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Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties

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[1] Source, transformation, and preservation mechanisms of dissolved organic matter (DOM) remain elemental questions in contemporary marine and aquatic sciences and represent a missing link in models of global elemental cycles. Although the chemical character of DOM is central to its fate in the global carbon cycle, DOM characterizations in long-term ecological research programs are rarely performed. We analyzed the variability in the quality of 134 DOM samples collected from 12 Long Term Ecological Research stations by quantification of organic carbon and nitrogen concentration in addition to analysis of UV-visible absorbance and fluorescence spectra. The fluorescence spectra were further characterized by parallel factor analysis. There was a large range in both concentration and quality of the DOM, with the dissolved organic carbon (DOC) concentration ranging from less than 1 mgC/L to over 30 mgC/L. The ranges of specific UV absorbance and fluorescence parameters suggested significant variations in DOM composition within a specific study area, on both spatial and temporal scales. There was no correlation between DOC concentration and any DOM quality parameter, illustrating that comparing across biomes, large variations in DOM quality are not necessarily associated with corresponding large ranges in DOC concentrations. The data presented here emphasize that optical properties of DOM can be highly variable and controlled by different physical (e.g., hydrology), chemical (e.g., photoreactivity/redox conditions), and biological (e.g., primary productivity) processes, and as such can have important ecological consequences. This study demonstrates that relatively simple DOM absorbance and/or fluorescence measurements can be incorporated into long-term ecological research and monitoring programs, resulting in advanced understanding of organic matter dynamics in aquatic ecosystems.

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1. Introduction

[2] There are three key reasons why the study of dissolved organic material (DOM) can contribute to understanding ecosystem function in diverse terrestrial and aquatic environments. First, the flux of DOM derived from plants and soils is a significant term in terrestrial carbon budgets and as a result is a dominant linkage between terrestrial and aquatic ecosystems (Figure 1). Second, within freshwater and marine ecosystems, DOM typically represents the largest pool of detrital organic carbon and greatly exceeds the organic carbon present in living biomass; in fact, dissolved organic carbon (DOC) is arguably is the most important intermediate in the global carbon cycle [*Battin et al.*, 2008]. Thus, the production, loss and transport of DOM are important terms in the carbon budget. Finally, in both terrestrial and aquatic systems, the DOM pool is highly reactive and influences ecosystem function by controlling microbial food webs and through many biogeochemical reactions, such as binding with hydrous metal oxides in soils [e.g., *Kaiser et al.*, 2004] or acting as electron shuttles under anoxic conditions in lakes [*Fulton et al.*, 2004]. Figure 1 summarizes different processes involving DOM occurring in diverse ecosystems along a generalized hydrologic flow path from mountain range to coastal zone.

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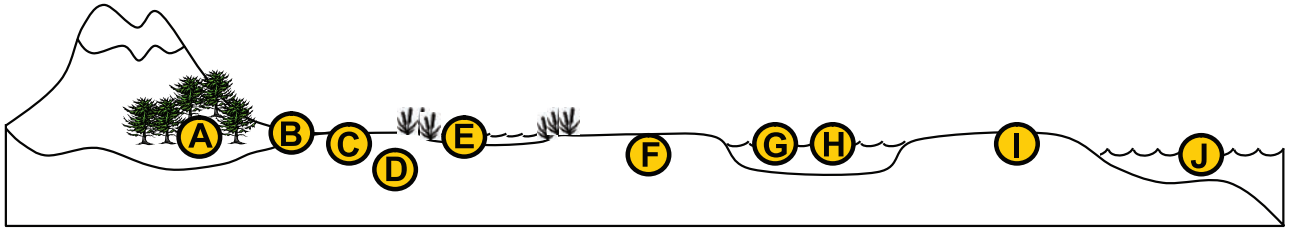

	DOM process	Working hypothesis	Example questions
A	Biomass leaching (litter fall, senescent plant materials, roots, exudation from wetland plants, etc…)	DOM quantity and quality varies with species, and is dependent on the release process and primary productivity.	How does nutrient availability influence the longer term DOM leaching and quality?
B	Biodegradation & microbial degradation	DOM components termed as labile are subject to microbial processing at an early stage after production compared to more refractive compounds.	How does DOM quality influence biodegradation and co-metabolism and how does physico-chemical speciation affect bioavailability?
C	Mineralization and incorporation into microbial biomass	DOM quality will control its microbial availability.	How does DOM quality affect microbial community structure?
D	Physicochemical transformations (soil sorption, partitioning processes, etc)	Low molecular weight hydrophobic organic components partition into DOM while more aromatic components of DOM adsorb strongly on oxide and other mineral surfaces.	How does DOM quality control such processes and subsequent bioavailability and red-ox processes?
E	Release of recently fixed C (Aquatic plants, emergent vegetation, roots, plankton, microbial mats, etc…)	DOM released from plants varies in quantity and quality with species, ecosystem type and environmental stress.	How does DOM quality from plant exudates vary seasonally and control microbial loop energetics?
F	Chemical interactions (complex formation between and among bio-and geo-polymers)	Complex formation between DOM components will result in products with different biogeochemical characteristics compared to the parent compounds (e.g. tannin-protein complexes).	How does DOM quality control complex formation and how do such complexes affect carbon cycling?
G	Photodegradation/Photolysis	Photochemical processes control DOM concentration and bioavailability in aquatic ecosystems.	How does DOM quality affect photolysis rates and photoproducts formation?
H	Zooplankton sloppy feeding and microbial oxidation, etc…	Sloppy feeding releases significant amounts of DOM which in turn can be used to support the microbial loop.	How does the DOM quality vary seasonally with primary productivity?
I	Soil/sediment leaching	Soils/sediments produce much of the DOM in pore-waters through leaching and microbial oxidation of SOM.	Which is the relative contribution of soil vs. roots to pore-water DOM inputs and quality?
J	Precipitation/flocculation	Double layer compression affects DOM quality with increasing salinity.	Which DOM components control flocculation and how does flocculation affect DOM quality and bioavailability?

Figure 1. Summary of different processes involving DOM occurring in diverse ecosystems along a generalized hydrologic flow path from mountain range to coastal zone. Examples of some of the current hypotheses and questions that can be addressed by measurements of DOM quality are described.

[3] DOM is composed of different classes of organic compounds with differing reactivity and ecological roles [e.g., *Maie et al.*, 2005; *Marschner and Kalbitz*, 2003]. For example, some compound classes known to occur in DOM, such as carbohydrates and proteins, are thought to serve as substrates supporting microbial growth. The aromatic carbon fraction of DOM, associated with the fulvic and humic acid moieties, is responsible for attenuating harmful UV light in aquatic ecosystems.

[4] Within a given ecosystem, the importance of these different DOM interactions may vary, with some processes only being important in one zone or interface within an ecosystem (Figure 1). For example, production of photo-products occurs in the photic zone of lakes, streams, rivers and ocean surface waters and sorption of DOM onto iron and aluminum oxides occurs in stream sediments and in specific soil horizons. Further, the quantity and quality of DOM are dynamic, responding to individual storm events and exhibiting seasonal variation tied to ecosystem dynamics [*Maie et al.*, 2006a; *Lu et al.*, 2003]. For example, in an alpine lake in the Rocky Mountains, *Hood et al.* [2003] found that during snowmelt, fulvic acids accounted for about 70% of the DOC and were derived from alpine plants and soils, whereas in midsummer, fulvic acid accounted for only 30% of the DOC and algal production in the lake became a major source of fulvic acid.

[5] Because DOM quality (i.e., composition) reflects the dynamic interplay between DOM sources and biogeochemical reactions, the hydrologic regime, land cover and corresponding management practices can have a significant impact on DOM biogeochemistry, as indicated in Figure 1. It is hypothesized that many, if not most, biogeochemical processes affecting the production, transport and fate of DOM will in one way or another be dependent upon DOM quality, examples of which are presented in Figure 1.

[6] For the purposes of computing the carbon budgets of ecosystems, measurements of the total concentration of DOM may seem to be sufficient and can be achieved by measuring the concentration of dissolved organic carbon. However, simple measurement of TOC alone can constrain the understanding of dominant processes driving seasonal, interannual or spatial patterns in concentration; addition of DOM quality measurements can aid in the explanation of concentration trends.

[7] The sources and chemical character of DOM (i.e., its quality) can be addressed through various analytical approaches such as spectroscopic methods that can be readily incorporated into monitoring programs and serve as a bridge between process understanding and monitoring of biogeochemical trends. These methods are primarily based on UV-visible (UV-Vis) and fluorescence measurements that have been widely used and reported in the literature [e.g., *de Souza Sierra et al.*, 1994, 1997; *McKnight et al.*, 2001; *Blough and Del Vecchio*, 2002; *Stedmon et al.*, 2003; *Cory and McKnight*, 2005]. Measurements range from specific absorbance values and ratios to the modeling of 3-D fluorescence components. Optical properties measurements of DOM have been successfully applied in some seasonal and larger-scale field studies, such as the study of an alpine catchment by *Hood et al.* [2003], during flushing of small watersheds [*Hood et al.*, 2006], and longer-term monitoring efforts in South Florida estuaries [*Jaffé et al.*, 2004; *Maie et al.*, 2006a]. Measurements that can be made on small volume, filtered water samples of the UV-Vis and fluorescence spectra of chromophoric DOM provide information primarily about chemical properties of DOM.

