Optical properties of dissolved organic matter (DOM): Effects of biological and photolytic degradation

Angela M. Hansen,* Tamara E. C. Kraus, Brian A. Pellerin, Jacob A. Fleck, Bryan D. Downing, Brian A. Bergamaschi
U.S. Geological Survey, California Water Science Center, 6000 J Street, Sacramento, CA 95819, USA

Abstract

Advances in spectroscopic techniques have led to an increase in the use of optical properties (absorbance and fluorescence) to assess dissolved organic matter (DOM) composition and infer sources and processing. However, little information is available to assess the impact of biological and photolytic processing on the optical properties of original DOM source materials. Over a 3.5 month laboratory study, we measured changes in commonly used optical properties and indices in DOM leached from peat soil, plants, and algae following biological and photochemical degradation to determine whether they provide unique signatures that can be linked to original DOM source. Changes in individual optical parameters varied by source material and process, with biodegradation and photodegradation often causing values to shift in opposite directions. Although values for different source materials frequently overlapped, multivariate statistical analyses showed that unique optical signatures could be linked to original DOM source material, with 17 optical properties determined by discriminant analysis to be significant (p < 0.05) in distinguishing between DOM source and environmental processing. These results demonstrate that inferring source material from optical properties is possible when parameters are evaluated in combination even after extensive biological and photochemical alteration.

Dissolved organic matter (DOM) plays a central role in aquatic environments. Although quantifying DOM amount (commonly by measuring DOC concentration) is important, it is also important to characterize DOM composition because its chemical make-up determines how it reacts in the environment (Liang and Singer 2003; Minor et al. 2014). For example, a portion of the DOM pool is a source of bioavailable organic matter that supports aquatic food webs, attenuates light in the water column, and mobilizes and transports pollutants. In addition, a variety of studies have demonstrated that DOM composition can be used to infer the sources of DOM, which can help inform drinking water and watershed management (e.g., McKnight et al. 2001; Stedmon et al. 2003; Kraus et al. 2011).

Both DOM amount and composition vary spatially and temporally due not only to its proximity to source material but also to its exposure to environmental processing (Hood et al. 2005; Coble 2007; Helms et al. 2008). Under some conditions sorption and the formation of colloids and even precipitation can transfer DOM into the particulate pool (POM), however the two main processes affecting DOM amount and composition in aquatic environments are biodegradation and photodegradation (Kiefer et al. 1990; Miller and Moran 1997; Del Vecchio and Blough 2002). Both of these processes can lead to the conversion of DOM to inorganic compounds (i.e., CO₂) and its subsequent loss from the water column, and to the alteration of DOM chemical composition.

Biodegradation—which can occur in both the photic and aphotic zone—typically leads to the rapid loss of labile, low molecular weight (LMW) aliphatic material, including proteins, carbohydrates, and organic acids (Mopper and Schultz 1993; Wetzel et al. 1995; Moran and Zepp 1997). However, it can also be accompanied by the production of high molecular weight (HMW) aromatic material, such as fulvic and humic acids, through alteration of existing compounds and/or production of new compounds by heterotrophs (Repeta et al. 2002; Stepanauskas et al. 2005). Photodegradation in aquatic environments can significantly impact DOM cycling and alter
its bioavailability and fate. Studies have shown that photodegradation in aquatic environments can both alter DOM from larger molecules to smaller labile photoproducts that are then removed from the DOM pool either by volatilization of carbon gases or by rapid microbial consumption (Mopper and Schultz 1993; Moran and Zepp 1997) or can transform labile compounds to higher molecular weight refractory material (Benner and Biddanda 1998; Obernosterer et al. 1999).

Optical measurements of absorbance and fluorescence are increasingly used to track DOM composition and to infer DOM source and processing due to cost and speed advantages over molecular level analyses (e.g., Coble 2007; Fellman et al. 2010; Gabor et al. 2014). Common parameters and indices derived from optical data include the absolute absorbance or fluorescence intensity at a specific wavelength, ratios of different wavelengths, carbon-normalization of optical properties, and the slopes across specific regions of the optical spectrum. These parameters are often used in multiparameter statistical approaches such as parallel factor analysis (PARAFAC) (e.g., Stedmon and Markager 2005; Fellman et al. 2008; Kowalczuk et al. 2009), principal component analysis (PCA) (e.g., Baker et al. 2008; Miller and McKnight 2010; Fleck et al. 2014), or discriminant analysis (DA) (Spencer et al. 2007) to deconvolve complex optical signals into specific components.

While optical measurements and derived indices are commonly used to infer sources and processing of DOM, few studies (Pellerin et al. 2010) use original DOM source materials to evaluate the effects of biological and photolytic alteration on DOM optical properties (Supporting Information Table S1). In this study, changes in DOM optical properties of source materials (leachates from peat soil, algae, and three types of plants) were investigated following biological and photochemical degradation during a 3 months incubation period. The effect of photoexposure was assessed on samples at various stages of biological degradation to capture the coupled effects that occur in natural environments (e.g., where DOM is intermittently irradiated when in the photic zone). In particular, the effects on commonly used indices derived from optical measurements were evaluated to determine the potential for degradation to alter or obscure inferences on source materials. Samples were analyzed for dissolved organic carbon (DOC) concentration, absorbance, and fluorescence. In addition to examining changes in commonly cited optical properties, we used PCA and DA to determine whether if evaluated in combination, these properties could distinguish between original DOM source material even following alteration by biological and photochemical processes.

**Methods**

**Collection and preparation of source material**

End-member DOM source materials common to the Sacramento-San Joaquin Delta (California, U.S.A.) (peat soil, wetland plants, and algae) were selected to represent primary sources of DOM entering the water column (Hansen 2014). Peat soil (euic, thermic Typic Medisaprists) formed primarily from tules and reeds with minor contributions from mixed-origin alluvial deposits, was collected from Twitchell Island, located in the west-central part of California’s Sacramento-San Joaquin Bay Delta. Soil was air dried, homogenized, and passed through a 2 mm sieve to remove large pieces of plant and mineral material, followed by oven drying at 60°C to constant weight. Three plant biomass samples [white rice (Oryza sativa), tule (Schoenoplectus acutus), and cattail (Typha spp.)] were collected as described in Pellerin et al. (2010). Briefly, above ground plant material (including both leaves and stems) collected from the field was gently rinsed with organic-free deionized water, air dried, and stored sealed in plastic bags. Prior to leaching, plant material was cut into < 2.5 cm pieces, homogenized, and oven-dried at 60°C to constant weight.

Soil and plant leachates were prepared by adding approximately 5 g of dried biomass from each plant type and 1000 g of soil to 4 L of organic-free water. Samples were placed on a shaker for 4 h at room temperature (21°C). Following centrifugation to settle the particles, the supernatant was filtered through precombusted 47-mm diameter, 0.3-μm nominal pore-size glass fiber filters (Advantec MFS model GF7547mm; Advantec MFS, Dublin, California, U.S.A.).

We also used a commercially available diatom (Thalassiosira weissflogii, 6–20 μm × 8–15 μm) commonly found in the Sacramento-San Joaquin Bay Delta, that was produced in a closed-system photobioreactor design that allows microalgae to be grown in laboratory-sterile conditions (Reed Mariculture, Campbell, California). To extract organic matter from the algae, the culture was frozen and thawed, then sonicated (to lyse cells) in a Teflon® bottle. Following centrifugation for 20 min at 2000 rpm, the supernatant was filtered through 0.3 μm glass fiber filters.

The resulting soil, plant, and algae leachates had DOC concentrations of 35–45 mg C L⁻¹, and were stored refrigerated at 2°C prior to the start of the experiments (started within 24 h).

**Biodegradation**

Soil, plant, and algae leachates were inoculated with 3% (v/v) unfiltered surface water collected from the American River near California State University Sacramento (California, U.S.A.) to reintroduce a microbial community. The low DOC concentration of the inoculum (c.2 mg C L⁻¹) and low inoculum-to-sample ratio had no measureable effect on leachate DOC concentrations and composition. Additionally, an inorganic nutrient solution composed of NH₄Cl, KNO₃, and KH₂PO₄ was added (0.1% of sample volume) to eliminate potential N and P limitation, resulting in concentrations of 9.5 mM NH₄Cl, 9.8 mM KNO₃, and 2.0 mM KH₂PO₄ at the beginning of the experiment.

