then centrifuged at 900*g* for 25 min and filtered through 0.40-μm polycarbonate filters. Concentration of C_{TS} was determined as described below and the quantity adsorbed was calculated by difference from the initial solution.

2.3. Chemical analysis

Concentration of C_{TS} was determined using a Shimadzu TOC 5000 analyzer. UV absorbance spectra were obtained with an Agilent 8453 diode-array detector spectrophotometer and a 1-cm quartz cuvette. To minimize self-absorption effects, DOM solutions were diluted, as necessary, to have absorbance values <0.100 at 254 nm [17]. Fluorescence measurements were taken with a Hitachi F-4500 spectrofluorimeter. Instrumental parameters were excitation and emission slits, 5 nm; response time, 8 s; and scan speed, 240 nm min^{-1}. The fluorescence spectra were obtained by setting the excitation range from 240 to 400 nm and the emission range from 300 to 500 nm in 3-nm increments. The humification index (HIX) value was calculated as $HIX = (\sum I_{435\to480})/(\sum I_{300\to345})$, where I is the fluorescence intensity at each wavelength and the excitation wavelength is 254 nm [17]. This index determines the extent of organic matter humification by quantifying the red-shifting of fluorescence emission that occurs and is a measure of the complexity and the condensed (aromatic) nature of the dissolved organic matter.

High-performance size-exclusion chromatography was used to estimate the apparent molecular weight (MW_{AP}) distributions of DOM. The system consisted of a Hewlett Packard 1100 high-performance liquid chromatography unit equipped with a Waters Protein Pak 125 stainless steel HPSEC column [18].

2.4. PARAFAC

The basis for the use of PARAFAC to decompose fluorescence spectra has been fully described elsewhere [8–10]. PARAFAC was conducted with MATLAB version 7.1, Release 14 (Mathworks, Natick, MA) using PLS_Toolbox version 4.0 (Eigenvector Research, Manson, WA). A non-negativity constraint was applied to each dimension to allow only chemically relevant results, since negative concentrations and florescence intensities are chemically impossible, assuming that quenching and inner filter effects are negligible. Subtraction of deionized-water blank spectra from each sample spectrum was used to remove Raman scatter lines. Rayleigh scatter lines were removed by replacing the fluorescence intensity values with missing values in the region immediately adjacent to where emission and excitation wavelengths were equal. Regions where the emission wavelength was less than the excitation wavelength were set to zero. PARAFAC models with two to seven components were computed for the data set and determination of the correct number of components was assessed by the core consistency diagnostic score, which should be close to 100% for appropriate models [10].

3. Results and discussion

3.1. DOM chemical properties

The MW_{AP} and HIX of the five DOM extracts are shown in Table 1. The pre-sorption MW_{AP} value of about 410 g mol^{-1} of the DOM derived from water extracts of soil O-horizons found in this study were somewhat lower than those determined from a water-extracted oak horizon forest floor layer, which was reported to have a mean number-averaged molecular weight of 750 g mol^{-1} [19]. The pre-sorption MW_{AP} values of 72 to 123 g mol^{-1} for the tree-leaf-derived DOM are lower than the 312 to 905 g mol^{-1} reported for DOM extracted from crop species [18]. The pre-sorption HIX values for the DOM extracts of the two soil O-horizons were about 3.8, which was similar to a HIX value reported for a water-extracted DOM from a mineral soil [17]. The pre-sorption leaf tissue DOM HIX values of about 0.5 were much lower than those found for the soil organic horizons (Table 1). The MW_{AP} and HIX data are consistent in suggesting that the plant-derived DOM from tree species are smaller and less humified than DOM from soil organic horizons.

Table 1
The apparent molecular weight (MW_{AP}) and humification index (HIX) of the dissolved organic matter from the soil organic horizons and leaf tissues prior to and after sorption (mean ± standard deviation)

Treatment/material	MW_{AP} (g mol^{-1})	HIX
	Pre-sorption	
O-Hardwood	397 ± 19	4.04 ± 0.40
O-Softwood	421 ± 6	3.69 ± 0.38
Oak	123 ± 4	0.66 ± 0.05
Pine	131 ± 1	0.44 ± 0.02
Spruce	72 ± 1	0.48 ± 0.05
	Post-goethite	
O-Hardwood	156 ± 5[a]	0.80 ± 0.10[b]
O-Softwood	146 ± 14[b]	0.74 ± 0.01[b]
Oak	108 ± 20[c]	0.29 ± 0.05[a]
Pine	100 ± 1[a]	0.24 ± 0.02[a]
Spruce	60 ± 1[a]	0.43 ± 0.04[c]
	Post-gibbsite	
O-Hardwood	118 ± 13[a]	0.74 ± 0.10[a]
O-Softwood	115 ± 4[b]	0.73 ± 0.07[b]
Oak	97 ± 6[c]	0.31 ± 0.05[a]
Pine	81 ± 28[c]	0.28 ± 0.01[b]
Spruce	46 ± 1[a]	0.44 ± 0.02[c]

[a] Difference between the pre-sorption and post-sorption means significant at the $p = 0.05$ level.
[b] Difference between the pre-sorption and post-sorption means significant at the $p = 0.01$ level.
[c] Pre-sorption and post-sorption means not significantly different.

