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Characterizing Dissolved Organic Matter Using PARAFAC Modeling of Fluorescence Spectroscopy: A Comparison of Two Models

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We evaluated whether fitting fluorescence excitation−emission matrices (EEMs) to a previously validated PARAFAC model is an acceptable alternative to building an original model. To do this, we built a 10-component model using 307 EEMs collected from southeast Alaskan soil and streamwater. All 307 EEMs were then fit to the existing model (CM) presented in Cory and McKnight (*Environ. Sci. Technol.* **2005**, *39*, 8142−8149). The first approach for evaluating whether the EEMs were fit well to the CM model was an evaluation of the residual EEMs, and we found 22 EEMs were fit poorly by the CM model. Our second measure for verifying whether EEMs were fit well to the CM model was a comparison of correlations between the percent contribution of PARAFAC components and DOM measurements (e.g., dissolved nutrient concentrations), and we found no significant difference ($p > 0.05$) between the two models. These results support the approach of fitting EEMs to an existing model when DOM is collected from similar environments, which can potentially reduce some of the problems when building an original PARAFAC model. However, it is important to recognize that some of the sensitivity or ecological interpretative power may be lost when fitting EEMs to an existing model.

Introduction

Conventional analysis of aquatic dissolved organic matter (DOM) has focused on bulk measurements because of the complex nature of DOM and the analytic and interpretive difficulties associated with characterizing DOM fractions. In spite of these constraints, advances in fluorescence spectroscopy enable the rapid and precise characterization of DOM. Fluorescence measurements of DOM can generate the two-dimensional fluorescence index (FI) and three-dimensional, excitation−emission matrices (EEMs; *1, 2*), which can be used to distinguish DOM source material and monitor seasonal changes in the chemical quality of aquatic DOM (*3*).

Fluorescence EEMs can be analyzed using parallel factor analysis (PARAFAC), a multiway decomposition method that decomposes the fluorescent signal of DOM into unique fluorescent groups whose abundance is related to DOM precursor material. PARAFAC has its origin in psychometrics (*4*), but interest has grown steadily over the past decade in chemometrics (*5*) and most recently with the analysis of DOM fluorescence (*6, 7*). Studies have used PARAFAC analysis of EEMs to characterize DOM in environments ranging from Antarctic lakes with no higher plants (*1*) to forest soils in Maine (*8*), with as many as 13 components being identified in a single model (*1*). PARAFAC provides a quantitative and more complete analysis of EEMs than traditional peak picking methods, and additional information, such as oxidation state and amino acid content, can be obtained from the EEMs.

PARAFAC analysis of fluorescence EEMs is an increasingly popular tool for characterizing DOM *(9, 10)*; however, a large number of EEMs are typically collected (e.g., ref *1*, 379 EEMs; ref *7*, 1276 EEMs) to build an original model. Although a tutorial on PARAFAC modeling of EEMs was recently introduced (*11*), there is still a range of potential problems such as component selection and interpretation of the model. As an alternative to building an original PARAFAC model, scientists have fit EEMs to a previously validated model, such as in Miller et al. (*12*), when 54 EEMs were fit to the PARAFAC model presented in Cory and McKnight (*1*). One of the problems with this approach is that the EEMs could be fit poorly to the existing model, which could potentially lead to inaccurate interpretation of the fit EEMs. To our knowledge, whether this is an appropriate approach has not been evaluated.

The overall aim of this study was to evaluate whether fitting EEMs to a previously validated PARAFAC model is an acceptable alternative to building an original model. Here we report on our evaluation of this approach and provide measures for comparing the two models. We built our own model using 307 sample EEMs (herein referred to as the Alaskan (AK) model) collected from soil and streamwater. All 307 EEMs used to build the AK model were then fit to the previously validated model of Cory and McKnight ((*1*); herein referred to as the CM model). To determine if the EEMs were fit well by the CM model, we evaluated the residual EEMs. Our second measure for verifying whether EEMs were fit well to CM model was to compare components from both PARAFAC models with DOM measurements (e.g., dissolved nutrient concentrations). This approach was designed to test the hypothesis that regardless of the model used to fit the EEMs, we would obtain the same chemical/ecological information from the EEMs as long as the EEMs were described accurately by the model. This approach will further allow us to relate chemical/ecological information to PARAFAC components.