[8] While information on DOM composition can be obtained relatively easily through optical methods, less is known on how specific optical characteristics can be applied as proxies for ecological assessments of biogeochemical processes such as DOM bioavailability and photoreactivity. However, some literature reports have provided useful correlations in this regard. For example, a DOM study in the Arctic [*Cory et al.*, 2007] reported a good relationship between fluorescence properties and photolability of DOM. Other studies showed that disinfection byproduct formation [*Weishaar et al.*, 2003] and photoproduction of CO [*Stubbins et al.*, 2008] were strongly correlated with the DOM aromatic C content. DOM aromaticity is closely correlated with several UV-Vis and fluorescence parameters [*Weishaar et al.*, 2003; *McKnight et al.*, 2001; *Cory and McKnight*, 2005]. Similarly, correlations between DOC biodegradation with optical properties (e.g., specific absorbance at 280 nm [*McDowell et al.*, 2006]) and between the abundance of protein-like fluorophores and DOM bioavailability [*Balcarczyk et al.*, 2008] have been reported. While further research to establish reliable photoreactivity and bioavailability proxies is needed, the existing evidence that optical characteristics of DOM correlate with specific DOM quality parameters is highly encouraging.

[9] In order to evaluate the potential use of DOM quality measurements in ecologically oriented monitoring programs, we compared the organic carbon concentration and optical characteristics of DOM from diverse aquatic ecosystems across many biomes, predominantly from the Long Term Ecological Research (LTER) station network. We studied sets of samples from LTER sites that allowed us to compare the spatial patterns in DOM quality along hydrologic and biogeochemical gradients within and across a range of ecosystems. The samples were analyzed in two laboratories and included an interlaboratory comparison of fluorescence measurements.

[10] This study intended to demonstrate that there are significant shifts in DOM quality with space and time, within and across ecosystem types, which can be captured by the analysis of DOM optical properties. Our results demonstrated that this study encompassed the significant variation in both DOM quantity and quality that may be encountered in freshwater and marine surface waters. Many of the observed spatial and/or temporal shifts in DOM quality at a given site were consistent with previously characterized variation in landscape features or hydrologic events. There was no significant correlation between DOM quantity and quality, suggesting that analysis of DOM quantity does not fully capture the variation in DOM cycling and reactivity within or among sites, which was an additional question this study intended to address. Statistical analysis of the DOM quality parameters across all sites demonstrated that the variation in the fluorescence signature of the samples was mainly attributed to variation in DOM source and biogeochemical processes controlling redox state. Overall, these results provide new insights and new questions, and show that by deciphering the ''clues in the chemistry'' we can gain greater understanding of the changing role of DOM in natural and managed ecosystems.

2. Materials and Methods

2.1. Sample Collection and Processing

[11] Surface water samples were collected from 10 different North American biomes, in streams, lakes, and estuarine environments by volunteer participants from 15 U.S. institutions including 12 LTER sites (Figure 2) and shipped refrigerated to the University of Colorado and Florida International University for processing and analysis. Therefore, this sample set is highly diverse, including DOM samples from across a climatic gradient, different biomes, ecosystem types, fresh to estuarine to coastal marine waters and with a large range of autochthonous, allochthonous, and anthropogenic influences. Surface water samples were collected in dark, low-density polyethylene bottles which had been previously cleaned by soaking in 0.5 mol L^{-1} HCl followed by 0.1 mol L^{-1} NaOH for 24 h each. Water samples were filtered through precombusted (470°C for 4 h) GF/F glass fiber filters (nominal pore size, 0.7 μm; Whatman International Ltd., Maidstone, England) and stored under refrigeration until analysis. All samples were analyzed within 3 weeks after sampling. Reanalyses after 2 months of storage, did not show any significant spectral changes. Additional water quality parameters such as DOC, total nitrogen (TN) and total phosphorus (TP) were also measured on all samples following methods of the FCE-LTER (http://fcelter.fiu.edu/) The DOC and TN results were used to calculate a C:N ratio.

2.2. Optical Measurements

[12] Bulk water samples were submitted for fluorescence and UV-Vis absorption analyses after filtration using stan-

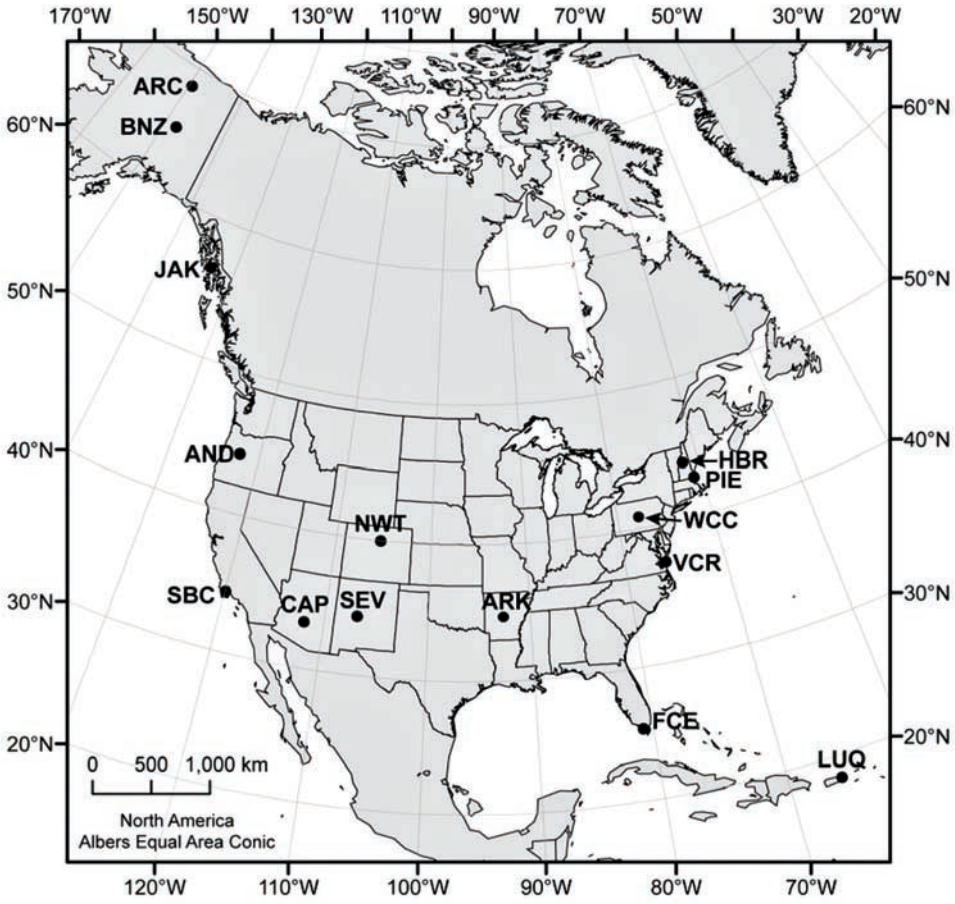

Figure 2. Map of sampling locations. The Long Term Ecological Research (LTER) sites (available at www.lternet.edu/sites/) are AND, Andrews; ARC, Arctic; BNZ, Bonanza Creek; CAP, Central Arizona-Phoenix; FCE, Florida Coastal Everglades; HBR, Hubbard Brook; LUQ, Luquillo; NWT, Niwot Ridge; PIE, Plum Island Ecosystem; SBC, Santa Barbara Coastal; SEV, Sevilleta; VCR, Virginia Coast Reserve. Non-LTER sites: ARK, Arkansas Rivers; JAK, Juneau, Alaska; WCC, White Clay Creek.

dard procedures reported in the literature [*McKnight et al.*, 2001; *Jaffé et al.*, 2004]. Briefly, UV-Vis absorption spectra were measured with a Shimadzu UV-2102PC spectrophotometer between 250 and 800 nm in a 1cm quartz cuvette to determine the specific UV absorbance (SUVA) at 254 nm ($SUVA_{254}$). The $SUVA_{254}$ parameter is defined as the UV absorbance at 254 nm measured in inverse meters (m^{-1}) divided by the DOC concentration (mg L^{-1}) [*Weishaar et al.*, 2003]. The UV spectral slope (S) was obtained by fitting the absorption data to a simple exponential equation [*Blough and Del Vecchio*, 2002]. The *S* parameter is known to be sensitive to baseline offsets; therefore, to correct for this, the average absorbance from 700 to 800 nm was subtracted from each spectrum [*Blough and Del Vecchio*, 2002].

[13] Fluorescence spectra were measured with a Jobin-Yvon-Horiba (France) Spex Fluoromax-3 fluorometer equipped with a 150-W continuous output xenon arc lamp. Two single emission fluorescence scans were obtained at excitation wavelengths of 313 nm and 370 nm. For each scan, fluorescence intensity was recorded at emission wavelengths ranging from 330 to 500 nm and from 385 to 550 nm, respectively. The band pass was set at 5 nm for excitation and emission wavelengths. From the 313 nm scan the maximum emission intensity (F_{max}) and maximum emission wavelength (λ_{max}) were determined [*Donard et al.*, 1989; *de Souza Sierra et al.*, 1994, 1997]. From the 370 nm scan a fluorescence index (FI) was calculated [*McKnight et al.*, 2001]. Originally, the fluorescence index was introduced as a ratio of emission intensities at 450 and 500 nm at an excitation wavelength of 370 nm [*McKnight et al.*, 2001]. However, after correcting fluorescence intensity values for inner filter effects [*McKnight et al.*, 2001] and for instrument bias (R. M. Cory et al., Effect of instrument-specific response on the analysis of fulvic acid fluorescence spectra, submitted to *Limnology and Oceanographic Methods*, 2008) a shift of emission maximum to longer wavelengths was observed, and thus the fluorescence index was modified to use the ratio of fluorescence intensities at 470 and 520 nm, instead of 450 and 500 nm [*Cory and McKnight*, 2005; Cory et al., submitted manuscript, 2008]. To compare to the FI values obtained by the FIU laboratory, FI values produced by the UC group were calculated from the fully corrected EEMs analyzed for parallel factor analysis (PARAFAC) (see below). FI values were obtained by

dividing the emission intensity at 470 nm by the emission intensity at 520 nm for λ_{ex} = 370 nm.