3.2. PARAFAC of DOM fluorescence spectra

The data array, consisting of 45 spectra [five materials × three treatments (goethite and gibbsite sorption and pre-sorption solutions) × three replicates] were analyzed by PARAFAC. The core consistency diagnostic values were 92.1% for the five-component model and 76.8% for the six-component model, indicating that the five-component model is likely the most appropriate. The excitation and emission spectral loadings of the five components are shown in Fig. 1. Components one and two have spectral loadings that likely arise from proteins containing the amino acids tyrosine and tryptophan, respectively, which have been found frequently in DOM materials [11]. Components three to five have spectral loadings that have been assigned in the literature to humic-substance-like molecules [11,12]. These components have multiple excitation maxima and a single emission maximum, a pattern that has been reported for humic- and fulvic-like DOM substances of aquatic estuary samples [11].

The modeled concentrations of the five components present in the initial, pre-sorption DOM extracts are shown in Table 2. These concentration values are based on their fluorescence signal contribution, rather than on molar concentration. Determining concentration on a molar basis would require knowledge of fluorescence quantum efficiencies, which are unknown. Distribution of the components in the two soil O-horizon-derived DOM were dominated by the three components corresponding to humic-substance-like materials. Conversely, the three plant-derived DOM were dominated by the two components that reflect protein-like materials. It is important to note the considerable content of each component in all five DOM sources, which emphasizes the continuum nature of natural organic matter.

3.3. DOM adsorption and chemical properties

The quantity of DOM sorbed onto goethite and gibbsite was normalized by weight and is shown in Fig. 2. The DOM had greater affinity for gibbsite, with average sorption of 1.90 ± 0.47 mg C_{TS} g^{-1}, as compared to 0.72 ± 0.32 mg C_{TS} g^{-1} for goethite.

Sorption was also dependent on the DOM source, which indicated that the chemical nature of DOM is also important in determining the extent of sorption. Although this study is limited in the number of samples, sorption is qualitatively higher for the soil O-horizons, which are higher in pre-sorption MW_{AP} and HIX values than for the leaf tissue DOM samples (Fig. 2 and Table 1). This pattern toward increased sorption with greater MW_{AP} and humification extent has been well documented for other DOM materials [19] and humic substances [1,2,4].

The chemical properties of the equilibrium solution after sorption implied the extent of fractionation. Soil O-horizon DOM molecules were significantly fractionated by both goethite and gibbsite; both minerals exhibited a preference for the higher MW_{AP} fraction of DOM. This resulted in the lower MW_{AP} of the post-sorption equilibrium solution (Table 1). The fractionation of the leaf tissue DOM was significant for spruce DOM, but not for oak. Pine DOM was significantly fractionated by goethite, but not by gibbsite. The HIX values indicated a statistically significant preference for sorption of the more humified DOM fraction in all of the samples, except for spruce DOM (Table 1).

3.4. Adsorption and PARAFAC

The PARAFAC model of the concentration of components was utilized to describe the sorption-driven DOM fractionation process. The percentage reduction in concentration for

Table 2
Fluorescence-signal-based concentrations of the five components modeled by PARAFAC of the dissolved organic matter extracted from the soil organic horizons and leaf tissues prior to sorption (arbitrary intensity; mean ± standard deviation)

Material	Component 1	Component 2	Component 3	Component 4	Component 5
O-Hardwood	1090 ± 28	1230 ± 110	8640 ± 157	5160 ± 119	1600 ± 16
O-Softwood	788 ± 74	672 ± 107	5160 ± 79	1831 ± 118	1540 ± 172
Oak	3410 ± 88	1230 ± 52	1060 ± 127	2720 ± 62	275 ± 42
Pine	8680 ± 440	2240 ± 75	832 ± 10	1130 ± 29	1755 ± 4
Spruce	5710 ± 427	2240 ± 75	1321 ± 72	548 ± 28	1940 ± 37