Methods

Field Methods and Laboratory Analysis. The soil and streamwater samples included in this study were collected from coastal temperate watersheds located near Juneau,

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Alaska. A 250-mL grab sample of streamwater was collected from a range of watersheds including coniferous forest, wetland-dominated, and high alpine basins. Soil−water samples were collected from 25-cm-deep piezometers located within three distinct soil types: bog, forested wetland, and upland forest (*13*). Thus, dissolved organic carbon (DOC) concentration ranged from 0.5 to 60 mg C L^{-1} for the EEMs included here. A subset of samples used to build the model of Fellman et al. (*13*) was included in the AK model. All water samples were field filtered through precombusted, glass fiber filters (nominal pore size 0.7 μm) and stored in the refrigerator until analysis within 72 h of collection.

Concentrations of DOC and total dissolved nitrogen (TDN) were analyzed via high-temperature combustion on a Shimadzu TOC/TN-V analyzer, and ion chromatography (Dionex ICS-1500 and 2500) was used to measure inorganic N concentrations (NH_4−N, NO_3−N, and NO_2−N). Total dissolved phosphorus (TDP) was measured with a 10-cm quartz cell using a persulfate digest combined with the ascorbic acid method (*14*). Biodegradable DOC (BDOC) was calculated as the difference in DOC before and after a 30-day laboratory incubation as described by Fellman et al. (*13*).

Fluorescence Spectroscopy and PARAFAC Modeling. Fluorescence EEMs used to build the AK model were measured on a Fluoromax-3 fluorometer (Jobin Yvon Horiba) with a xenon lamp following the procedures of Hood et al. (*15*). EEMs were created by measuring fluorescence intensity across excitation wavelengths 240−450 nm (every 5 nm) and emission wavelengths 300−600 nm (every 2 nm). The excitation and emission slit widths were 5 nm. Water samples were diluted with Milli-Q water to avoid inner filter effects (*16*) and EEMs were corrected for instrument bias and Raman normalized using the area under the water Raman peak at excitation 350 nm.

The complete data set used in the AK PARAFAC model included 307 EEMs. PARAFAC modeling was conducted with MATLAB using the PLS_toolbox version 3.7 following the procedures of Stedmon et al. (*6*). The AK model identified a total of ten unique components and was validated using core consistency diagnostics and split-half validation (see S1 in Supporting Information (SI)). PARAFAC components were reported as a percentage of total DOM fluorescence by using the concentration scores (expressed as F_{max}) for each modeled component and dividing that by the total fluorescence of all PARAFAC components.

For the PARAFAC model comparison, we fit all 307 EEMs used in the AK model to the previously validated CM model described by 13 unique components (see S2 in SI). We fit our EEMs to the CM model because it was developed using EEMs containing DOM from diverse sources (e.g., terrestrial and microbially-derived) collected across redox gradients in natural aquatic systems. EEMs were fit to the CM model using the N-way toolbox for MATLAB by running PARAFAC with the "fixed mode option", where the excitation and emission modes were fixed (determined from the CM model, see S3 MATLAB code in SI). EEMs used to build both PARAFAC models were measured on a Fluoromax-3 fluorometer using nearly identical procedures. Fluorescence scans performed on Quinine Sulfate Reference Material showed congruent measurements from fixed wavelength pairs for both fluorometers as the emission spectra deviated less than 10% across a range of wavelengths (see S4 in SI). Therefore, we feel confident that any differences observed between the two PARAFAC models were not driven by instrumental differences.

To determine if EEMs were fit well by the CM model, we compared the maximum intensity in each of the measured EEMs with the maximum intensity in the corresponding residual EEM (*9*). If the maximum intensity of the residual EEM was <10% of the maximum intensity in the modeled EEM, it was determined that the EEM was accurately modeled by PARAFAC. This approach accounts for areas of negative intensity in the residual EEMs due to overfitting because we considered all values (whether positive or negative) in the residual EEMs.