Leachates with added inoculum and nutrients were dispensed in triplicate to 1 L acid-washed, precombusted (460°C), amber glass bottles. The 1 L source bottles were
incubated in the dark at 21°C for 111 d and continuously aerated using filtered lab air to prevent anoxic conditions. Condensation traps were used to allow air to escape while minimizing sample loss from evaporation; lost volume (typically <2% of sample volume) was replaced with organic-free water before each subsample collection. On days 0, 3, 7, 14, 28, and 111, subsamples (100 mL) were collected from each source bottle and vacuum filtered through 0.3 μm glass fiber filters before analysis.

**Photoexposure**

In addition to the subsample collected for biodegradation as previously described, a second 100 mL subsample was collected from each 1 L source bottle on days 3, 14, and 111 to examine the effects of photolytic alteration on DOC amount and composition. The first set of unfiltered biodegraded subsamples was placed in precombusted 125 mL amber glass bottles and wrapped in aluminum foil before irradiation to serve as dark controls. The second set of unfiltered biodegraded subsamples was placed in acid-washed 500 mL sealed optically transparent quartz tubes for photoexposure. The quartz tubes were positioned on their side under irradiation to maximize the surface area of sample exposed; water depth in each tube was 5 cm. Both sets were irradiated for 4 h in a solar simulator equipped with 12 ultraviolet UVA 340 fluorescent bulbs (Q-Lab) which provide a spectral shape similar to that of natural sunlight in the wavelength region of 295–365 nm, the most important wavelengths for environmental photoreactions involving DOM (Stubbins et al. 2010). Light output (0.45 W m⁻²) from the solar simulator was verified using a hyperspectral radiometer (HyperOCR; Atlantastic, NS, CAN). The 4 h of solar simulator irradiation equated to approximately 13 h of solar irradiance at latitude 38.1068N and longitude 121.6465W, representing conditions at the Sacramento-San Joaquin Bay Delta water surface in San Francisco Bay in late July (sun 0.13 W m⁻²). Following light exposure, samples were vacuum filtered through 0.3 μm glass fiber filters prior to analysis. Note that samples were not filtered prior to photoexposure, thus some biodegradation likely occurred during the 4-h irradiation period.

All samples for DOC concentration were preserved by acidification to approximately pH 2.0 with high purity H₂SO₄ and analyzed within 7 d, whereas absorbance spectra and fluorescence excitation emission matrices (EEMs) were run on nonacidified samples within 8 h of filtration.

**Analytical measurements**

Measurements of DOC concentration and optical properties were performed at the U.S. Geological Survey (USGS) Organic Matter Research Laboratory (OMRL) in Sacramento, California. DOC concentration (mg C L⁻¹) was determined by high-temperature catalytic combustion using a Shimadzu TOC-VCSH total organic carbon analyzer (Shimadzu Scientific Instruments, Columbia, Maryland), according to a modified version of method EPA 415.3 (U.S. Environmental Protection Agency 2005). The accuracy and precision of the measurements were within 5% as indicated by an internal standard (caffeine), laboratory replicates, and matrix spikes. The long-term method detection limit for DOC concentration was 0.30 mg C L⁻¹ based on three times the standard deviation of a low concentration standard measured over the annual cycle.

Absorbance spectra and fluorescence matrices were simultaneously collected on filtered samples at room temperature (21°C) in an acid-cleaned 1 cm quartz cuvette using a spectrophotometer equipped with a CCD detector (Aqualog®; Horiba Instruments, New Jersey, U.S.A.). Excitation and absorbance scans were performed using a double-grating monochrometer, a 150 Watt Xenon lamp with a 5 nm bandpass, and a 1 s integration time at wavelengths of 230–600 nm. Emission spectra were collected with a CCD at approximately 1.64 nm (4 pixel) intervals at wavelengths of 250–600 nm. To limit the effects of photobleaching during analysis, excitation and absorbance wavelengths were scanned from low to high energy (red to UV), reducing UV exposure of the sample. Spectral correction procedures included instrument correction, baseline correction, normalization to the daily water Raman peak area (Murphy et al. 2011), and the removal of Rayleigh scatter lines. Concentration-related inner filter effects were corrected as described by Gu and Kenny (2009). Fluorescence data are expressed in Raman-normalized intensity units (RU) (Murphy et al. 2010). We report absorbance (Aλ) as the unitless measurement obtained directly from the spectrophotometer. Samples with A₂₅₄ greater than 3.0 were diluted and reanalyzed to ensure linearity in the wavelengths of interest. For more details on method detection limits and quality control, see Supporting Information.

To make concentration-independent comparisons across sources (i.e., examine composition), carbon-normalized (specific) absorbance and fluorescence was calculated by dividing the intensity of the response at a given wavelength or wavelength pair by the sample DOC concentration. The specific UV absorbance at 254 nm (SUVA₂₅₄) has been shown to be strongly correlated with the hydrophobic organic acid fraction of DOM (Spencer et al. 2012) and is a useful proxy for DOM aromatic content (Weishaar et al. 2003) and molecular weight (Chowdhury 2013). We report SUVA at a particular wavelength (e.g., 254 nm) in units of L mg-C⁻¹ m⁻¹ using the decadal absorption coefficient. Other commonly cited DOC-normalized absorbance wavelengths (e.g., SUVA₃₅₀, SUVA₄₅₀) were also examined (Table 1).

Spectral slopes were calculated in MATLAB R2013b (MathWorks, Natick, Massachusetts, U.S.A.) for several wavelength ranges (275–295, 290–350, and 350–450) using nonlinear least-squares fit for each spectral range (Del Vecchio and Blough 2002; Boss and Zaneveld 2003). Spectral slope ratio (SR) was also calculated as S₂₇₅–₂₉₅ divided by S₃₅₀–₄₀₀ (Helms et al. 2008).

DOC-normalized fluorescence for commonly reported peaks [e.g., SpC (ex340/em440), SpT (ex275/em340)] is reported in
Table 1. Description of commonly used compositionally based absorbance and fluorescence optical properties.

| Absorbance measurements | Calculation | Purpose | Reference |
|-------------------------|-------------|---------|-----------|
| Specific ultraviolet absorbance at 254 nm \([SUVA_{254}] (L \text{ mg-C}^{-1} \text{m}^{-1})\) | Absorption coefficient at 254 nm divided by DOC concentration | Absorbance per unit carbon. Typically a higher number is associated with greater aromatic content | Weishaar et al. (2003) |
| Specific ultraviolet absorbance \([SUVA (280, 350, 370 \text{ nm})] (L \text{ mg-C}^{-1} \text{m}^{-1})\) | Absorption coefficient at a given wavelength in the ultraviolet region divided by DOC concentration | Absorbance per unit carbon. Typically a higher number is associated with greater aromatic content | Chin et al. (1994) |
| Specific visible absorbance \([SVA (412, 440, 480, 510, 532, 555 \text{ nm})] (L \text{ mg-C}^{-1} \text{m}^{-1})\) | Absorption coefficient at a given wavelength in the visible region divided by DOC concentration | Absorbance per unit carbon. Typically a higher number is associated with greater aromatic content | |
| Spectral slopes \((S_{275-295}, S_{290-350}, S_{350-400}) (\text{nm}^{-1})\) | Nonlinear fit of an exponential function to the absorption spectrum over the wavelength range | Typically higher \(S\) values indicate low molecular weight material and/or decreasing aromaticity | Blough and Del Vecchio (2002), Helms et al. (2008) |
| Spectral slope ratio \((S_{275-295} : S_{350-400}) (\text{nm}^{-1})\) | Spectral slope \(S_{275-295}\) divided by spectral slope \(S_{350-400}\) | Shown to be negatively correlated to DOM molecular weight and to generally increase on irradiation | Helms et al. (2008) |