[14] For excitation emission matrix (EEM) fluorescence measurements in combination with PARAFAC, samples were analyzed with a Jobin-Yvon-Horiba (France) Spex Fluoromax-3 fluorometer following the procedures outlined by *Cory and McKnight* [2005]. Briefly, emission scans were acquired at excitation wavelengths (λ_{ex}) between 240 and 450 nm at 10 nm intervals. The emission wavelengths were scanned from 350 to 550 nm at 2 nm intervals. All fluorescence spectra were acquired in ratio mode whereby the sample (emission signal, S) and reference (excitation lamp output, R) signals were collected and the ratio (S/R) was calculated. The ratio mode eliminates the influence of possible fluctuation and wavelength dependency of excitation lamp output. Samples having absorbance greater than 0.05 absorbance units (1.0 cm quartz cell) at the lowest excitation wavelength (240 nm) were diluted with Milli-Q water to avoid interference from the inner-filter effect [*Lakowicz*, 1999]. Approximately half of the samples were diluted to avoid the inner-filter effect and dilution factors ranged from two to 20. Sample intensities were corrected for the dilution factor in the postprocessing. All postprocessing of the data was done in Matlab (version 13.1). Several postacquisition steps were involved in the correction of the fluorescence spectra (Cory et al., submitted manuscript, 2008). Each sample EEM underwent spectral subtraction with a Milli-Q water blank to remove most of the effects due to Raman scattering. Instrument bias related to wavelength-dependent efficiencies of the specific instrument's optical components (gratings, mirrors, etc.) were then corrected by applying multiplication factors, supplied by the manufacturer, for both excitation and emission wavelengths for the range of observations. Finally, the fluorescence intensities in all sample EEMs were normalized to the area under the Milli-Q water Raman peak (λ_{ex} = 350 nm) collected daily in order to compare intensities among samples collected over time following the protocol of *Stedmon et al.* [2003]. The ability of the manufacturer supplied correction factors to remove instrument bias was evaluated by analyzing and correcting an emission spectrum for quinine sulfate, a well-characterized fluorophore with a fluorescence quantum efficiency close to 1.0 [*Velapoldi and Mielenz*, 1980]. The corrected quinine sulfate spectrum had the same emission peak maximum as the NIST reference spectrum for quinine sulfate and also overlapped nearly perfectly with the NIST reference spectrum for quinine sulfate [*Velapoldi and Mielenz*, 1980].

[15] All sample EEMs were fit to the 13-component PARAFAC model generated by *Cory and McKnight* [2005]. Determination of the goodness of the fit was done by visual comparison of the measured, modeled and residual (measured minus modeled) EEM using the following criteria. First, the measured and modeled EEMs had to exhibit strong agreement in the shape, excitation and emission maxima position and intensities of all peaks. Second, assuming the first criterion was met, a satisfactory fit was established if the residual EEM primarily contained noise. A residual EEM predominately of noise is characterized by lack of typical DOM emission curves (peaks) and intensities centered around zero. Statistical processing of optical data was performed using the Statistical Discovery Software JMP (version 5).

3. Results and Discussion

3.1. Variations in DOM Quantity (TOC) and Quality (SUVA and FI)

[16] The DOC concentrations (TOC) in the data sets from the diverse study areas ranged from over 30 mgC/L to less than 1 mgC/L (Figure 3). Many data sets that included only stream or other surface water samples had DOC concentrations that were less than 6 mgC/L. The two data sets with extremely low average DOC concentrations were from a coastal site, the Santa Barbara (SBC) coastal area, and from a small northeastern stream, White Clay Creek (WCC), averaging 0.5 mgC/L and 1.0 mgC/L, respectively. The data sets which had the greatest DOC concentrations were tundra and wetland areas with saturated soils, in both the Arctic (ARC and BNZ) or in the subtropics (FCE). For the ARC data set, the highest DOC concentrations occurred in soil interstitial waters. The study areas for which the DOC concentrations ranged from low to as high as 15 mgC/L included coastal areas and their inflow rivers (VCR, PIE and LUQ). Also included in this group were an agriculturally impacted stream (ARK) and a New England forest area (HBR), which encompassed soil interstitial waters and stream samples. Collectively, these sample sets represent not only a great diversity in biomes, but also the range in patterns of DOC concentration that may be encountered within a larger ecosystem study.

[17] As shown in Figure 3, these data sets also represent a range in DOM composition and quality, as measured by optical properties such as SUVA and FI. Both of these two parameters are rather simple and rapid to determine, and our results also show that they are quite robust and reproducible. As such, the UC and FIU groups used this sample set for an intercalibration for the FI measurements, resulting in a robust correlation coefficient (r^2) of 0.87 (Figure 4). The average difference in FI value between the two different measurements was 0.05, about five times greater than analytical error on the Fluoromax fluorometers for the FI measurement. There are at least two reasons a stronger correlation was not obtained for this interlab comparison. First, the CU samples were diluted (if needed) to obtain a decadic absorption coefficient of less than or equal to 0.05 cm^{-1} at 254 nm, to avoid the inner-filter effect, where as the FIU group did not dilute and applied the inner-filter correction to the emission intensities (see section 2). In addition, the CU determinations of the FI obtained from the corrected EEM spectra, while the FIU determinations were measured directly from a single emission scan.

[18] The FI values in Figure 4 fall slightly above the 1:1 line, indicating a systematic bias toward higher FI values obtained by the CU analysis compared to the FIU measurement. To gain insight into the nature of this bias, the difference in FI value for a given sample was plotted against the TOC concentration, absorbance at 254 nm, and SUVA value (data not shown). While no relationships were identified between the CU and FIU difference in FI value and parameters charactering concentration and absorbance for a given sample, it was clear that the greatest discrepancies in FI values were obtained for samples having the lowest TOC

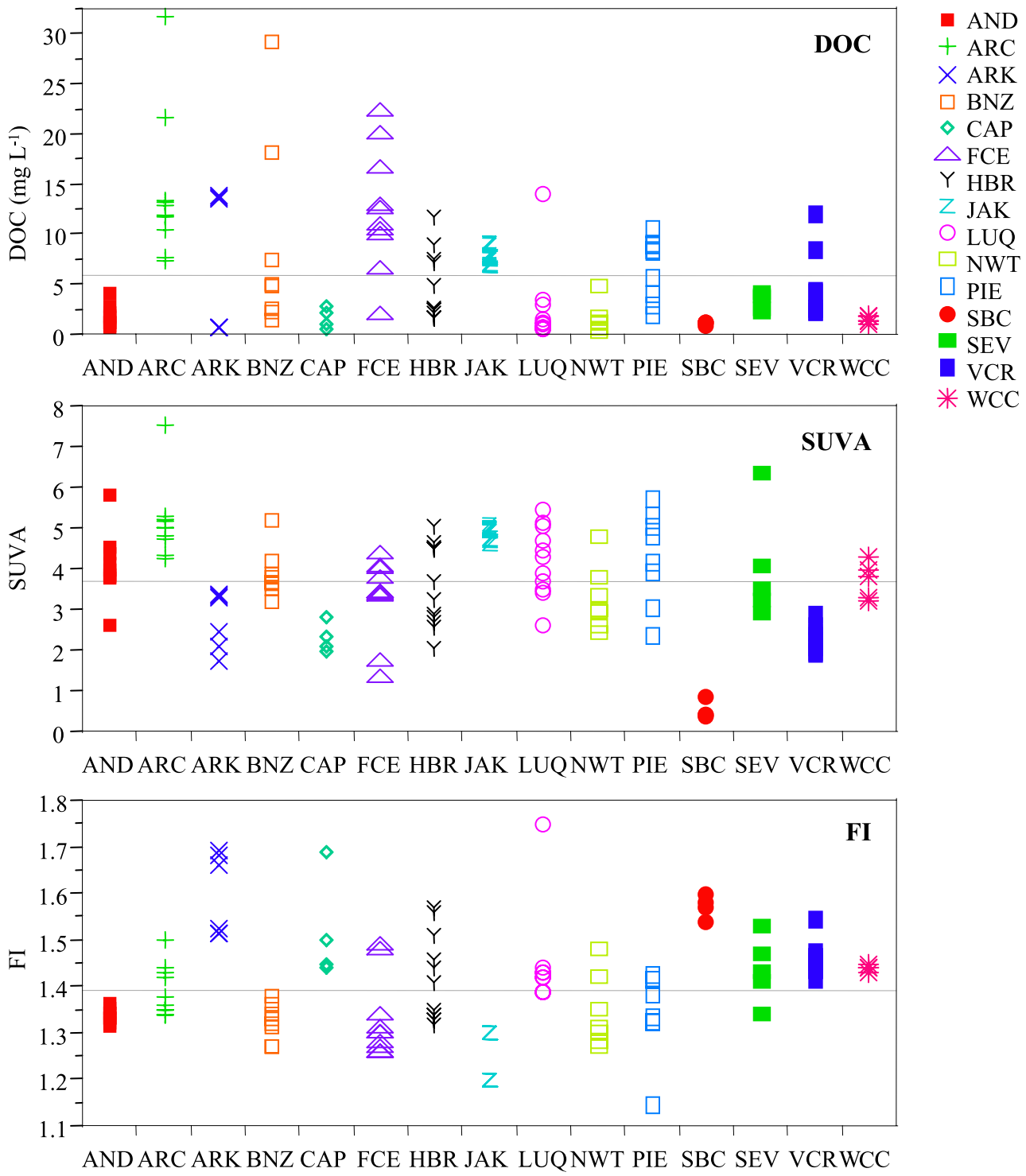

Figure 3. Distribution and range of dissolved organic matter (DOM) quantity (dissolved organic carbon, or DOC concentration) and DOM quality (specific UV absorbance, or SUVA, and fluorescence index, or FI) for all samples from the studied sites.