Quinone-like fluorophores with varying redox state account for >50% of the total DOM fluorescence in natural waters (*1*). Thus, it is possible to use PARAFAC analysis of DOM fluorescence to study the redox reactivity of DOM in natural environments. We used the oxidation state of fluorescent DOM to further evaluate the EEMs fit to the CM model by calculating a redox index (RI) for both the AK and CM model. The RI was calculated following the approach of Miller et al. (*12*), although here we used only three reduced (SQ1−3) and two oxidized (Q2−3), quinine-like fluorophores.

Pyrolysis-GC/MS. We compared PARAFAC components to pyrolysis-GC/MS products to investigate links between the fluorescing constituents and other chemical characteristics of DOM. Py-GC/MS provides a fingerprint of organic matter constituents by the thermal degradation of organic macromolecules into smaller molecular units, which are then separated by gas chromatography and mass spectrometry. Water samples for py-GC/MS were prepared by concentrating the DOM using a Labconco freeze drier (FreezeZone 2.5) at −50 °C. Py-GC/MS was conducted with a CDS model 2500 pyrolyzer and autosampler combined with a gas chromatograph/mass spectrometer (GC/MS) following the methods of White et al. (*17*). A total of nine individual pyrolysis products were selected and compared to the Wiley 275 spectral library. These nine compounds were grouped by probable source into the following classes: lignin (2-methoxy phenol), phenol (phenol), aromatics (dimethylbenzene and trimethylbenzene), polysaccharide (benzofuran, furfural, and methyl furfural) and N containing compounds (indole and pyridine). The pyrolysis products were evaluated as a relative abundance and were not individually quantified on a concentration basis.

Results

Alaskan PARAFAC Model. We identified a total of 10 components (AK1−10) within the 307 EEMs included in the AK model (Table 1). All 10 components identified by our model have been previously identified as either part of a PARAFAC model or through peak picking (visual inspection of EEMs to locate fluorophores) of EEMs (*1, 7, 18, 19*). Five components identified in the AK model corresponded to quinone-like components identified in the CM model and two of the components resemble the amino acid fluorophores (*19*). Components AK2 and AK6 have fluorescence characteristics similar to UV humic-like material derived from terrestrial sources (*7*) and component AK8 resembles humic-like material that can be derived from both terrestrial and marine sources (*19*).

A comparison of the AK and CM models (CM 1−13) found eight components had similar fluorescence characteristics (Figure 1). Quantitative similarity for these common components was statistically confirmed using Tucker congruence coefficients (see S5 in SI) and linear correlations between PARAFAC concentration scores and the corresponding components for both models (Table 1). Interestingly, the weakest correlations among the eight components common to both models were found between the tyrosine-like and tryptophan-like components.

Evaluating EEMs Fit to the CM Model. The first approach for evaluating whether EEMs were fit well to the CM model was an evaluation of the residual EEMs, and we found 22 out of the 307 EEMs fit to the CM model were modeled poorly. Nine of the EEMs modeled poorly were soil−water from upland forest sites, 12 were soil−water collected from forested wetland sites, and the last EEM was streamwater collected from a wetland site. A comparison of the measured, modeled,

TABLE 1. Fluorescence Characteristics and Correlation Coefficients between Components Identified in both the AK Model and the CM Model[a]

Alaskan model (ex/em)	Cory and McKnight (*1*) (ex/em)	description	R^2	*P* value
Comp 1 (240/450−470)	Comp 2 (250/450)	Q2	0.86	<0.001
Comp 2 (330/456)	Comp 1 (340/458)	humic-like	0.94	<0.001
Comp 3 (290/510)	Comp 5 (295/516)	SQ1	0.85	<0.001
Comp 4 (240/384)	Comp 12 (250/388)	Q3	0.76	<0.001
Comp 5 (340/410−440)	Comp 9 (345/410)	SQ3	0.95	<0.001
Comp 6 (240/414)	missing from model	humic-like		
Comp 7 (275/462)	Comp 7 (270/462)	SQ2	0.95	<0.001
Comp 8 (295/416)	missing from model	humic-like		
Comp 9 (270/306)	Comp 13 (280/<350)	tyrosine-like	0.54	<0.001
Comp 10 (280/336)	Comp 8 (270/<350)	tryptophan-like	0.70	<0.001
protein-like	protein-like		0.87	<0.001

[a] Correlation coefficients are from linear regression analysis between the PARAFAC scores of the corresponding components for all sample EEMs (n = 307) in the two models. Protein-like fluorescence is the sum of tyrosine-like and tryptophan-like components. Q2 and Q3 are oxidized, quinone-like fluorophores. SQ1−3 are reduced, semi-quinone-like fluorophores.