| Fluorescence measurements | Calculation | Purpose | Reference |
|--------------------------|-------------|---------|-----------|
| Specific fluorescence at various peaks \([spA, spB, spC, spD, spM, spN, spT, spZ] (RU L \text{ mg-C}^{-1})\) | Fluorescence at a given ex/em pair divided by DOC concentration | Fluorescence per unit carbon | |
| Peak ratio (A:T) | The ratio of Peak A (ex260/em450) to Peak T (ex275/em304) intensity | An indication of the amount of humic-like (recalcitrant) vs. fresh-like (labile) fluorescence in a sample | Baker et al. (2008), Cory et al. (2010) |
| Peak ratio (C:A) | The ratio of Peak C (ex340/em440) to Peak A (ex260/em450) intensity | An indication of the amount of humic-like vs. fulvic-like fluorescence in a sample | Coble (1996), Burdige et al. (2004), Para et al. (2010), Helms et al. (2013) |
| Peak ratio (C:M) | The ratio of Peak C (ex340/em440) to Peak M (ex300/em390) intensity | An indication of the amount of diagenetically altered (blue-shifted) fluorescence in a sample | |
| Peak ratio (C:T) | The ratio of Peak C (ex340/em440) to Peak T (ex275/em304) intensity | An indication of the amount of humic-like (recalcitrant) vs. fresh-like (labile) fluorescence in a sample | Baker et al. (2008) |
units of RU L mg-C$^{-1}$. Four fluorescent DOM compositional indicators—fluorescence index (FI), humification index (HIX), freshness index ($\beta$:$\alpha$), and biological index (BIX)—were also calculated as described in Table 1.

### Statistical analyses

Statistical analyses including correlations, principal component analysis (PCA), and discriminant analysis (DA), were performed using JMP version 11.0 (SAS Institute 2013) on commonly used compositionally based absorbance and fluorescence optical parameters (see Table 1). Data treatment consisted of averaging the three replicates, and PCA and DA data were log10-transformed.

Correlations between individual qualitative parameters were examined to gain insight into whether these measurements were related (Supporting Information Tables S2a,b). Because highly significant correlations ($p < 0.0001$) were found even when $R^2$ values were low, we used an $R^2$ value of 0.65 or greater as a threshold to indicate whether measurements showed a strong relationship between parameters, meaning they were likely tracking similar DOM pools as they underwent environmental processing, or a poor correlation ($R^2 < 0.65$) suggesting these parameters were not strongly linked, and thus were likely tracking distinct pools of DOM that changed independently during environmental processing.

Unlike PCA where the primary aim is dimension reduction resulting in the clustering of similar objects based on correlating variables, DA is a classification method used to predict the assignment of a sample to a group based on known responses by maximizing between-group variance relative to within-group variance. Ten unique groups were assigned based on source material (soil, rice, cattail, tule, algae) and treatment (biodegradation, photoexposure). The forward stepwise selection method for inclusion of significant variables was adopted, and the tolerance level was set at 0.05.

We also ran PARAFAC and determined that a five-component model fit this data set; these components showed distinct trends following biological and photochemical exposure (see Supporting Information for PARAFAC results). However, PARAFAC components are unique to the data set on which the analysis is run. This issue has been
Results and discussion

DOM compositional parameters

DOM compositional parameters measured from optical properties include both absorbance and fluorescence measurements. Some parameters are carbon-normalized by dividing by DOC concentration (e.g., SUVA254, SUVA350, SVA440, SpT, SpC); some are ratios between fluorescence wavelengths (e.g., Fl, HIX, β/z), or slopes (e.g., S275−295) that reflect the shape of the absorbance spectrum, and finally, some parameters report the relative proportions of different DOM fluorophores (% PARAFAC component loadings; see Supporting Information).

Absorbance

SUVA254

SUVA254, the absorption of light at 254 nm per unit of carbon, has been shown to be a useful proxy for DOM aromatic content (Weishaar et al. 2003) and can also be indicative of molecular weight (Chowdhury 2013). Surface water SUVA254 values typically range from 1.0 L mg-C\(^{-1}\) m\(^{-1}\) to 6.0 L mg-C\(^{-1}\) m\(^{-1}\). Although higher values than 6.0 have been reported for interstitial waters dominated by a strong terrestrial signature (Jaffe et al. 2008), others have reported that these higher values can be due to absorption at 254 nm from iron, colloids, or other constituents in the sample (Weishaar et al. 2003; Hudson et al. 2007).

The initial SUVA254 value for the peat soil leachate was 3.0 L mg-C\(^{-1}\) m\(^{-1}\) (Fig. 2a), similar to previously reported values for DOM derived from peatlands (Fleck et al. 2004; Olefeldt et al. 2013). Initial SUVA254 values for the plant and algae leachates were below 1.0 L mg-C\(^{-1}\) m\(^{-1}\), which is comparable to crop and aquatic plant leachate values reported by Pellerin et al. (2010). These initial low SUVA254 values for the plant and algae leachates reflect that a large portion of this newly leached DOM includes low molecular weight, aliphatic compounds that do not absorb at 254 nm.

Biodegradation increased SUVA254 values in all samples (Fig. 2a; Table 2), consistent with biodegradation preferentially removing aliphatic, low molecular weight DOM (e.g., Moran et al. 2000; Obernosterer and Benner 2004; Pellerin et al. 2010). The only exception to this trend occurred on day 3 when SUVA254 values were higher relative to days 0 and 7 in rice, tule, and algae, but lower in soil, possibly reflecting a transient DOM pool made up of degradation byproducts that was then itself consumed and/or transformed (Stedmon and Cory 2014). By day 111, SUVA254 values for the three plant leachates were close to or even greater than the soil leachate (2.9−4.1), while algae-derived DOM remained distinctly low (1.7 L mg-C\(^{-1}\) m\(^{-1}\); Fig. 2a).

Compared with biodegradation alone, photoexposure of degraded DOM had less effect on SUVA254 values especially during the first few weeks of incubation. Even at day 14 SUVA254 values dropped less than 10% (0.2 L mg-C\(^{-1}\) m\(^{-1}\)) in all samples following photoexposure. Changes in SUVA254 values due to photoexposure were fairly consistent across

Results and discussion

While the focus of our study was on the optical properties of DOM, changes in the bulk DOC concentration are also important to understanding the impacts of biological and photolytic processes on aquatic organic matter. Within the first 3 d of biodegradation, DOC concentrations in the plant and algae leachates decreased by more than 75% to less than 10 mg-C L\(^{-1}\) (Fig. 1). In contrast, DOC concentrations in the soil leachate did not decrease in the first 3 d. The rate of DOC loss due to biodegradation was lower over the remainder of the incubation. By day 111 DOC concentrations in the plant and algae leachates had decreased 91−97% in the plant and algae samples and by approximately 20% in the soil leachate compared with initial conditions. The lower rate of DOC loss in the soil leachate compared with the plant and algae leachates is likely indicative of a more recalcitrant, high molecular weight DOM pool than in leachates from fresh plant litter and algae. There was little to no measurable change (<3%) in DOC concentration following irradiation in all samples on days 3, 14, and 111.

Fig. 1. Dissolved organic carbon (DOC) concentration (mg/L) over time for the five different DOM sources: soil, rice, cattail, tule, and algae (Weiss.). Solid lines connect data collected on days 0, 3, 7, 14, 28, and 111 following biodegradation (bio), while dotted lines connect data for biodegraded samples that were photoexposed (bio+photo) on days 3, 14, and 111.
DOM source over time, reducing it by 10–16% in all samples on day 111 (Fig. 2a; Table 2).

On evaluation of the effects of long-term biodegradation of DOM, both with and without photoexposure, it is evident by day 111 that SUVA_{254} values of fresh plant-derived DOM begin to resemble soil-derived SUVA_{254} values, suggesting that higher SUVA_{254} values (>3 L mg-C^{-1} m^{-1}) cannot be used to infer whether riverine DOM is from degraded plant inputs or older DOM incorporated into soil. However, even after 111 d of biodegradation with and without photoexposure, algae maintained low SUVA_{254} values (<1.7 L mg^{-1} m^{-1}) that are only overlapping with fresh, unaltered plant-derived DOM (Fig. 2a; Table 2).