concentration and lowest absorbance values. This may suggest that applying the inner-filter correction to very dilute samples introduces error to the FI value, likely owing to the difficulty of obtaining an accurate absorbance spectrum from very dilute samples. Further comparison between FI values for samples analyzed on a wider range of fluorometers demonstrated that despite a discrepancy in the absolute FI value of the same sample analyzed on multiple instruments, the same trend among a sample set was obtained independently of the fluorometer used (Cory et al., submitted manuscript, 2008). Thus, while it may remain difficult to compare absolute FI values among

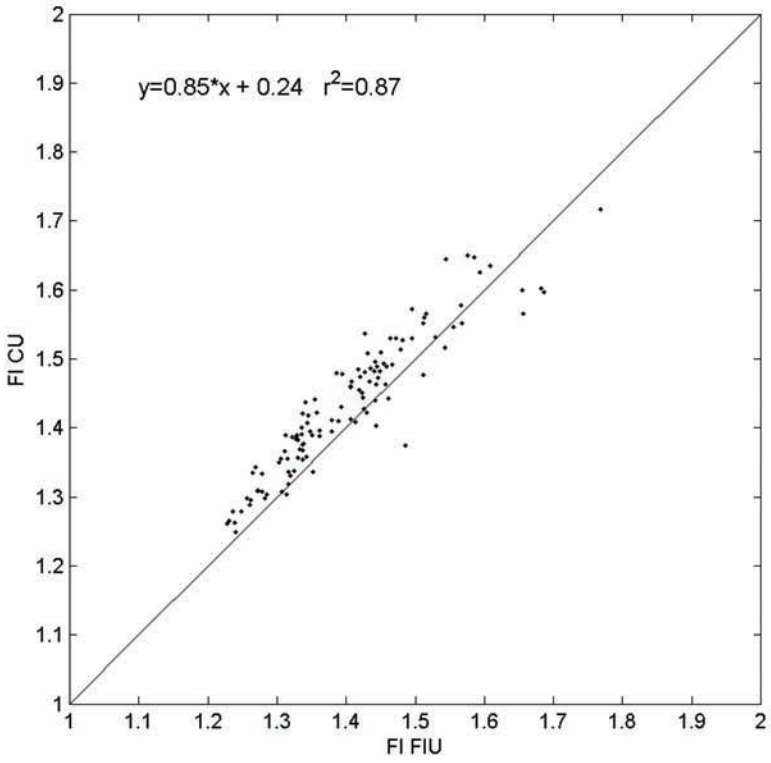

Figure 4. Results of interlaboratory comparison between the FI. Solid line represents 1:1 correlation. CU and FIU indicate University of Colorado and Florida International University.

different studies employing different fluorometers and analytical procedures, the FI trend is robust.

[19] The range in SUVA and FI values at a given site are likely linked to variation in landscape features within the study areas. For example, among the data sets with DOC concentrations less than 6 mgC/L, the samples from the alpine-subalpine catchment in Colorado (NWT) showed a large range in SUVA and FI, reflecting the increase in SUVA and decrease in FI with greater contribution of terrestrially derived DOM in the subalpine lakes surrounded by the subalpine forest relative to the alpine lakes [*Hood et al.*, 2003, 2005].

[20] The DOM quality results provide a different perspective on differences and similarities among the study areas compared to the DOM quantity data. Although SBC and WCC had similar low DOC concentrations, their DOM quality clearly contrasted. The SBC data set had the lowest SUVA values and higher FI values indicative of microbial sources. Whereas, the WCC data set had average SUVA values and lower FI values, reflecting an influence of terrestrial organic matter inputs from the forested catchment through which the stream flows. The tundra and wetland data sets with high DOC concentrations (ARC, BNZ and FCE) generally exhibited little variation in SUVA and FI values, which were in the range indicative of terrestrially derived organic matter. Similarly, the ARC soil interstitial waters had the highest SUVA and lowest FI values of the entire data set, consistent with an expectation that soil-water-derived DOM should exhibit the strongest terrestrial signature. The study areas within an intermediate DOC concentration range (VCR, PIE, LUQ, ARK, and HBR) also showed a range in DOM quality, based on optical properties, which were consistent with known variations in site characteristics. For example, in the LUQ data set the sample from a costal site near a wastewater treatment plant discharge had a low SUVA and a high FI value compared to the rest of the coastal sites, suggesting that proximity to the wastewater discharge enhanced the microbial contribution (and optical property signature) of this sample.

[21] One important observation is that in this data set there was not a statistically significant relationship between DOC concentration and either of the two DOM quality parameters (SUVA and FI; Figures 5a and 5b). In contrast, the two DOM quality parameters were significantly (at 99% confidence level) linearly correlated (e.g., Figures 6a and 6b). In general, lower SUVA values were associated with higher FI values (Figure 6a). The relationship between these parameters is anchored at the low SUVA–high FI end of the spectrum by data from the study areas where microbial inputs dominate, including the SBC, ARK sites, as well as the one sample adjacent to a wastewater treatment plant in the LUQ data set. The other end of the spectrum is broader and includes data from many study areas with significant terrestrial inputs (including BNZ, FCE, JAK, PIE, and NWT).

[22] There was a clear but somewhat less significant relationship (95% confidence level) between FI and the ratio of DOC:TN in the samples (Figure 6b). This relationship is also anchored at the low C:N high FI end by the data from the SBC area, reflecting the lower C:N ratio in DOM derived from microbial biomass. The other end of the spectrum for this relationship is broad and dominated by

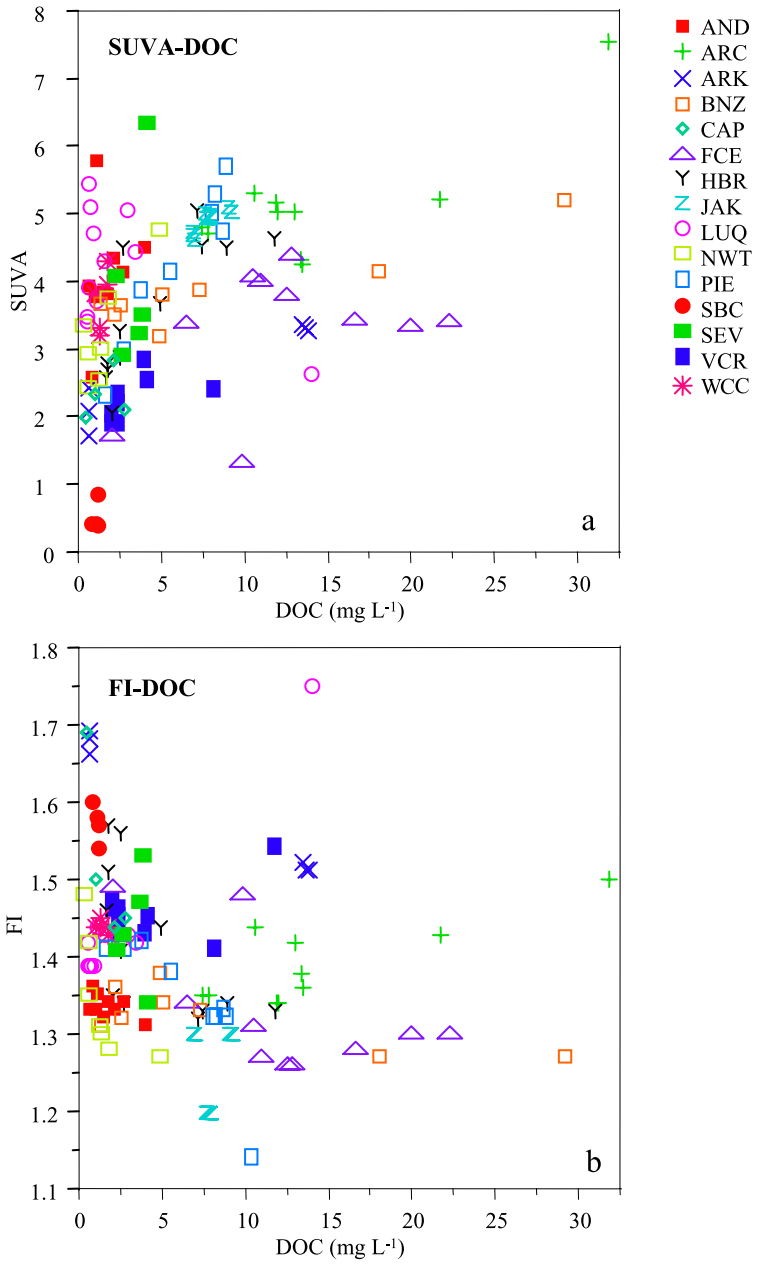

Figure 5. Crossplots between (a) DOM quality (SUVA and FI) and (b) quantity (DOC) parameters for all samples from the studied sites.

data from study areas with terrestrial DOM inputs (JAK, BNZ, and PIE).