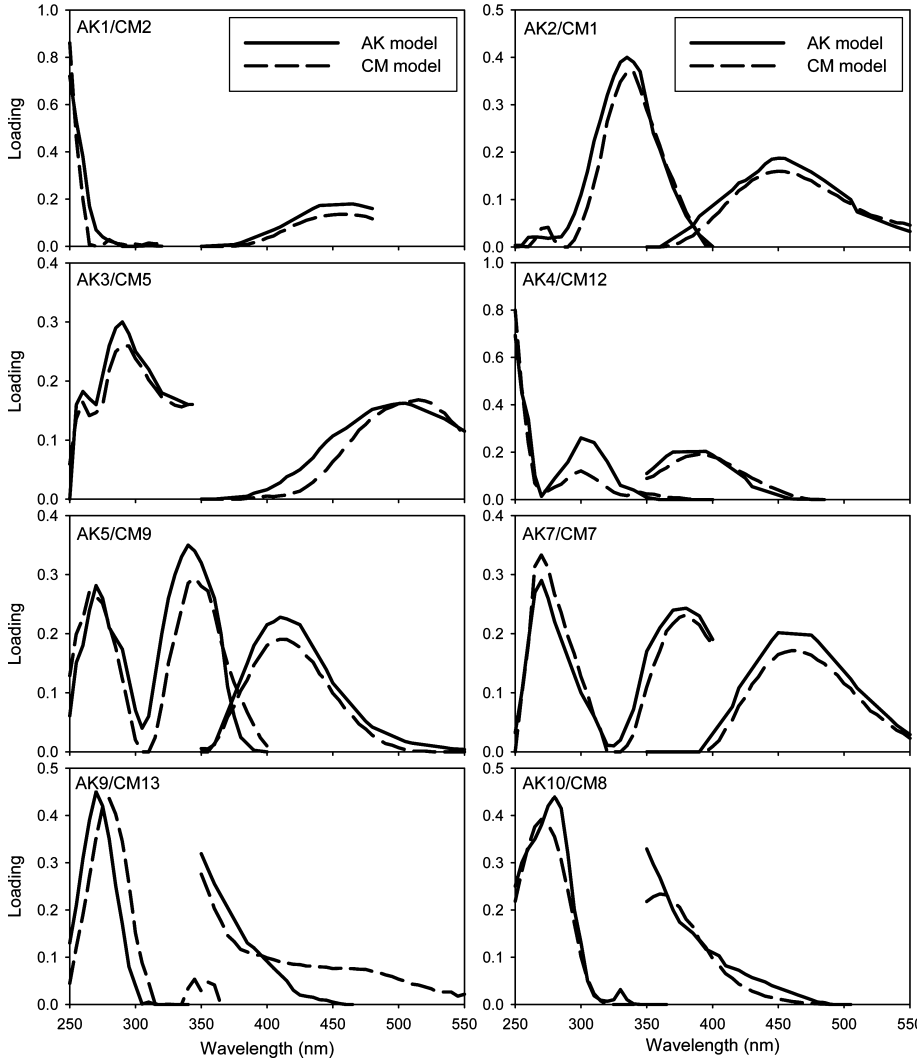

FIGURE 1. Comparison of the spectral properties for the eight PARAFAC components common to both the AK and CM models.

and residual EEMs for a typical soil−water sample showed that the residual EEM contained mostly noise, confirming that the model accounted for the majority of DOM fluorescence for most of the measured sample EEMs (Figure 2a). Evaluating the residual EEM from one of the soil−water samples poorly modeled by the CM model shows the presence of additional humic fluorophores, one of which is located at approximately 295 nm excitation and 426 nm emission and represents component 8 of the AK model (Figure 2b).