**Spectral slopes (S_{275-295}, S_{290-350}, S_{350-400})**

Spectral slopes and slope ratios have been related to the relative molecular weight and aromaticity of DOM (Chin...
| Soil | Cattail | Tule | Rice | Wells | Soil | Cattail | Tule | Rice | Wells | Soil | Cattail | Tule | Rice | Wells |
|------|---------|------|------|------|------|---------|------|------|------|------|---------|------|------|------|
| Specific Absorbance | | | | | | | | | | | | | | |
| Specific VIS absorbance | | | | | | | | | | | | | | |
| Spectral Slope | | | | | | | | | | | | | | |
| Specific Fluorescence | | | | | | | | | | | | | | |
| Peak Ratio (A:T) | | | | | | | | | | | | | | |
| Peak Ratio (C:A) | | | | | | | | | | | | | | |
| Peak Ratio (C:M) | | | | | | | | | | | | | | |
| Fluorescence Index | | | | | | | | | | | | | | |
| Freshness Index | | | | | | | | | | | | | | |
et al. 1994; Helms et al. 2008), with lower values generally indicative of higher molecular weight DOM. Previous studies have reported $S_{275-295}$ values in the range of 0.020–0.030 nm$^{-1}$ and 0.010–0.020 nm$^{-1}$ for ocean and coastal waters respectively (Del Vecchio and Blough 2002), 0.014–0.018 nm$^{-1}$ for wetlands (Helms et al. 2008), and 0.012–0.023 nm$^{-1}$ for terrestrial systems (Spencer et al. 2012). Spectral slope values for the three wavelength ranges examined here ($S_{275-295}$; $S_{290-350}$; $S_{350-400}$) decreased during biodegradation in all sources with the exception of all plant $S_{290-350}$ values and algae $S_{350-400}$ values which increased; soil slope values in all three wavelength ranges generally remained unchanged (Table 2). These decreases were predominantly related to the loss of the labile pool of LMW, aliphatic DOM, however some microbial production of HMW, aromatic DOM may have occurred as well. Markedly, the algae leachate $S_{275-295}$ was initially very high (0.055 nm$^{-1}$) in contrast to the plant and soil leachates (0.014–0.021 nm$^{-1}$), but decreased rapidly in the first 3 d of biodegradation to 0.013 nm$^{-1}$ and continued to resemble the plant leachates through the end of the experiment (Fig. 2e).

Light exposure increased $S_{275-295}$ and $S_{290-350}$ values in all five sources, indicating either (1) loss of higher molecular weight DOM due to disaggregation or bond cleavage or (2) an increase in LMW photoproducts due to condensation reactions (Obernosterer et al. 1999; Stepanauskas et al. 2005). Previous studies reported an increase in these spectral slope values after irradiation and attributed it to the transformation of high-molecular weight dissolved lignin to highly oxidized low-molecular weight lignin photoproducts (Opsahl and Benner 1998; Obernosterer and Benner 2004; Helms et al. 2008). However, this does not explain the increase measured in the algae leachate which does not contain lignin. In contrast to spectral slope calculated for the lower UV wavelengths, photoexposure decreased $S_{350-400}$ values; this is also again consistent with the findings of Helms et al. (2008), as well as other studies (Benner and Biddanda 1998; Obernosterer et al. 1999; Tranvik and Bertilsson 2001), where the decrease was associated with phototransformation of LMW material into more humic substances.

**Slope ratio ($S_R$ : $S_{275-295}$/ $S_{350-400}$)**

The slope ratio $S_R$ ($S_{275-295}$/ $S_{350-400}$) has been linked to shifts in DOM molecular weight and photobleaching (Helms et al. 2008), with steeper slope ratios reflective of greater amounts of low molecular weight compounds. Previous studies have reported $S_R$ values for a variety of aquatic environments including 0.76–1.79 in wetlands (Helms et al. 2008, 2013), 0.70–2.40 in lake waters (Zhang et al. 2009) and 1.6–3.4 in ocean water (Stubbins et al. 2012; Catalá et al. 2015). In this study, initial $S_R$ values ranged between 0.47 and 0.81 for plant and soil leachates and generally showed little change following biodegradation (Table 2; Supporting Information Fig. S1d). Initial algae $S_R$ values were an order of magnitude higher at 58.59. As noted earlier, $S_{275-295}$ of the freshly leached algae was quite high (0.055) and the $S_{350-400}$ was quite low (0.001) which resulted in this unusually high $S_R$ and demonstrates that the value may not be representative of a true exponential fit, however algae $S_R$ returned to commonly reported values (1.54) within 14 d.

For all DOM sources, exposure to light increased $S_R$ by up to 100%. These considerable changes in $S_R$ following photoexposure were also observed by Helms et al. (2008) and Spencer et al. (2009), and have been attributed to a shift from HMW to LMW compounds. Following photoexposure, soil $S_R$ values remained distinctly lower than plant and algae values for all of the measured time points (0.95 vs. 1.11–1.42), suggesting that an $S_R$ value greater than 1.00 in natural waters could be used to indicate the DOM was primarily derived from plant or algae sources. However, in the absence of photoexposure an $S_R$ value less than 1.00 could be associated with any of these sources.

**Fluorescence**

Humic (degraded) material is generally associated with fluorescence Peaks A, C, D, M, and recently identified Peak Z (Fleck et al. 2014); while the protein-like fraction is generally associated with fluorescence in the lower UV region (Peaks B, T, and N). However, we would like to emphasize that although the use of the term “protein-like” or “amino acid-like” primarily originates from the fact that three aromatic amino acids—tryptophan, tyrosine, and phenylalanine—fluoresce in this lower UV region when measured in pure form (Cory and McKnight 2005), recent studies have clearly shown that other non-protein compounds fluoresce in the low-UV regions (Hernes and Benner 2003; Coble 2007; Hernes et al. 2009). Specifically, undegraded polyphenols known to be present in vascular plant leachates fluoresce in this region (Begg and Summers 2011; Aiken 2014). Therefore, these components may be more appropriately described as reflective of “fresh” organic matter and will thus be referred to hereafter as “fresh-like” DOM.

**Fluorescence index (FI)**

The fluorescence index (FI) has been used in a wide range of studies to distinguish DOM derived from terrestrial sources (degraded plant and soil organic matter; lower values) vs. microbial sources (extracellular release and leachate from bacteria and algae; higher values) (McKnight et al. 2001; Cory et al. 2007, 2010). FI values in natural waters typically range between 1.2 and 1.8 (e.g., Jaffe et al. 2008; Wilson and Xenopoulos 2009; Cory et al. 2010; Carpenter et al. 2013; Helms et al. 2013; Fleck et al. 2014).

The initial FI value for the peat soil leachate was 1.6 and showed little change with biodegradation (Fig. 2b; Table 2). Although initial plant and algae leachate FI values were similar to peat soil (1.6–1.9), these values increased with microbial processing, such that by day 111 they exceeded 2.0. Values for the algae leachate showed by far the greatest
change over time with final values close to 3.5. The particularly high FI value for the algae leachate supports the idea that higher FI values can be used to indicate DOM derived from phytoplankton production in the water column that has undergone bacterial processing. Other studies reporting values that exceed the typical FI range of 1.2–1.8 have been observed in leachates from cyanobacteria intracellular organic matter (Korak et al. 2015) and wastewater effluent organic matter (Dong and Rosario-Ortiz 2012).

Although photoexposure did not greatly affect the FI values of the plant and algae leachates during the first week of biodegradation when there was rapid loss of the bioavailable DOM pool, later on photoexposure reduced the FI in these sources to the more typically reported range of 1.6–1.8 (Fig. 2b). A similar decrease in FI following photoexposure was also observed in the soil leachate reducing it from 1.6 to 1.4. This reduction due to photoexposure resulted in plant and algae FI values that were similar to the FI values of soil-derived DOM prior to photoexposure (1.6). This reveals that photoexposure can mask the DOM source signal gleaned from the FI by making plant- and algae-derived DOM resemble that of soil-derived DOM.