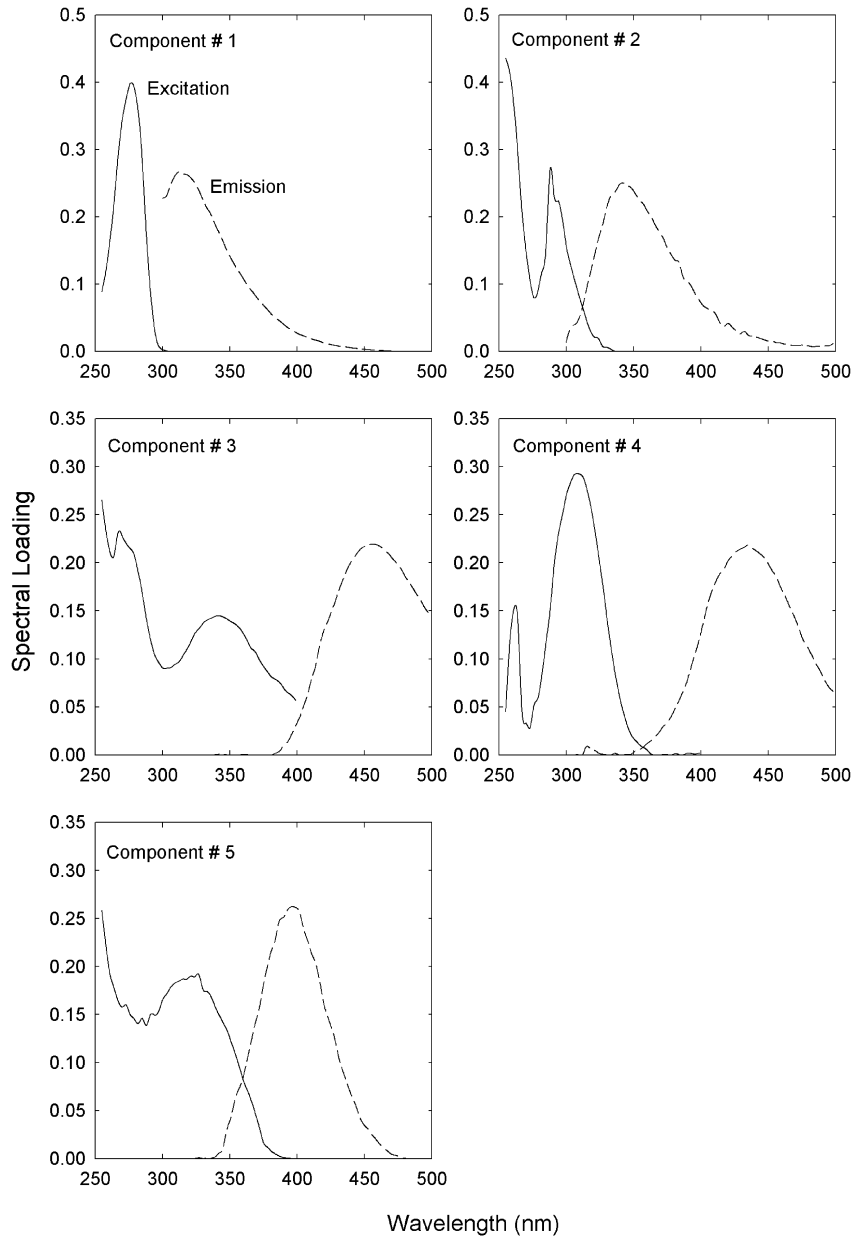

Fig. 1. Excitation and emission loadings from the five-component PARAFAC model of dissolved organic matter derived from soil O-horizons of hardwood and softwood stands and leaf tissues of red oak, white pine, and blue spruce tree species.

the five components after sorption was calculated as {[(pre-sorption concentration − post-sorption concentration)/pre-sorption concentration] × 100} and is shown in Table 3. Component one, which indicates tyrosine-like compounds, showed the least sorption among the five PARAFAC components, with an average reduction in concentration of 29% in the post-sorption solution. Component one did not significantly sorb to goethite for any of the DOM extracts, although the two soil O-horizons and oak DOM did significantly sorb to gibbsite. Tryptophan-like component two significantly sorbed onto both goethite and gibbsite for all DOM molecules except for pine DOM, exhibiting an overall average concentration reduction of 52% upon sorption. Components three, four, and five (humic-substance-like materials) for the two soil O-horizon DOMs significantly sorbed to both minerals, with an average concentration reduction of 91% (Table 3). For all leaf-tissue-derived DOM, components three and four showed a significant reduction in concentration for both minerals, with a 67% average reduction in concentration. Among the leaf-tissue-derived materials, only the pine extract DOM showed a significant reduction in concentration of component five (Table 3).