3.2. Variation in Dominant DOM Fluorophores

[23] While SUVA and FI are easily determined DOM quality parameters that allow comparisons to be made among these diverse aquatic ecosystems, more detailed DOM quality information can be obtained through EEM-PARAFAC analyses of water samples. Although this recently developed approach [*Stedmon et al.*, 2003] is undoubtedly more involved and technically challenging, it has been applied in a number of field studies [*Fulton et al.*, 2004; *Cory et al.*, 2007; *Stedmon et al.*, 2003, 2007a, 2007b; *Stedmon and Markager*, 2005; *Hunt and Ohno*, 2007; *Yamashita et al.*, 2008]. Once the fluorometer is set up and calibrated with appropriate standards, such as aquatic fulvic acids available from the IHSS, the EEM-PARAFAC is able to provide detailed information on fluorescence properties, and thus the quality of DOM for large sample sets. The range in variation in the distribution of components is shown in the results for several different DOM samples from HBR and NWT and two from the FCE in Figure 7. The 13 DOM components of the PARAFAC model used [*Cory and McKnight*, 2005] were grouped into oxidized quinone-like components (Group I), reduced quinone-like components (Group II), amino acid-like components (Group III), and unknown components (Group IV). On the basis of this classification, not only is the DOM

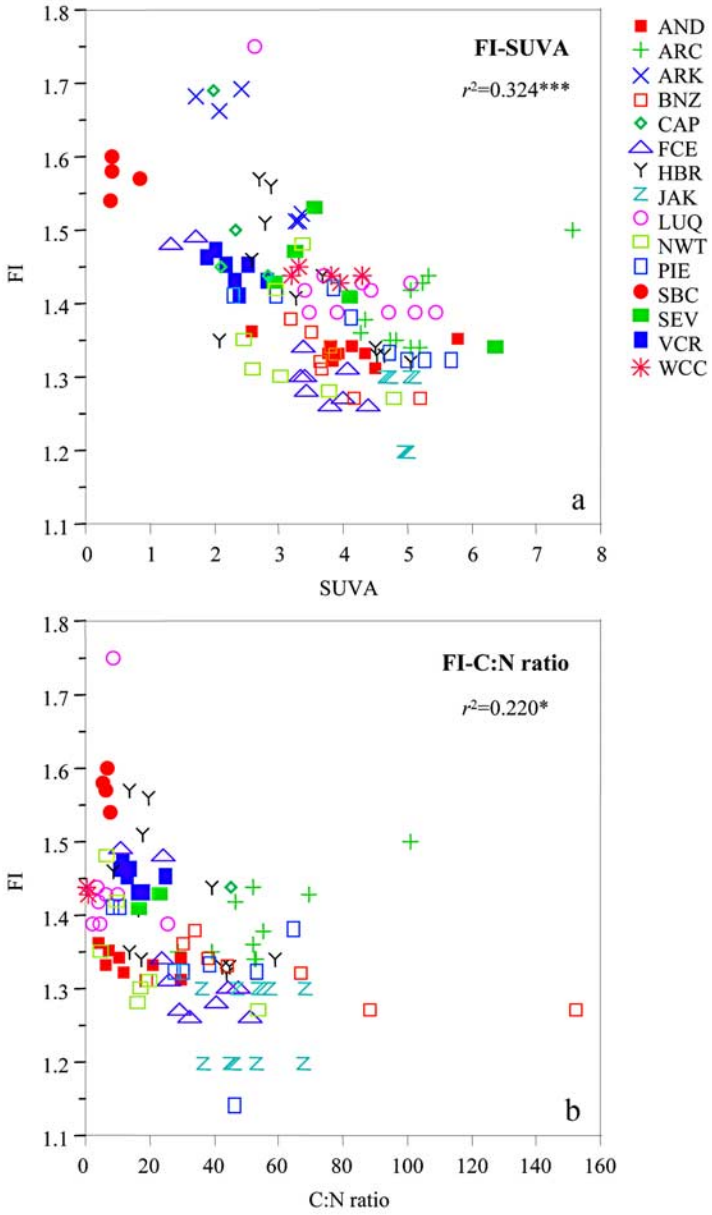

Figure 6. Crossplots between (a) DOM quality (SUVA and FI) and (b) parameters (C:N and FI) for all samples from the studied sites. Asterisks indicate significant at the 95% confidence level; triple asterisks indicate significant at the 99% confidence level.

quality different between different biomes, but significant variation can be observed within watersheds or ecosystems (see Figure 7). For example, for the two different EEM-PARAFAC results for Florida Bay it is clear that the nearshore site (T7) has a DOM quality that is significantly different from the more offshore site (T11), reflected by a much larger relative abundance of Group IV and Group II (likely humic DOM) at site T7 compared to a significantly enhanced Group III (protein-like DOM) at site T11. These differences are likely the result of a more significant DOM input from mangroves at T7 compared to inputs from sea grass/plankton communities at T11. These differences are further enhanced through hydrological processes and primary productivity variations on a seasonal basis as shown below [*Maie et al.*, 2006a].

[24] When the PARAFAC results on the relative distribution of the components for all the samples were analyzed by principle component analysis (PCA), the data points were broadly distributed along the first two axes (PC1 and PC2; Figure 8). As shown, the PC1 axis represents variations in PARAFAC components 1, 5, and 10 on the positive scale and in components 3, 8, and 13 on the negative scale. Components 1, 5, and 10 have been linked with plant/soil-derived humic substances, while components 3, 8, and 13 have been associated with microbially derived humic substances (component 3) and amino acids (components 8 and 13) [*Cory and McKnight*, 2005]. These results suggest that the PC1 axis is separating on the basis of variation in the predominant source of the organic matter: terrestrially versus microbially derived material. The PC2 axis more

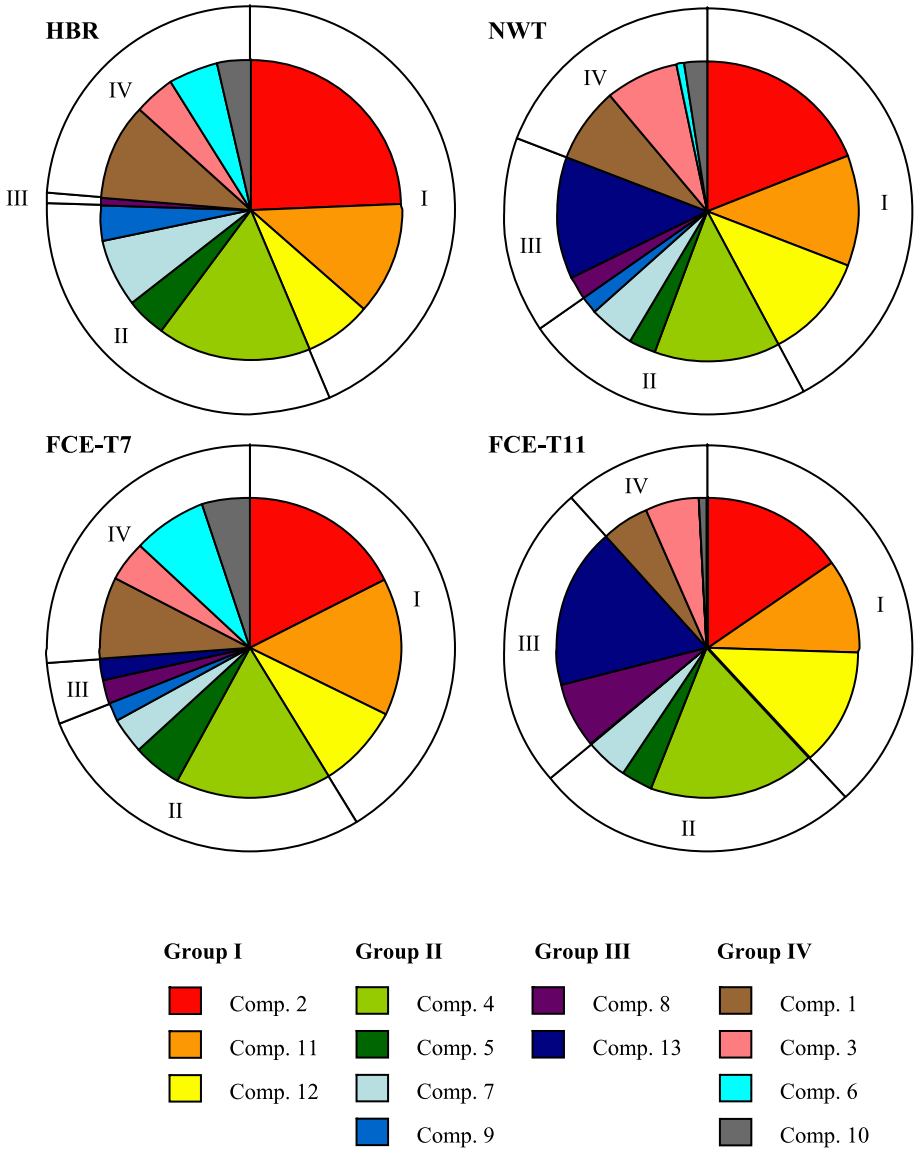

Figure 7. Examples of excitation emission matrix (EEM)–parallel factor analysis (PARAFAC) results using a 13-component model for selected samples from three LTER sites (HBR, NWT, and two sites from the FCE).

strongly represents variation in the samples controlled by the dominance of the reduced quinone-like PARAFAC components 7, 9, and 10 on the positive scale, and by oxidized quinone-like PARAFAC components 2, 12 and 6 on the negative scale [*Cory and McKnight*, 2005], suggesting that variation along this axis may be linked to relative redox state of the or organic matter. Thus, the weightings of the PARAFAC components on the first two principal components suggest that variation in organic matter sources (PC1) and biogeochemical processes such as those that control redox state (PC2) have the largest influence on DOM fluorescence.