**Humification index (HIX)**

The humification index (HIX) has been used as an indicator of source, diagenesis and sorptive capacity (Zsolnay et al. 1999; Ohno 2002) and is based on the idea that as humification of DOM proceeds, the ratio of hydrogen to carbon decreases, shifting the emission spectra of the fluorescing molecules toward the longer wavelengths with higher values indicating an increasing degree of humification. HIX values commonly range between 0.6 and 0.9 (e.g., Ohno 2002; Chen et al. 2011; Fleck et al. 2014).

At the start of the incubation, soil HIX values were distinctly higher than all other sources (1.0 vs. 0.2–0.4), and these high values for soil remained unchanged during the 111 d of biodegradation (Fig. 2c; Table 2). In contrast, HIX values in all other sources increased over time as microbial processing consumed the labile portion of the DOM pool. By day 111 values were 0.8–0.9 for the plant and algae leachates. Photoexposure had little to no effect on soil HIX (decreased <2%), but decreased HIX by approximately 8–18% in plant and algae sources. This reduction increased the difference in HIX values between soil derived DOM and the other DOM sources.

Because the soil HIX remained distinctly higher than plant and algae HIX under both biological and photolytic exposure, these results suggest that if the HIX in natural waters is less than 0.9 one can infer that DOM is derived from relatively recent plant and/or algae inputs. However, additional soil samples should be included in future studies.

**Freshness index (β\(\times\)) and biological index (BIX)**

The freshness index (β\(\times\)) is associated with the contribution of recently produced DOM (Parlanti et al. 2000; Wilson and Xenopoulos 2009), while the biological index (BIX) (Huguet et al. 2009), a modified and renamed variation of β\(\times\), has been similarly described as an indicator for the presence of autochthonous (microbial-derived) DOM. Both of these parameters are obtained by nearly the same calculation (Table 1), and essentially reflect the ratio of fresh-like DOM to humic-like DOM. In this study, β\(\times\) and BIX were very highly correlated (\(R^2 = 0.97\); \(p < 0.0001\)); therefore results only from β\(\times\) will be discussed.

β\(\times\) values measured in this study ranged from 0.5 to 0.9, which are similar to the previously reported values of 0.8–0.9 by Fleck et al. (2014) for rice field drainage waters (Fig. 2d; Table 2). β\(\times\) in all plant leachates increased from 0.5–0.8 to 0.7–0.9 during the first 3 d of incubation, while algae decreased (from 0.8 to 0.7) and soil remained relatively unchanged (0.5). Following this early trend, there was a general decrease in β\(\times\) in plant- and algae-derived DOM as they underwent further microbial processing, however the magnitude and rate of this decrease differed among sources.

The effects of photodegradation on the β\(\times\) varied depending on source and degree of microbial processing (e.g., Day 3 vs. 14 vs. 111). The clearest effect was seen on day 111, when photoexposure increased β\(\times\) values for plant and algae leachates but decreased soil values (Fig. 2d; Table 2). After 111 d of biodegradation followed by photoexposure, soil-derived DOM had β\(\times\) values that were lower than the other sources by 20–40% (0.5 vs. 0.6–0.8). The opposite response of β\(\times\) to irradiation for the soil-derived DOM, which is already highly degraded in the natural environment, vs. that of the plant- and algae-derived DOM, demonstrates that even after more than 3 months of microbial processing, fundamental differences remain between these DOM pools. Further, the β\(\times\) index, which is normally associated with microbial processing, is also clearly sensitive to photochemical alteration. Even in the absence of photoexposure, although β\(\times\) values tended to decrease over time as the DOM became by definition “less fresh,” values for algae- and plant-derived leachates were highly variable and overlapping.

**Peak ratios**

**C:T and A:T ratios**

There are a number of fluorescent peak ratios published in the literature, here we describe the four most commonly reported (C:T, A:T, C:A, C:M; Supporting Information Fig. S3). Like the freshness and biological indices, the ratios of C:T and A:T are used to describe the relative amount of humic-like DOM vs. fresh-like DOM, with higher values indicating a higher proportion of degraded material. There was a strong correlation between the C:T and A:T ratios (\(R^2 = 0.94\); \(p < 0.0001\); Supporting Information Tables S2a,b).

Initial C:T (Fig. 2f) and A:T ratios were 0.1–0.3 for plant and algae samples, while soil was distinctly higher at 6.6 and 11.4, respectively (Table 2). Following biodegradation, soil
ratios increased only slightly, while plant and algae ratios increased more than 10-fold to 3.2–4.9 for C:T and 2.7–4.1 for A:T. In contrast, photoexposure decreased these ratios substantially in all sources. Even with the opposing effects of biodegradation (increase) and photodegradation (decrease), these results suggest that if C:T is greater than approximately 5.0 and A:T is greater than 4.5 in natural waters, there is likely a greater relative contribution of soil-derived DOM than plant- or algae-derived DOM (Supporting Information Fig. S3).

**C:A ratio**

Although Peaks C and A are both commonly associated with humic-like material, they have been reported to vary independently, thus suggesting they represent decoupled pools of DOM (Kothawala et al. 2012). In this dataset, there was no clear indication that the intensity of the DOM fluorescence response for Peaks A and C were affected differently by biodegradation, however, photoexposure did result in a shift in the C:A ratio (Supporting Information Fig. S3c). The initial C:A ratio for plants and algae leachates undergoing biodegradation was distinctly higher than soil, ranging between 0.9 and 1.1 vs. 0.6 (Table 2). Following biodegradation alone, on days 3 and 7 relative to other days, there was a decrease in the C:A ratio in the plant- and algae-derived DOM, which resulted from a more rapid loss of Peak C fluorescence compared with Peak A during the initial period of incubation (data not shown). In contrast, by day 111, the percent loss of these two pools compared with initial conditions was either similar resulting in no change in the C:A ratio (rice, cattail), or was greater for Peak A than Peak C resulting in an increase in the C:A ratio (tule, algae). The soil C:A ratio remained largely unaffected by microbial processing.

Photodegradation lead to a decrease in the C:A ratio, particularly in the plant and algae leachates (Supporting Information Fig. S3c). This resulted from a greater percent loss of fluorescence intensity at Peak C vs. Peak A (data not shown). As a result, exposure to light diminished the distinction between the C:A ratio of these different DOM sources. Nevertheless, the soil C:A ratio remained lower at 0.5 following photodegradation compared with the other sources, suggesting that a C:A ratio below 0.6 can be associated with terrestrial soil-derived DOM.

**C:M ratio**

The ratio of Peaks C to M was originally used to distinguish DOM derived from terrestrial vs. marine environments (Burdige et al. 2004; Para et al. 2010; Helms et al. 2013). We found that soil C:M was approximately 1.0 and remained relatively unaffected by microbial processing or photoexposure (Supporting Information Fig. S3d). Initial plant leachate C:M ranged from 1.2 to 2.2 with the rice leachate having the highest values, while the algae was lower at 0.8. Declines in the C:M ratio occurred in all of the plant and algae leachates, such that toward the end of the experiment plant-derived DOM resembled the soil-derived DOM, but the algae remained distinctly lower than all other biodegraded samples. However, photoexposure generally increased the C:M ratio of all sources, resulting in overlapping values, suggesting that this fluorescence ratio is less promising at discriminating DOM origin in cases where both biological and photolytic processing is occurring.

**Correlations between parameters**

High correlation was observed among specific (DOC-normalized) absorbance data within their respective regions of the light spectrum [i.e., ultraviolet (UV), $R^2 = 0.70–0.98; p < 0.0001$; and visible (VIS), $R^2 = 0.87–1.00; p < 0.0001$]. The poor correlation ($R^2 < 0.61$) between the lower UV (254–280 nm) and higher VIS wavelengths (412–555 nm) was expected, as the wavelengths at which an organic molecule absorbs light are determined by differences in their chemical structure; thus these regions appear to be tracking different, albeit overlapping, pools of DOM. All spectral slope values ($S_{275-295}; S_{290-350}; S_{350-400}; S_5$) showed poor correlation with each other and with all other parameters indicating they are tracking different pools of DOM.