Our second measure for verifying whether EEMs were fit well to the CM model was a comparison of correlations between the percent contribution of PARAFAC components with DOM measurements for both PARAFAC models. The

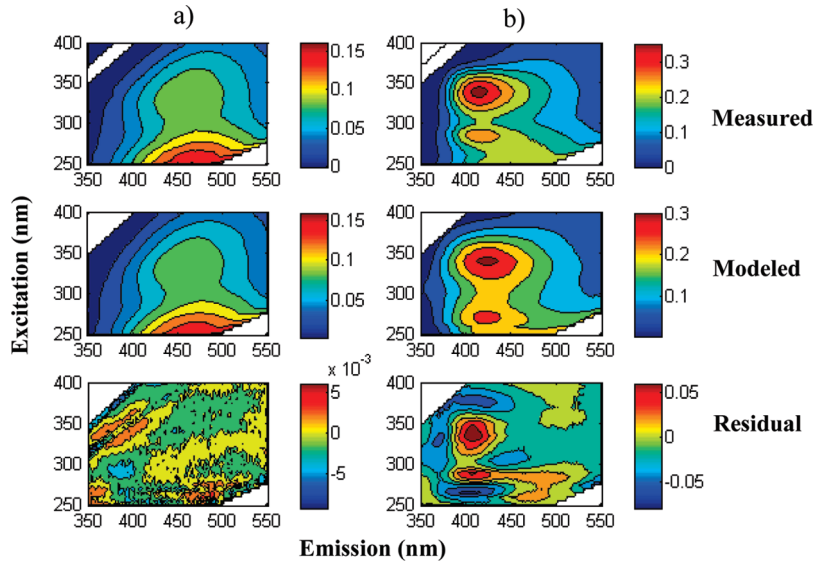

FIGURE 2. Comparison of the measured (top), modeled (middle), and residual (bottom) EEMs for two soil−water samples: (a) EEM modeled well and (b) EEM modeled poorly by the CM model. Intensities are in Raman units.

TABLE 2. Correlation Coefficients between Dissolved Nutrient Concentrations and the Percent Relative Contribution of PARAFAC Components for Samples Included in the Alaskan (AK) and Cory and McKnight (CM) Model (AK/CM)[a]

Component	DOC	TDN	DIN	DON	TDP	% BDOC
tyrosine-like	ND	0.26[b]/0.26[b]	0.05/0.06	0.23[b]/0.21[b]	0.18[b]/0.11	0.47[b]/0.30[b]
tryptophan-like	ND	0.34[b]/0.31[b]	0.10/0.10	0.39[b]/0.13	0.39[b]/0.36[b]	0.58[b]/0.46[b]
protein-like	ND	0.42[b]/0.40[b]	0.14/0.12	0.44[b]/0.31[b]	0.52[b]/0.44[b]	0.81[b]/0.60[b]
AK 1/CM 2	0.29[b]/0.28[b]	0.27(−)[b]/0.11(−)	0.08(−)/0.06(−)	0.21(−)[b]/0.10(−)	0.41(−)[b]/0.21(−)[b]	ND

[a] Protein-like fluorescence is the sum of tyrosine-like and tryptophan-like components. ND indicates value not determined and (−) indicates a negative relationship. [b] $P < 0.05$.

TABLE 3. Correlation Coefficients between the Percent Relative Contribution of PARAFAC Components and py-GC/MS Products for Samples Included in the Alaskan (AK) and Cory and McKnight (CM) Model (AK/CM)[a]