[25] Although the entire data set is broadly distributed along the two axes, the data from several sites are located in only one quadrant. For example, the data from SBC are located in the quadrant corresponding to negative values for PC1 and positive values for PC2, indicating that the DOM in these samples is more reduced with strong microbial character [*Cory and McKnight*, 2005]. In contrast, samples from the FCE site are distributed among all quadrants except the quadrant characterized by components linked to more reduced, terrestrially derived organic matter [*Cory and McKnight*, 2005], suggesting that while there is a range in source and relative oxidation state of the DOM in these samples, the DOM character is not as strongly influenced by more reduced, terrestrially derived organic matter as other sites.

[26] A closer analysis of individual sampling locations reveals that in some cases the variability within one of the sampled ecosystems is controlled primarily by only one of the two PCA components. For example, a narrow sample cluster range along PC1 for a particular area suggests a limited range of DOM sources (e.g., ARC, SEV, and SBC), with a wider range along PC2 indicating a greater degree of

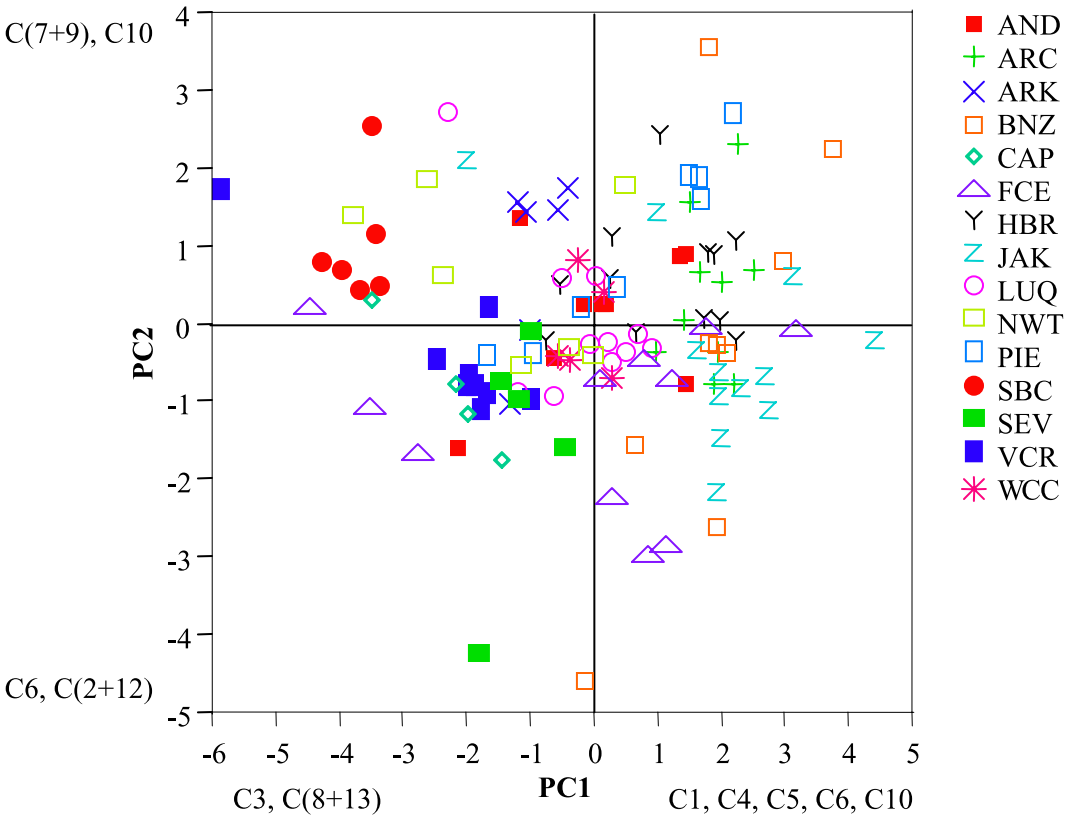

Figure 8. Principal component analysis of all EEM-PARAFAC data from all samples of the studied sites using a 13-component model.

variation in redox state among the sites sampled within that area. In contrast, some sampled ecosystems revealed a wide range both along PC1 and PC2 suggesting both variations in both DOM source and biogeochemical processes that control redox state (e.g., FCE, PIE, VCR, and BNZ). This approach in assessing DOM quality differences seems to work successfully using bulk water samples and optical properties measurements, and can add important biogeochemical information to long-term ecological monitoring programs.

3.3. Statistical Variability of DOM Quality Within a Study Area

[27] Long-term and/or regionally extensive sampling grids in water quality monitoring programs for the assessment of DOM dynamics can generate large data sets that need to be treated statistically in order to identify significant trends or tendencies. The results of two such analyses for a sampling grid for the FCE ranging from freshwater marsh to fringe mangrove to sea grass–dominated estuarine sites are presented in Figures 9a and 9b. All optical data indicative of DOM quality (λ_{max}, FI, SUVA, and S values) were included in the analysis shown in Figure 9a and compared to similar results using only EEM-PARAFAC generated data for the same samples (Figure 9b).

[28] The dendrogram in Figure 9a clearly groups the samples into three main clusters. The first cluster represents mainly the sites from Taylor Slough (TSPH2, 3, 6, and 7) and the freshwater marsh sites of Shark River Slough (SRS2 and 3). In comparison to the marl-dominated soils of Taylor, the Shark sites are peat environments and cluster separately from the latter. Within the Taylor subcluster, the freshwater marsh sites (TSPH2 and 3) are separated from the mangrove estuary sites (TSPH6 and 7), leaving TSPH3 as an intermediate between the truly freshwater and estuarine sites. In fact, TSPH3 is characterized by mainly freshwater marsh vegetation, but already features some dwarf mangroves. The second cluster is composed of the two mangrove-dominated estuarine sites from Shark River Slough, which is in agreement with the subcluster observed for the Taylor mangrove sites (TSPH6 and 7). The third cluster consists of the three Florida Bay sites (TSPH9 to 11) which are mainly influenced by sea grass communities and to a lesser extent by mangroves.

[29] When EEM-PARAFAC data is used for the same samples, the clustering was sharpened (Figure 9b). While the overall classification of the sites remained similar, the three main clusters are somewhat rearranged. Now, cluster one includes all freshwater marsh sites and cluster two all of the mangrove influenced sites, including TSPH3, but keeping the estuarine Taylor sites in a separate subcluster. Finally, cluster three continues to be for the Florida Bay samples with the most offshore, least terrestrially influenced site (TSPH11) separated from the central and NE Bay sites. On the basis of the vegetation cover and geomorphology of the FCE sites, this clustering in regards to DOM quality seems to make sense. It seems clear that both approaches reflect logical results in regards to DOM sources, but the

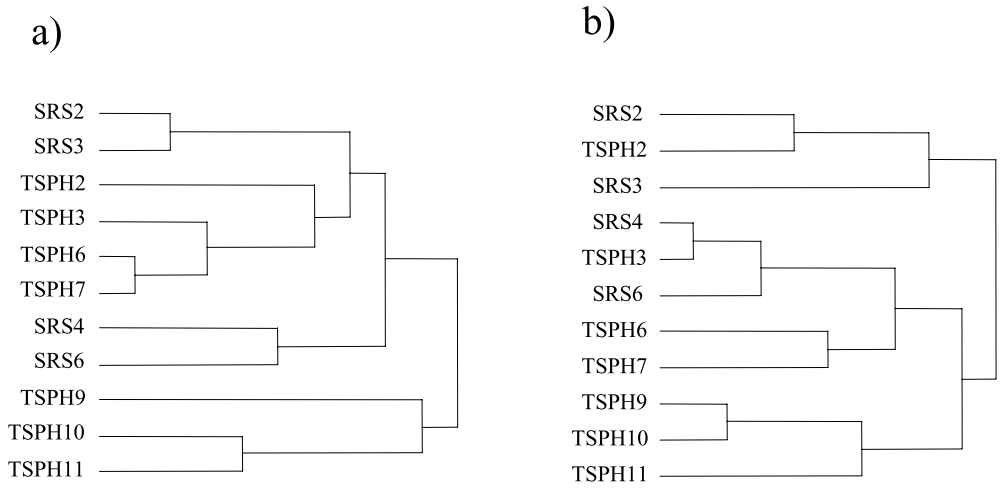

Figure 9. Dendrograms for DOM quality data for all FCE-LTER sites as determined by (a) SUVA, FI, maximum fluorescence wavelength, and *S* values and (b) EEM-PARAFAC components based on a 13-component model. SRS, Shark River Slough; TSPH, Taylor Slough sites.

EEM-PARAFAC is likely to be more sensitive to DOM quality differences.

3.4. Spatial and Temporal Variations in DOM Quality

[30] While differences in DOM quality can be induced by a variety of physical/chemical processes and ecological drivers, spatial and seasonal changes can also exert significant influence on DOM dynamics [e.g., *Maie et al.*, 2006a; *Lu et al.*, 2003]. As such DOM quality measurements can be used to identify key gradients within the study areas. For example, the HBR area in New England contains an important elevation gradient in the forested catchment which has been shown to affect DON dynamics [*Dittman et al.*, 2007] and could potentially affect DOM quality. Shown in Figure 10 are the SUVA and FI parameters plotted against elevation (in meters). Both DOM quality parameters vary consistently with elevation change by almost 50% for SUVA and by over 0.2 units for the FI. These variations suggest a significant change in DOM quality along this elevation gradient, where the aromaticity (SUVA) and the associated terrestrial component of the DOM decrease with decreasing elevation. It is apparent that along this gradient the microbial contribution to the DOM pool increases consistently.