Similar to absorbance where wavelengths in proximity to one another are correlated, the humic-like peaks (SpA, SpC, SpM, SpD, SpZ; $R^2 = 0.76–0.90; p < 0.0001$) were strongly correlated to each other and the fresh-like peaks (SpB, SpT; $R^2 = 0.65; p < 0.0001$) were similarly auto-correlated, which indicates within these two group that these parameters are tracking largely overlapping pools of DOM. However, the poor correlation between parameters associated with humic-like material and those associated with fresh-like material upholds the notion that these fluorescence regions are tracking different pools of DOM.

We expected to see stronger correlations between SUVA$_{254}$, FI, and HIX as these parameters all have been linked to molecular weight, aromaticity and bioavailability (Hayase and Tsubota 1985; Carder et al. 1989; Weishaar et al. 2003; Helms et al. 2008; Spencer et al. 2009), but our results suggest these parameters are not redundant ($R^2 < 0.52$). None of the fluorescent peak ratios (C:T, A:T, A:C, C:M) or $\beta_2$, which focuses on ratios of humic to fresh material, showed strong relationships with any of the other qualitative parameters, with the exception of C:T and A:T ($R^2 = 0.94; p < 0.0001$, also discussed above), and the HIX with C:T ($R^2 = 0.73; p < 0.0001$). This lack of correlation underscores the complex nature of DOM, and highlights that each of these indices tracks a unique, albeit overlapping, subset of the bulk DOM pool.

**Multivariate statistical analyses**

**Principal component analysis (PCA)**

PCA was run on 30 absorbance and fluorescence parameters and 45 sample types. Together, principal components 1 and 2 (PC1 and PC2, respectively) explained 71.4% of the
PC1, which explained 49.7% of the total variance, showed strong positive loadings for parameters associated with humic-like (e.g., SpC, SpD, SpZ) and HMW, aromatic moieties (e.g., SUVA254, SUVA280). PC2, which explained 21.7% of the total variance, showed strong positive loadings for parameters representing fresh-like material (SpB, SpT, β:α) (Fig. 3b).

Graphical representations of PCA output enables us to visualize how the 45 samples relate to each other based on categories of interest, which in our case includes source (peat soil, rice, cattail, tule, algae), treatment (biodegradation vs. photoexposure), and time (T0 through T111) (Fig. 3a). The soil leachate exhibited a high positive score on the PC1 axis and a high negative score on PC2, indicating this source is associated with the presence of HMW, aromatic compounds which further supports the notion that PC1 is linked to more degraded DOM. The minimal loss of DOC in the soil leachate over time during the incubation experiment confirms these samples contained little to no fresh/labile DOM even at T0. Initial (T0) plant and algae sources had a high negative PC1 loading indicating the predominance of fresh, LMW, low aromatic-containing, labile DOM (Fig. 3a). By day 111 these leachates shifted to a high positive PC1 loading, indicating loss of this DOM fraction and/or increases in the more degraded, humic fractions of DOM. This trajectory followed what is expected as DOM undergoes degradation: a shift from fresh-like to humic-like material. However, in most cases the effects of photoexposure caused the signature of the plant- and algae-derived DOM to look less degraded, as they shifted to lower PC1 values, suggesting their composition resembled DOM from earlier times. For example, the photoexposed day 111 plant and algae DOM exhibited similar PC1 and PC2 loadings to the day 28 plant and algae sources (see black arrows in Fig. 3a). This further illustrates the complexity in interpreting

**Table 3.** The 17 optical parameters quantitatively determined by discriminant analysis to be the most significant ($p < 0.05$) in distinguishing between DOM source and environmental processing. See text for details.

| Parameter | $p$-value |
|-----------|-----------|
| SUVA$_{254}$ | 0.003 |
| SUVA$_{350}$ | 0.001 |
| SUVA$_{412}$ | 0.003 |
| S$_{275-295}$ | <0.0001 |
| S$_{290-350}$ | <0.0001 |
| SpB | 0.001 |
| SpC | <0.0001 |
| SpD | 0.002 |
| SpM | <0.0001 |
| SpN | <0.0001 |
| SpT | <0.0001 |
| SpZ | <0.0001 |
| C:A | <0.0001 |
| C:M | <0.0001 |
| Fl | 0.027 |
| HIX | <0.0001 |
| β:α | <0.0001 |

**Fig. 3.** Principal component analysis (PCA) loadings plot (a) and scores plot (b) for 30 of the compositionally based absorbance and fluorescence parameters (see Table 1). Points represent replicate means (n = 3) for each source, day, and treatment; colors represent treatment (red, biodegradation; blue, photoexposure of biodegraded sources); letters represent source (S, soil; R, rice; C, cattail; T, tule; W, algae); numbers represent incubation day (0, 3, 7, 14, 28, 111). Large ellipses indicate identifiable sample groupings associated primarily with biodegradation. Photoexposed day 111 plant and algae DOM (far right ellipse) exhibits similar PC1 and PC2 loadings to the day 28 biodegraded-only plant and algae sources.
DOM composition when it has undergone the simultaneous effects of biodegradation and photoexposure.

**Discriminant analysis (DA)**

The 17 optical parameters presented in Table 3 were quantitatively determined by DA to be the most significant ($p < 0.05$) parameters in distinguishing between DOM source and environmental processing; the remaining 13 parameters were not found to significantly contribute to this model. The discriminant plot of the standardized canonical shows clear horizontal separation of the soil leachate (Groups 1, 2) from the plant and algae leachates (Groups 3–10) in the first canonical variable (Can1) (Fig. 4). Thus Can1 quantifies the degree to which the soil and remaining sources differ, which we attribute to differences in DOM composition. Can1 accounted for the majority (46.5%) of the overall variance in DOM composition and was highly significant ($p < 0.0001$). Can2 accounted for a smaller variance (32.9%), but still remained highly significant ($p < 0.0001$) and resulted in a distinct vertical separation of the biodegraded samples (Groups 1, 3, 5, 7, 9) from those that had undergone biodegradation and photoexposure (Groups 2, 4, 6, 8, 10).

The biplot rays display which parameters have the most influence on maximizing between-group variance while minimizing within-group variance.

Twelve of the 17 statistically significant qualitative indicators identified by DA were fluorescence properties, and included nearly all of the DOC-normalized specific fluorescence peaks, with the exception of SpA, while only three of the absorbance wavelengths were found to be significant suggesting that the fluorescence measurements contain more information that reveals DOM original source material. Less surprising was the DA selection of absorbance and fluorescence ratios and indices which were originally developed to characterize compositional shifts in DOM.

**Conclusions and recommendations**

The goal of this study was to better characterize changes in commonly used qualitative DOM optical properties following biological and photochemical degradation of original DOM source materials and then to investigate in which combination these parameters can discriminate between DOM source and processing. Individual optical properties changed extensively as DOM composition was altered during both biodegradation and photodegradation, particularly in the plant and algae leachates.
algae leachates, and these changes frequently resulted in overlapping optical parameter values which made it impossible to identify original source material. While these indices were not necessarily developed as indicators of specific sources (e.g., plant vs. algae), they are often used to infer generic sources (e.g., terrestrial vs. aquatic) or properties associated with sources (e.g., humic vs. nonhumic) in many studies. Results from DA demonstrated that qualitative optical parameters are more successful when evaluated in combination, rather than individually, to identify unique optical signatures that can be linked to original DOM source even after extensive biological and photochemical alteration.

Results of this study highlight the challenge of interpreting DOM source material when confounding environmental processes impact qualitative indicators. Careful consideration should be taken when characterizing DOM that has undergone environmental processing in natural waters, as not all qualitative parameters investigated here were successful in extricating one signal (biological vs. photochemical) from another or in consistently discriminating among source material (plant type, soil, algae). However, some interesting and potentially informative trends were observed.