component	% N containing compounds	% phenol	% aromatics	% lignin	% polysaccharide
AK 1/CM 2	ND	0.08(−)/0.08(−)	0.10/0.10	0.44[b]/0.37[b]	0.01/0.01
AK 2/CM 1	ND	0.79[b]/0.75[b]	0.58(−)[b]/0.47(−)[b]	0.10(−)/0.08(−)	0.01/0.01
AK 3/CM 5	ND	0.62(−)[b]/0.51(−)[b]	0.42[b]/0.38[b]	0.17[b]/0.10	0.01/0.01
AK 4/CM 12	ND	0.54[b]/0.33[b]	0.41(−)[b]/0.38(−)[b]	0.06(−)/0.09(−)	0.01/0.01
AK 5/CM 9	ND	0.66(−)[b]/0.56(−)[b]	0.63[b]/0.42[b]	0.09/0.10	0.04/0.03
AK 6/NA	ND	0.06(−)/NA	0.08/NA	0.10(−)/NA	0.24[b]/NA
AK 7/CM 7	ND	0.60(−)[b]/0.65(−)[b]	0.46[b]/0.48[b]	0.07/0.07	0.01/0.01
AK 8/NA	ND	0.75[b]/NA	0.49(−)[b]/NA	0.08(−)/NA	0.01/NA
protein-like	0.60[b]/0.41[b]	ND	ND	ND	ND

[a] Protein-like fluorescence is the sum of tyrosine-like and tryptophan-like components. ND indicates value not determined, (−) indicates a negative relationship, and NA indicates component missing from the CM model. [b] $P < 0.05$.

percent contribution of protein-like components were significantly correlated with TDN, DON, and TDP concentrations for the AK model (Table 2). However, these components were not as effective at predicting DON and TDP concentrations for the CM model. The percent contribution of component AK1, which is the dominant humic-like component in the AK model, and the corresponding component in the CM model (CM2) were both positively correlated with DOC and negatively correlated with TDP concentration. The percent contributions of protein-like components were significantly correlated with percent BDOC for samples fit to both the AK and CM models (Table 2).

An evaluation of py-GC/MS products found that the N containing compounds were significantly correlated with protein-like fluorescence for both PARAFAC models (Table 3). The percent contribution of phenol and aromatics were significantly correlated with 6 different components in the AK model and the corresponding components in the CM model. Percent lignin was significantly correlated with AK1 and AK3, but only component CM2 in the CM model. Additionally, percent polysaccharide was significantly correlated with AK6. An evaluation of the correlation coefficients generated from comparing the percent contribution of PARAFAC components with dissolved nutrient concentrations, BDOC and py-GC/MS products for both models found no significant difference (t test, $p > 0.05$) between the AK and CM models.

Fluorescence EEMs fit to the CM model were further evaluated using the oxidation state of fluorescent DOM. The RI calculated from the CM model accounted for 76% of the variation in the RI calculated in the AK model for all EEMs taken together (Figure 3a). However, when the 22 samples

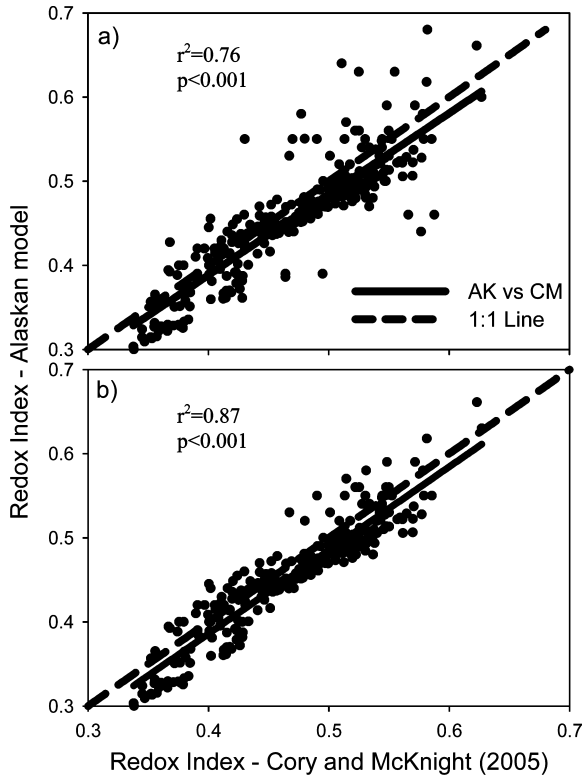

FIGURE 3. Regression models describing the relationship between the redox index calculated from the PARAFAC components corresponding to the two models with (a) all EEMs included in the CM model and (b) removing the 22 EEMs modeled poorly by the CM model using the residual EEM approach of Mladenov et al. (*9*).