[31] Another example is that of seasonal DOM quality variations in the Florida coastal Everglades (FCE). Here we present different examples for FI variations in two seasonally influenced estuaries (Shark River–SRS4 and Taylor River–TSPH7) and the seasonal variation in the EEM-PARAFAC-derived protein-like and humic-like components for Florida Bay (2 year monthly average for 28 sampling stations throughout the Bay), as shown in Figures 11a and 11b, respectively. The FI was found to vary seasonally, ranging from terrestrially influenced DOM at low values of 1.24 to strongly microbial influenced values of as high as 1.43 for site TSPH7, located at the mouth of the Taylor River to Florida Bay. FI values were lowest during the wet season owing to high water discharge and associated terrestrial DOM and highest during the dry season when the influence of Florida Bay waters is maximized. High FI values at that time are associated with high sea grass and microbial primary productivity [*Maie et al.*, 2006a] (see also Figure 11b). The seasonal trends in FI for station SRS4, located several miles inland from the Shark River delta into the Florida Shelf, were quite similar to those of TSPH7 by depicting high and low values for dry and wet seasons respectively. The range was, however, lower, reflecting a less intense microbial influence during the summer for SRS4 owing to limited tidal exchange with the Florida Shelf [*Jaffé et al.*, 2004]. The seasonal changes at the end of the wet and start of the dry season were also less drastic for SRS4 compared to TSPH7 owing to a more significant freshwater discharge at the former.

[32] The seasonal variability for the protein-like components as determined through EEM-PARAFAC for Florida Bay is shown in Figure 11b. Their highest relative abundance during the peak summer months suggests that such DON components are controlled by primary productivity, most likely from sea grass communities and plankton as previously suggested [*Maie et al.*, 2006a]. In contrast,

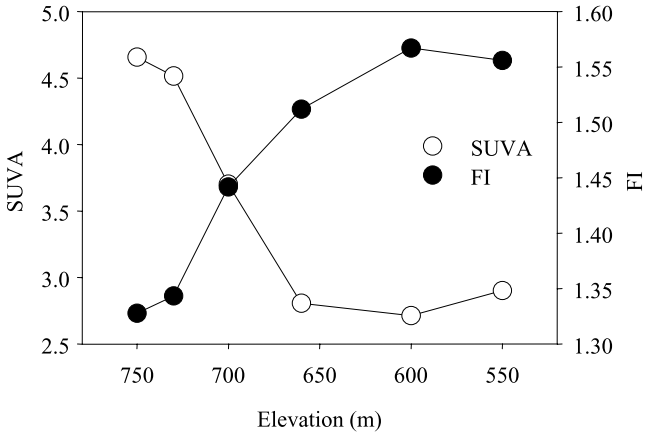

Figure 10. Variation in DOM quality as determined by optical properties (SUVA and FI) for an elevation gradient at the HBR-LTER.

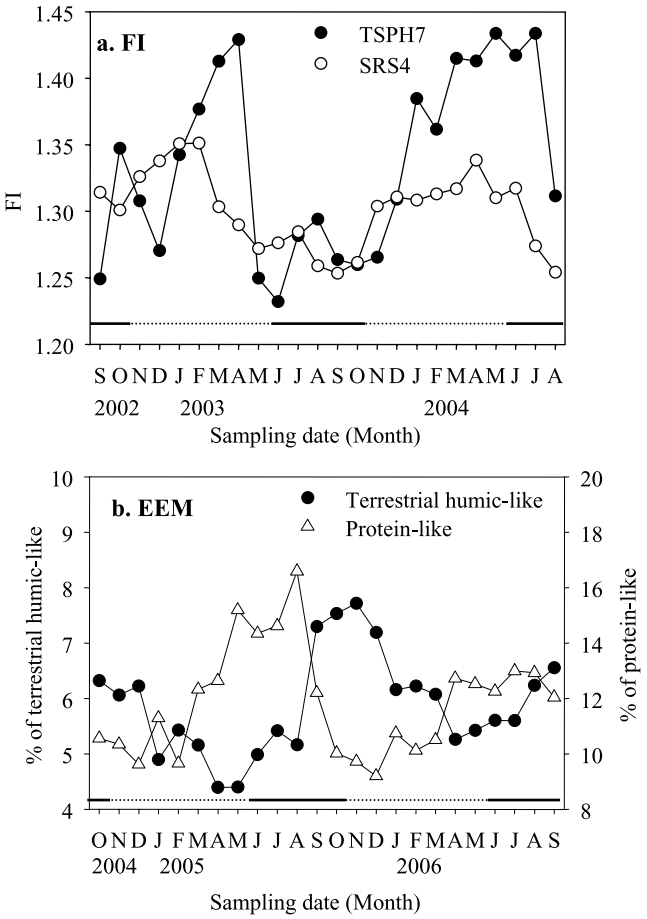

Figure 11. Examples of seasonal variability in DOM quality as determined by optical properties for (a) fluorescence index at two mangrove estuarine sites at the Shark (SRS4) and Taylor (TSPH7) rivers in the FCE-LTER and (b) EEM-PARAFAC results for a terrestrial humic-like component and a protein-like component for Florida Bay.

humic-like EEM-PARAFAC based components are controlled by hydrological processes as peak water discharge from the Everglades to Florida Bay frequently occurs during the fall. Thus, the % terrestrial humic-like components peak during the September to November period.

[33] While the temporal changes presented here are based on monthly samplings, *Hood et al.* [2006] reported on DOM quality changes on hourly timescales. These authors reported changes in the DOM aromaticity based on FI values in a stream at AND throughout a storm event. These DOM quality differences allowed them to assess the hydrology dynamics of surface runoff versus groundwater contributions to this stream. It is clear from the data presented in this section that DOM quality changes can occur on varying spatial scales and on both long- and short-term temporal scales. While much of the DOM compositional changes are induced by biophysical controls [e.g., *Gonsior et al.*, 2008; *Hood et al.*, 2006; *Maie et al.*, 2006b], such changes in composition likely result in changes in photoreactivity, bioavailability, chelating capacity and nutrient cycling and can affect carbon fluxes and consequentially ecological drivers if not accounted for.

4. Conclusions and Implications

[34] This study has shown that a wide range in DOM composition/quality characterizes surface waters in diverse aquatic ecosystems in different biomes. Further, there is not an overall relationship between DOM concentration and DOM quality. There are relationships among simple optical DOM quality measures, such as SUVA and FI, which likely vary as result of different biophysical controls [*Battin et al.*, 2008], biotic sources and biogeochemical processing of the DOM. In contrast, DOC concentrations may be more strongly influenced by hydrologic dilution, for example. These results provide examples of how the incorporation of DOM quality determinations, in the form of simple optical properties, in field studies can advance our understanding of the environmental dynamics and ecological significance of this organic substrate. Further, if simple optical parameters are not enough, more detailed characterization, which also requires no concentration/preparative scale effort, can be performed on the basis of model databases of a large set of related samples (>100 filtered 20 mL whole water samples) using EEM-PARAFAC modeling.

[35] The significant relationship between rather easily determined optical properties such as FI and the C:N ratio of DOM is promising for using such spectroscopic tools to estimate DON fluxes during different periods or seasons in ecosystems. In many systems, DON can represent a significant portion of the total dissolved N [*Bronk*, 2002] and detailed molecular characterization studies have shown that a major portion of this DOM is in the form of proteins [*Maie et al.*, 2006b]. Thus, the incorporation of optical properties of DOM as well as more advanced measurements such as EEM-PARAFAC, may help understand the coupling of C and N cycles in watersheds and test several of the hypotheses for increasing DOM transport put forward to explain current trends.

[36] Large data sets will also benefit from statistical analyses using principle components with the aim of further assessing DOM quality differences based not only on source but also on diagenetic processing. Preliminary results from such PARAFAC based databases have suggested that there is a good possibility in establishing the identity of DOM components that can be used as geochemical proxies for the assessment of bioavailability [*Balcarczyk et al.*, 2008], photoreactivity [*Cory et al.*, 2007] chelating power [*Ohno et al.*, 2008; *Yamashita and Jaffé*, 2008] and other biogeochemically important characteristics of DOM. The development of such proxies needs further attention. In times where the environment in general, and aquatic ecosystems in particular, are under high stress from climate change, land use changes, urbanization, pollution and other anthropogenically induced disturbances which have a significant impact on the biogeochemical cycles, the scientific community needs to address these issues without delay using analytical tools that are available to them now. In order to understand global biogeochemical cycles, it is crucial to characterize DOM on the molecular level, identify sources, determine physical, chemical and biological transformations and assess its ultimate fate in a great variety of aquatic

ecosystems along climatic and geomorphological gradients. Such knowledge will allow us to better understand its ecological importance and assess effects in anthropogenically altered biogeochemical cycles.

[37] In the case of DOM biogeochemistry and its environmental dynamics, significant advances could be achieved by studying detailed DOM quality variations on spatial and temporal scales, along climate, nutrient, hydrological and land use gradients and with regards to carbon flux changes to both the atmosphere and the oceans. The application of optical DOM quality parameters for this purpose is ready and at a state of maturity to address such a challenging task and timely needs. There remain the challenges of capacity building, infrastructure and serious collaborative efforts and incentives in making this happen in a short time period.

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References

Balcarczyk, K., J. B. Jones Jr., R. Jaffé, and N. Maie (2008), Dissolved organic matter bioavailability and composition in streams draining catchments with discontinuous permafrost, *Biogeochemistry*, in press.