Highly labile DOM leached from plants and algae was consumed very rapidly indicating this material is not likely to persist in the environment, and thus except under specific conditions this bioavailable pool is not expected to make up a significant fraction of the DOM in natural waters. Based on results from this study, the presence of fresh labile material leached from plants and algae can be identified by the following parameter values: SUVA$_{254}$ < 2.5 L mg$^{-1}$ C$^{-1}$ m$^{-1}$; HIX < 0.9; C:T < 5.0; and A:T < 10.0. In contrast, soil-derived DOM optical properties were much more stable, as this material has already undergone long residence time in the environment where it has been exposed to microbial processing. However, photoexposure did change the optical signature of soil-derived DOM. Qualitative indicators that were most sensitive to identifying photodegraded soil-derived DOM included: C:T > 5.0; A:T > 10.0; freshness index ($\beta$) < 0.5.

In the natural environment, biodegradation and photoexposure can happen simultaneously. The sometimes opposing effects of biological and photochemically driven changes in DOM composition may confound source identification as seen in this dataset. For example biodegradation increased SUVA$_{254}$ values while photoexposure decreased SUVA$_{254}$ values. This effect of one degradation process masking the signal from the other was also observed in $S_{275-295}$, FI, HIX, C:A, C:T, A:T and RFE (See Supporting Information) suggesting that using these parameters alone can generate inconsistent and disparate results. Despite the significant changes in DOM composition following biological and photolytic degradation, several qualitative parameters were identified that were useful individually in resolving between DOM derived from peat soil vs. from plant and algae source material throughout the 111 d of environmental processing; these included $S_{350-400}$, A:T, C:T, HIX, as well as two PARAFAC components (See Supporting Information Table S3).

This study lent itself well to multivariate statistical analyses like PCA and DA as these modeling techniques can reveal meaningful information from structurally complex datasets. PCA demonstrated that when 30 absorbance and fluorescence parameters were evaluated in combination, the optical signature of the materials did not fall out clearly by source and environmental processing. As was seen when examining the individual parameters, optical signatures of the different sources overlapped over time, with the effects of biodegradation and photodegradation often acting in opposition. The trajectory in PCA space did however generally follow what is expected as DOM undergoes degradation: a shift from fresh-like to humic-like material.

DA was used to identify which qualitative indicators that, when used in combination, are the most promising for distinguishing DOM source and processing. Of the 30 qualitative indicators modeled, 17 were quantitatively determined by DA to be significant ($p < 0.05$). Absorbance parameters included SUVA$_{254}$, SUVA$_{350}$, SVA$_{112}$, $S_{275-295}$, and $S_{290-350}$, and fluorescence parameters included humic-like (SpC, SpM, SpD, SpZ) and fresh-like (SpB, SpT, SpN) DOC-normalized fluorescence peaks as well as peak ratios (C:A, C:M) and indices (FI, HIX, $\beta$).

Future recommendations for investigation include expanding the suite of DOM end-member source material. Here, the sources of DOM [peat soil, tule, rice, cattail, algae (T. weissfloggii)] were chosen to relate these results to DOM in surface waters of wetlands in the Sacramento-San Joaquin Delta. Clearly there are other important sources of DOM to aquatic systems (e.g., trees, submergent vegetation, organic matter from mineral soils, and other types of pelagic and benthic algae), which we expect will have different initial DOM composition and thus optical properties than the limited sources included in this study. In addition, anthropogenic sources of DOM (e.g., wastewater, urban run-off, oil and gas) represent a significant fraction of the bulk DOM pool in many systems, and also should be studied. In fact, oil and gas products fluoresce in the low-UV region and could be mistaken for “fresh-like” DOM.

The intensity and duration of light exposure should also be examined more closely to gain insight into how radiation photochemically alters DOM, thus impacting its bioavailability. Additionally, modifying the source treatment by performing simultaneous, sequential, and/or alternating combinations of degradation processes (e.g., photo$+$bio, bio$+$photo$+$bio$+$photo, etc.) and testing different exposure times could elucidate which fractions of DOM are bioactive vs. those that are photoactive as in Obernosterer and Benner (2004). It is also important to emphasize that there are other factors that can affect optical properties, such as nonlinear mixing behavior of different DOM sources, and variability in the fluorescence signature resulting from changes in solution chemistry (Yang and Hur 2014), not to mention adsorption and precipitation.
Future studies examining how optical properties of end-member DOM sources change as they are exposed to environmental processing will further our understanding of how to interpret optical measurements of water samples collected in the environment, which are typically made-up of a mixture of sources which have undergone variable degrees of microbial and photolytic processing.

References

Aiken, G. R. 2014. A chemist’s perspective, p. 75–122. In P. Coble, J. Lead, A. Baker, D. Reynolds, and R. G. M. Spencer [eds.], Aquatic organic matter fluorescence. Cambridge Univ. Press.

Baker, A., L. Bolton, M. Newson, and R. G. M. Spencer. 2008. Spectrophotometric properties of surface water dissolved organic matter in an afforested upland peat catchment. Hydrol. Process. 22: 2325–2336. doi:10.1002/hyp.6827

Beggs, K. M. H., and R. S. Summers. 2011. Character and chlorine reactivity of dissolved organic matter from a mountain pine beetle impacted watershed. Environ. Sci. Technol. 45: 5717–5724. doi:10.1021/es1042436

Benner, R., and B. Biddanda. 1998. Photochemical transformations of surface and deep marine dissolved organic matter: Effects on bacterial growth. Limnol. Oceanogr. 43: 1373–1378. doi:10.4319/lo.1998.43.6.1373

Blough, N. V., and R. Del Vecchio. 2002. Chromophoric DOM in the coastal environment, p. 509–546. In D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter. Academic Press.

Boss, E., and J. R. V. Zaneveld. 2003. The effect of bottom substrate on inherent optical properties: Evidence of biogeochemical processes. Limnol. Oceanogr. 48: 346–354. doi:10.4319/lo.2003.48.1_part_2.0346

Burdige, D. J., S. W. Kline, and W. H. Chen. 2004. Fluorescent dissolved organic matter in marine sediment pore waters. Mar. Chem. 89: 289–311. doi:10.1016/j.marchem.2004.02.015

Carreira, K. L., R. G. Steward, G. R. Harvey, and P. B. Ortner. 1989. Marine humic and fulvic-acids—their effects on bacterial growth. Limnol. Oceanogr. 34: 68–81. doi:10.4319/lo.1989.34.1.0068

Carpenter, K. D., and others. 2013. Sources and characteristics of organic matter in the Clackamas River, Oregon, related to the formation of disinfection by-products in treated drinking water, 78 p. U.S. Geological Survey Scientific Investigations Report 2013–5001.

Catalá, T. S., and others. 2015. Water mass age and ageing driving chromophoric dissolved organic matter in the dark global ocean. Global Biogeochem. Cycles 24: 917–934. doi:10.1002/2014GB005048

Chen, H., B. Zheng, Y. Song, and Y. Qin. 2011. Correlation between molecular absorption spectral slope ratios and fluorescence humification indices in characterizing CDOM. Aquat. Sci. 73: 103–112. doi:10.1007/s00027-010-0164-5

Chin, Y. P., G. Aiken, and E. OluVhughin. 1994. Molecular-weight, polydispersity, and spectroscopic properties of aquatic humic substances. Environ. Sci. Technol. 28: 1853–1858. doi:10.1021/es00060a015

Chowdhury, S. 2013. Trihalomethanes in drinking water: Effect of natural organic matter distribution. Water SA 39: 1–7. doi:10.4314/wsa.v39i1

Coble, P. G. 1996. Characterization of marine and terrestrial DOM in seawater using excitation emission matrix spectroscopy. Mar Chem 51: 325–346.