4. Conclusion

As observed in other studies [20,21], the water-extractable DOM compounds of plant tissue and soil contain fluorophores that are similar to those of proteins (components one and two) and humic substances (components three, four, and five). This suggests that the humic substances that have been traditionally used to describe soil organic matter fractions based on their

Table 3
The percentage decrease in fluorescence signal based concentrations of the five components modeled by PARAFAC of the dissolved organic matter extracted from the soil organic horizons and leaf tissues after sorption to goethite and gibbsite (% decrease; mean ± standard deviation)

Material/mineral	Component 1	Component 2	Component 3	Component 4	Component 5
			O-Hardwood		
Goethite	28.1 ± 9.6[a]	60.8 ± 6.1[b]	91.4 ± 0.6[d]	96.4 ± 0.4[d]	73.4 ± 0.2[d]
Gibbsite	59.5 ± 0.8[c]	84.6 ± 2.4[c]	95.2 ± 0.7[d]	99.8 ± 0.2[d]	87.0 ± 0.6[d]
			O-Softwood		
Goethite	35.4 ± 14.7[a]	60.7 ± 3.5[b]	92.5 ± 0.4[d]	95.9 ± 0.4[c]	86.9 ± 2.1[c]
Gibbsite	49.6 ± 14.2[b]	67.1 ± 8.9[b]	95.1 ± 0.4[d]	99.7 ± 0.1[c]	90.8 ± 1.3[c]
			Oak		
Goethite	9.5 ± 6.1[a]	48.2 ± 1.5[c]	53.4 ± 1.4[b]	86.1 ± 0.2[d]	47.5 ± 1.1[a]
Gibbsite	27.9 ± 2.4[b]	54.2 ± 0.1[c]	75.0 ± 2.7[b]	92.2 ± 1.1[d]	44.4 ± 1.4[a]
			Pine		
Goethite	15.2 ± 0.9[a]	17.6 ± 2.5[a]	58.4 ± 5.3[c]	84.5 ± 0.1[d]	67.2 ± 1.2[d]
Gibbsite	23.7 ± 3.6[a]	28.6 ± 0.7[a]	51.1 ± 0.7[c]	88.6 ± 0.1[d]	69.2 ± 2.2[d]
			Spruce		
Goethite	26.9 ± 3.9[a]	57.2 ± 2.4[c]	55.6 ± 1.8[c]	55.4 ± 13.8[b]	19.4 ± 7.6[a]
Gibbsite	12.7 ± 0.9[a]	42.3 ± 2.9[c]	46.1 ± 4.5[b]	62.8 ± 9.1[c]	3.7 ± 3.1[a]

[a] Pre-sorption and post-sorption concentration means not significantly different.
[b] Difference between the pre-sorption and post-sorption concentration means significant at the $p = 0.05$ level.
[c] Difference between the pre-sorption and post-sorption concentration means significant at the $p = 0.01$ level.
[d] Difference between the pre-sorption and post-sorption concentration means significant at the $p = 0.001$ level.

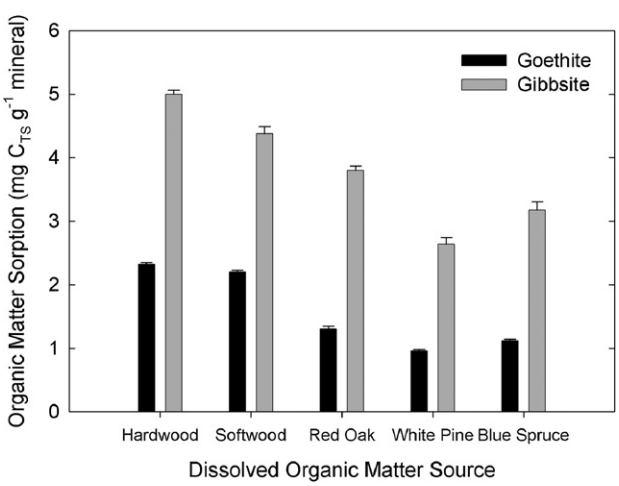

Fig. 2. Sorption of dissolved organic matter derived from soil O-horizons of hardwood and softwood stands and leaf tissues of red oak, white pine, and blue spruce tree species. Error bars indicate standard deviation, $n = 3$.

operational pH solubilities in basic extracts of soils are not composed of unique classes of compounds based on their physicochemical properties. The presence of "humic-like" components, as shown by PARAFAC, combined with the relatively low MW_{AP} of the water-extracted DOM extracts of both plant biomass and organic soil horizons (Tables 1 and 2), supports a new paradigm for the structure of humic substances recently proposed by Sutton and Sposito [22]. In this new model, humic substances are composed of low-molecular-weight components held collectively in associations through hydrophobic interactions and hydrogen bonding. Biomolecules, which certainly would be dominant in water extracts of plant biomass but were explicitly excluded in the traditional definition of humic substances, are clearly important components of humic substances. The presence of humic-like components in these water-extracted DOM from soil O-horizons and leaf tissue would be consistent with the new definition of humic substances [22]. PARAFAC of multidimensional fluorescence spectra allows direct determination of the differential affinities of the modeled DOM components (i.e., fluorophores) to soil mineral surfaces. This information may be a valuable management tool in assessing the potential sequestration of organic matter derived from a variety of carbon-rich soil amendments in both managed and unmanaged ecosystems.

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