Battin, T. J., L. A. Kaplan, S. Findlay, C. S. Hopkinson, E. Marti, A. I. Packman, J. D. Newbold, and F. Sabater (2008), Biophysical controls on organic carbon fluxes in fluvial networks, *Nat. Geosci.*, *1*, 95–100, doi:10.1038/ngeo101.

Blough, N. V., and Del R. Vecchio (2002), Chromophoric DOM in the coastal environment, in *Biogeochemistry of Marine Dissolved Organic Matter*, edited by D. A. Hansel, and C. A. Carlson, pp. 509–542, Academic, San Diego, Calif.

Bronk, D. A. (2002), Dynamics of DON, in *Biogeochemistry of Marine Dissolved Organic Matter*, edited by D. A. Hansel, and C. A. Carlson, pp. 153–247, Academic, San Diego, Calif.

Cory, R. M., and D. M. McKnight (2005), Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinines in dissolved organic matter, *Environ. Sci. Technol.*, *39*, 8142–8149, doi:10.1021/es0506962.

Cory, R. M., D. M. McKnight, P. L. Miller, Y. Chin, and C. Jaros (2007), Chemical characteristics of fulvic acids from Arctic surface waters: Microbial contributions and photochemical transformations, *J. Geophys. Res.*, *112*, G04S51, doi:10.1029/2006JG000343.

de Souza Sierra, M. M., O. F. X. Donard, M. Lamotte, and C. B. M. Ewald (1994), Fluorescence spectroscopy of coastal and marine waters, *Mar. Chem.*, *47*, 127–144, doi:10.1016/0304-4203(94)90104-X.

de Souza Sierra, M. M., O. F. X. Donard, and M. Lamotte (1997), Spectral identification and behavior of dissolved organic fluorescent material during estuarine mixing processes, *Mar. Chem.*, *58*, 51–58, doi:10.1016/S0304-4203(97)00025-X.

Dittman, J. A., C. T. Driscoll, P. M. Groffman, and T. J. Fahey (2007), Dynamics of nitrogen and dissolved organic carbon at the Hubbard Brook experimental forest, *Ecology*, *88*, 1153–1166, doi:10.1890/06-0834.

Donard, O. F. X., M. Lamotte, C. Belin, and M. Ewald (1989), High-sensitivity fluorescence spectroscopy of Mediterranean waters using conventional pulsed laser excitation source, *Mar. Chem.*, *27*, 117–136, doi:10.1016/0304-4203(89)90031-5.

Fulton, J. R., D. M. McKnight, C. M. Foreman, R. M. Cory, C. Stedmon, and E. Blunt (2004), Changes in fulvic acid redox state through the oxyline of a permanently ice-covered Antarctic lake, *Aquat. Sci.*, *66*, 27–46, doi:10.1007/s00027-003-0691-4.

Gonsior, M., B. M. Peake, W. J. Cooper, R. Jaffé, H. Young, A. E. Kahn, and P. Kowalczuk (2008), Spectral characterization of chromophobic dissolved organic matter (CDOM) in a fjord (Doubtful Sound, New Zealand), *Aquat. Sci.*, doi:10.1007/S00027-008-8067-4.

Hood, E., D. M. McKnight, and M. W. Williams (2003), Sources and chemical quality of dissolved organic carbon (DOC) across an alpine/subalpine ecotone, Green Lakes Valley, Colorado Front Range, United States, *Water Resour. Res.*, *39*(7), 1188, doi:10.1029/2002WR001738.

Hood, E., M. W. Williams, and D. M. McKnight (2005), Sources of dissolved organic matter (DOM) in a Rocky Mountain stream using chemical fractionation and stable isotopes, *Biogeochemistry*, *74*, 231–255, doi:10.1007/s10533-004-4322-5.

Hood, E., M. N. Gooseff, and S. L. Johnson (2006), Changes in the character of stream water dissolved organic carbon during flushing in three small watersheds, Oregon, *J. Geophys. Res.*, *111*, G01007, doi:10.1029/2005JG000082.

Hunt, J. F., and T. Ohno (2007), Characterization of fresh and decomposed dissolved organic matter using excitation-emission matrix fluorescence spectroscopy and multiway analysis, *J. Agric. Food Chem.*, *55*, 2121–2128, doi:10.1021/jf063336m.

Jaffé, R., J. N. Boyer, X. Lu, N. Maie, C. Yang, N. Scully, and S. Mock (2004), Sources characterization of dissolved organic matter in a mangrove-dominated estuary by fluorescence analysis, *Mar. Chem.*, *84*, 195–210, doi:10.1016/j.marchem.2003.08.001.

Kaiser, K., G. Guggenberger, and L. Haumaier (2004), Changes in dissolved lignin-derived phenols, neutral sugars, uronic acids, and amino sugars with depth in forested Haplic Arenosols and Rendzic Leptosols, *Biogeochemistry*, *70*, 135–151, doi:10.1023/B:BIOG.0000049340.77963.18.

Lakowicz, J. R. (1999), *Principles of Fluorescence Spectroscopy*, 698 pp., Springer, New York.

Lu, X. Q., N. Maie, J. V. Hanna, D. Childers, and R. Jaffé (2003), Molecular characterization of dissolved organic matter in freshwater wetlands of the Florida Everglades, *Water Res.*, *37*, 2599–2606, doi:10.1016/S0043-1354(03)00081-2.

Maie, N., C. Yang, T. Miyoshi, K. Parish, and R. Jaffé (2005), Chemical characteristics of dissolved organic matter in an oligotrophic subtropical wetland/estuarine ecosystem, *Limnol. Oceanogr.*, *50*, 23–35.

Maie, N., J. N. Boyer, C. Yang, and R. Jaffé (2006a), Spatial, geomorphological and seasonal variability of CDOM in estuaries of the Florida Coastal Everglades, *Hydrobiologia*, *569*, 135–150, doi:10.1007/s10750-006-0128-x.

Maie, N., K. Parish, A. Watanabe, H. Knicker, R. Benner, T. Abe, K. Kaiser, and R. Jaffé (2006b), Chemical characteristics of dissolved organic nitrogen in an oligotrophic subtropical coastal ecosystem, *Geochim. Cosmochim. Acta*, *70*, 4491–4506, doi:10.1016/j.gca.2006.06.1554.

Marschner, B., and K. Kalbitz (2003), Controls of bioavailability and biodegradability of dissolved organic matter in soils, *Geoderma*, *113*, 211–235, doi:10.1016/S0016-7061(02)00362-2.

McDowell, W. H., A. Zsolnay, J. A. Aikenhead-Peterson, E. G. Gregorich, D. L. Jones, D. Jödermann, K. Kalbitz, B. Marschner, and D. Schwesig (2006), A comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources, *Soil Biol. Biochem.*, *38*, 1933–1942, doi:10.1016/j.soilbio.2005.12.018.

McKnight, D. M., E. W. Boyer, P. K. Westerhoff, P. T. Doran, T. Kulbe, and D. T. Andersen (2001), Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity, *Limnol. Oceanogr.*, *46*, 38–48.

Ohno, T., A. Amirbahman, and R. Bro (2008), Parallel factor analysis of excitation-emission matrix fluorescence spectra of water soluble soil organic matter as basis for the determination of conditional metal binding parameters, *Environ. Sci. Technol.*, *42*, 186–192, doi:10.1021/es071855f.

Stedmon, C. A., and S. Markager (2005), Tracing the production and degradation of autochthonous fractions of dissolved organic matter by fluorescence analysis, *Limnol. Oceanogr.*, *50*, 1415–1426.

Stedmon, C. A., S. Markager, and R. Bro (2003), Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy, *Mar. Chem.*, *82*, 239–254, doi:10.1016/S0304-4203(03)00072-0.

Stedmon, C. A., D. N. Thomas, M. Granskog, H. Kaartokallio, S. Papadimitriou, and H. Kuosa (2007a), Characteristics of dissolved organic matter in Baltic coastal sea ice: Allochthonous or autochthonous origins?, *Environ. Sci. Technol.*, *41*, 7273–7279, doi:10.1021/es071210f.

Stedmon, C. A., S. Markager, L. Tranvik, L. Kronberg, T. Slätis, and W. Martinsen (2007b), Photochemical production of ammonium and transformation of dissolved organic matter in the Baltic Sea, *Mar. Chem.*, *104*, 227–240, doi:10.1016/j.marchem.2006.11.005.

Stubbins, A., V. Hubbard, G. Uher, C. L. Law, R. C. Upstill-Goddard, G. R. Aiken, and K. Mopper (2008), Relating carbon monoxide photoproduction to dissolved organic matter functionality, *Environ. Sci. Technol.*, *42*(9), 3271–3276, doi:10.1021/es703014q.

Velapoldi, R. A., and K. D. Mielenz (1980), *Standard Reference Materials: A Fluorescence Standard Reference Material– Quinine Sulfate Dihydrate*, *Spec. Publ. 260–64*, 122 pp., Natl. Bur. of Stand., Washington, D. C.

Weishaar, J. L., G. R. Aiken, B. A. Bergamaschi, M. S. Farm, R. Fujii, and K. Mopper (2003), Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon, *Environ. Sci. Technol.*, *37*, 4702–4708, doi:10.1021/es030360x.

Yamashita, Y., and R. Jaffé (2008), Characterizing the interactions between trace metal and dissolved organic matter using excitation-emission matrix and parallel factor analysis, *Environ. Sci. Technol.*, *42*, 7374–7379, doi:10.1021/es801357h.

Yamashita, Y., N. Maie, E. Tanube, and R. Jaffé (2008), Assessing the dynamics of dissolved organic matter in coastal environments by excitation emission matrix fluorescence and parallel factor analysis (EEM-PARAFAC), *Limnol. Oceanogr.*, *53*, 1900–1908.

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