modeled poorly by the CM model (determined by evaluating the residual EEMs) were removed from the linear regression, the CM RI accounted for 87% of the variation in the AK model RI (Figure 3b). Interestingly, the five-component RI averaged 0.46 for all 307 EEMs and the average seven-component RI of Miller et al. (*12*) was 0.47. This indicates that the simpler, five-component RI was an excellent indicator of the redox state of fluorescent DOM for these 307 EEMs, which is particularly useful because the two components not included in the five-component RI are not commonly observed fluorophores in other studies.

Discussion

The high similarity among the eight components common to both models indicates PARAFAC analysis of fluorescence EEMs is an effective method for characterizing aquatic DOM in natural environments. The weaker correlations and congruence coefficients between tyrosine-like and tryptophan-like fluorescence compared with the other humic-like components indicates there is considerable analytical variance in the measurement of protein-like fluorophores. This can occur as a result of Raman interference and/or the presence of humic material because humic fluorophores have been shown to interfere with amino acid fluorescence, particularly from fluorophores with low excitation wavelengths (*20*). Phenolic groups have also been shown to contribute to the fluorescence signal in the amino acid region (*21*). Additionally, the EEMs used to build the AK model encompass a broader range of wavelengths in the low emission and excitation ranges (range where proteins fluoresce) than in the CM model. Thus, the AK model likely captured more of the variability in the protein-like region relative to the CM model.

Evaluating EEMs Fit to the CM Model. Of the 307 EEMs fit to the CM model, 22 were modeled poorly as determined by evaluating the residual EEMs. One of the limitations of this approach for assessing EEM fit is that it does not consider shapes of unmodeled residual data. Thus, finding an independent measure for confirming EEM fit is preferable for fitting EEMs to an existing model. The fact that the percent contribution of PARAFAC components from both models explained a similar amount of the variability in DOM measurements (e.g., BDOC) suggests that regardless of the PARFAC model used, the EEMs provide similar chemical and/or functional information about the DOM. Thus, a rigorous comparison of correlations between the percent contribution of PARAFAC components and other DOM measurements provides an independent check confirming the fit of EEMs attached to the CM model. Our finding that removing the 22 samples modeled poorly by the CM model from the RI regression model (Figure 3b) improves the relationship underscores the importance of properly evaluating the residual EEMs. Therefore, we suggest that the combined approach of evaluating the residual EEMs and a comparison of correlations between the percent contribution of PARAFAC components and DOM measurements is a more thorough strategy for evaluating EEMs fit to an existing model than evaluating the residual EEMs alone.

Our finding that fitting EEMs to a previously validated PARAFAC model is an acceptable alternative to building an original model is important because if fluorescence spectra are measured properly, it will allow for easier comparisons between PARAFAC models and will allow scientists to say with more confidence that any differences observed in their data are not the direct result of differences in modeling methodology. One of the limitations of building an original PARAFAC model is that a large number of EEMs that contain DOM from a range of sources of diverse chemical quality are typically needed to decompose the fluorescence signal of DOM. We therefore suggest that fewer samples need to be collected if EEMs can be fit to a previously validated model built from samples where DOM was collected from a similar environment. Because there can be a large amount of variability in the amount of information contained in the individual EEMs (e.g., ref *6*, 90 EEMs, 5-component model; ref *7*, 1276 EEMs, 8-component model), it is difficult to recommend a minimum data set when building an original PARAFAC model. However, with most data sets, it is likely that 20−100 and preferably >100 EEMs are needed to increase natural variability and to simplify the model validation process (*11*).

There was no significant difference in correlation coefficients generated from comparing the percent contribution of PARAFAC components with DOM measurements for both models. However, the PARAFAC components from the CM model generally explained slightly less of the variation in DOM measurements. For example, there were five different examples when significant correlations were observed between DOM measurements and PARAFAC components for the AK model but not the CM model. Although we do recommend fitting EEMs to a previously validated PARAFAC model when DOM is collected from similar environmental conditions, this finding indicates that some of the sensitivity or ecological interpretative power may be lost when applying this approach.

Chemical Relationships with PARAFAC Components. The strong relationship between the percent contribution of PARAFAC components and aromatic/phenol content provides further evidence for the role of humic-like material of terrestrial origin in DOM fluorescence. Components AK 1, 2, and 7 were also negatively correlated with BDOC (data not shown) suggesting that these fluorophores are resistant to biological degradation. The finding that the three semi-

quinone-like components (AK 3, 5, and 7) were positively correlated with percent aromatics is consistent with the idea that reduced quinone-like components should contribute to the aromatic fraction of DOM (*1*). PARAFAC components were also poorly correlated with polysaccharide content, which was expected because carbohydrates do not typically exhibit fluorescence. This result further suggests that carbohydrates and fluorescent DOM do not vary in the same way within our soil and streamwater samples.

The significant correlation between component AK1/CM2 and DOC concentration is not surprising given that AK1 and CM2 are also correlated with lignin content and the majority of samples in this study were dominated by terrestrially derived DOM, as indicated by FI values of 1.2−1.4. These results are consistent with research showing that terrestrially derived DOM is generally enriched in lignin compared to microbial-like DOM (*2, 22*). However, for a set of samples with DOM that is more microbial-like in origin (e.g., FI > 1.5), there may not be a significant relationship between AK1/CM2 and DOC concentration because the majority of the organic matter contributing to the DOC concentration may not contain fluorophores such as AK1/CM2.

We found tryptophan-like fluorescence was correlated with dissolved N and P concentrations, which is consistent with research of coastal wetlands and estuaries (*21*). However, the fact that both protein-like components were only mildly correlated with dissolved N concentrations indicates that these components did not represent pure fluorophores, but rather represent mixtures of proteinaceous compounds with similar fluorescence characteristics. A comparison of the spectral properties for reference standards of tyrosine and tryptophan with the spectral properties of the tyrosine-like and tryptophan-like components we identified in the Alaskan model also support the idea that these two components did not represent pure dissolved amino acids (see S6 in SI). Therefore, we suggest that PARAFAC components may be effective indicators of relative C, N, and P content or function in the environment, but that they likely should not be used to predict nutrient or BDOC concentrations.

When To Build a New PARAFAC Model? Whether to fit EEMs to an existing PARAFAC model or build an original model remains a difficult question to answer and we do not provide a specific recommendation here. Although we do recommend fitting EEMs to an existing model, this should be conducted only after thorough evaluation of fit to the existing model and on a case by case basis. In this study, 21 out of the 22 EEMs fit poorly to the CM model were from soil−water collected from the upland forest and forested wetland sites. This finding indicates that these are not just outlying EEMs and that an original PARAFAC model could be built using only soil−water collected from southeast Alaskan soils. However, if the 22 EEMs identified as being fit poorly to the CM model were spread evenly among the wide range of study sites where DOM was collected, then these poorly fit EEMs could likely be regarded as outliers and potentially excluded from the data set.

There is great interest in building a universal PARAFAC model that could be widely available for researchers to fit their own EEMs too. Much remains to be learned about DOM fluorescence in natural environments and, at this point, a much larger data set of EEMs that contains a greater diversity in DOM source and chemical quality than we present here is required to build a universal model. The 21 EEMs fit poorly to the CM model that were from soil−water collected in the upland forest and forested wetland sites further demonstrates that DOM from different ecosystems around the globe still needs to be characterized. Therefore, we suggest that further DOM characterization is necessary prior to the development of a universal PARAFAC model and at this time, we recommend either building an original model or fitting EEMs to a previously validated model on a case by case basis.

Acknowledgments

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Supporting Information Available

S1, split-half validation of the 10-component AK model; S2, spectral properties for the CM model; S3, PARAFAC code for the N-Way toolbox in MATLAB; S4, emission spectra for quinine sulfate reference standard; S5, Tucker congruence coefficients for components common to both the AK and CM models; S6, comparison of spectral properties for protein-like fluorophores and reference standards. This material is available free of charge via the Internet at http://pubs.acs.org.

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