Coble, P. G. 2007. Marine optical biogeochemistry: The chemistry of ocean color. Chem. Rev. 107: 402–418. doi:10.1021/cr050350+

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

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

Cory, R. M., K. Mcneill, J. P. Cotner, A. Amado, J. M. Purcell, and A. G. Marshall. 2010. Singlet oxygen in the coupled photochemical and biochemical oxidation of dissolved organic matter. Environ. Sci. Technol. 44: 3683–3689. doi:10.1021/es902998y

Del Vecchio, R., and N. V. Blough. 2002. Photobleaching of chromophoric dissolved organic matter in natural waters: Kinetics and modeling. Mar. Chem. 78: 231–253. doi:10.1016/S0304-4203(02)00036-1

Dong, M. M., and F. L. Rosario-Ortiz. 2012. Photochemical formation of hydroxyl radical from effluent organic matter. Environ. Sci. Technol. 46: 3788–3794. doi:10.1021/es2043454

Downing, B. D. and others. 2009. Quantifying fluxes and characterizing compositional changes of dissolved organic matter in aquatic systems in situ using combined acoustic and optical measurements. Limnol Oceanogr-Meth 7: 119–131.

Fellman, J. B., E. Hood, and R. D. Boone. 2008. Fluorescence characteristics and biodegradability of dissolved organic matter in forest and wetland soils from coastal temperate watersheds in southeast Alaska. Biogeochemistry 88: 169–184. doi:10.1007/s10533-008-9203-x

Fellman, J. B., E. Hood, and R. G. M. Spencer. 2010. Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. Limnol. Oceanogr. 55: 2452–2462. doi:10.4319/lo.2010.55.6.2452

Fleck, J. A., D. A. Bossio, and R. Fujii. 2004. Dissolved organic carbon and disinfection by-product precursor release from managed peat soils. J. Environ. Qual. 33: 465–475. doi:10.2134/jeq2004.4650
Fleck, J. A., G. Gill, B. A. Bergamaschi, T. E. C. Kraus, B. D. Downing, and C. N. Alpers. 2014. Concurrent photolytic degradation of aqueous methylmercury and dissolved organic matter. Sci. Total Environ. 484: 263–275. doi: 10.1016/j.scitotenv.2013.03.107

Gabor, R. S., A. Baker, D. M. McKnight, and M. P. Miller. 2014. Fluorescence indices and their interpretation, p. 303–338. In P. Coble, J. Lead, A. Baker, D. Reynolds, and R. G. M. Spencer [eds.], Aquatic organic matter fluorescence. Cambridge Univ. Press.

Gu, Q., and J. E. Kenny. 2009. Improvement of inner filter effect correction based on determination of effective geometric parameters using a conventional fluorimeter. Anal. Chem. 81: 420–426. doi: 10.1021/ac801676j

Hansen, A. M. 2014. The effects of biodegradation and photodegradation on optical properties of dissolved organic matter in aquatic systems. M.S. thesis. California State Univ. Sacramento. Available from http://csws-dspace.calstate.edu/handle/10211.3/132751

Hayase, K., and H. Tsubota. 1985. Sedimentary humic-acid and fulvic-acid as fluorescent organic materials. Geochim. Cosmochim. Acta 49: 159–163. doi:10.1016/0016-7037(85)90200-5

Helms, J. R., A. Stubbins, J. D. Ritchie, E. C. Minor, D. J. Kieber, and K. Mopper. 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnol. Oceanogr. 53: 955–969. doi:10.4319/lo.2008.53.3.0955

Helms, J. R., A. Stubbins, E. M. Perdue, N. W. Green, H. Chen, and K. Mopper. 2013. Photochemical bleaching of oceanic dissolved organic matter and its effect on absorption spectral slope and fluorescence. Mar. Chem. 155: 81–91. doi:10.1016/j.marchem.2013.05.015

Hernes, P. J., and R. Benner. 2003. Photochemical and microbial degradation of dissolved lignin phenols: Implications for the fate of terrigenous dissolved organic matter in marine environments. J. Geophys. Res. Oceans 108, No. C9, 3291. doi:10.1029/2002JC001421

Hernes, P. J., B. A. Bergamaschi, R. S. Eckard, and R. G. M. Spencer. 2009. Fluorescence-based proxies for lignin in freshwater dissolved organic matter. J. Geophys. Res. Biogeosci. 114. doi: 10.1029/2009G000938

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

Hudson, N., A. Baker, and D. Reynolds. 2007. Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters—a review. River Res. Appl. 23: 631–649. doi:10.1002/rra.1005

Huguet, A., L. Vacher, S. Relexans, S. Saubusse, J. M. Froidefond, and E. Parlanti. 2009. Properties of fluorescent dissolved organic matter in the Gironde Estuary. Org. Geochem. 40: 706–719. doi:10.1016/j.orggeochem.2009.03.002

Jaffe, R., D. McKnight, N. Maie, R. Cory, W. H. Mcdowell, and J. L. Campbell. 2008. Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties. J. Geophys. Res. Biogeosci. 113: G04032. doi:10.1029/2008JG000683

Kieber, R. J., X. L. Zhou, and K. Mopper. 1990. Formation of carbonyl-compounds from UV-induced photodegradation of humic substances in natural-waters—fate of riverine carbon in the Sea. Limnol. Oceanogr. 35: 1503–1515. doi:10.4319/lo.1990.35.7.1503

Korak, J. A., E. C. Wert, and F. L. Rosario-Ortiz. 2015. Evaluating fluorescence spectroscopy as a tool to characterize cyanobacteria intracellular organic matter upon simulated release and oxidation in natural water. Water Res. 68: 432–443. doi:10.1016/j.watres.2014.09.046

Kothawala, D. N., E. Von Wachenfeldt, B. Koehler, and L. J. Tranvik. 2012. Selective loss and preservation of lake water dissolved organic matter fluorescence during long-term dark incubations. Sci. Total Environ. 433: 238–246. doi:10.1016/j.scitotenv.2012.06.029

Kowalczuk, P., M. J. Durako, H. Young, A. E. Kahn, W. J. Cooper, and M. Gonsior. 2009. Characterization of dissolved organic matter fluorescence in the South Atlantic Bight with use of PARAFAC model: Intertropical variability. Mar. Chem. 113: 182–196. doi:10.1016/j.marchem.2009.01.015

Kraus, T. E. C., B. A. Bergamaschi, P. J. Hernes, D. Doctor, C. Kendall, B. D. Downing, and R. F. Losee. 2011. How reservoirs alter drinking water quality: Organic matter sources, sinks, and transformations. Lake Reserv. Manag. 27: 205–219. doi:10.1080/07438141.2011.597283

Liang, L., and P. C. Singer. 2003. Factors influencing the formation and relative distribution of haloacetic acids and trihalomethanes in drinking water. Environ. Sci. Technol. 37: 2920–2928. doi:10.1021/es026230q

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. doi:10.4319/lo.2001.46.1.0038

Miller, W. L., and M. A. Moran. 1997. Interaction of photochemical and microbial processes in the degradation of refractory dissolved organic matter from a coastal marine environment. Limnol. Oceanogr. 42: 1317–1324. doi:10.4319/lo.1997.42.6.1317

Miller, M. P., and D. M. McKnight. 2010. Comparison of seasonal changes in fluorescent dissolved organic matter among aquatic lake and stream sites in the Green Lakes Valley. J. Geophys. Res. Biogeosci. 115: G00F12. doi:10.1029/2009JG000985

Minor, E. C., M. M. Swenson, B. M. Mattson, and A. R. Oyler. 2014. Structural characterization of dissolved
organic matter: A review of current techniques for isolation and analysis. Environ. Sci. Process. Impacts 16: 2064–2079. doi:10.1039/C4EM00062E

Mopper, K., and C. A. Schultz. 1993. Fluorescence as a possible tool for studying the nature and water column distribution of dissolved organic matter. Mar. Chem. 41: 229–238. doi: 10.1016/0304-4203(93)90124-7

Moran, M. A., and R. G. Zepp. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. Limnol. Oceanogr. 42: 1307–1316. doi:10.4319/lo.1997.42.6.1307

Moran, M. A., W. M. Sheldon, and R. G. Zepp. 2000. Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. Limnol. Oceanogr. 45: 1254–1264. doi:10.4319/lo.2000.45.6.1254

Murphy, K. R., K. D. Butler, R. G. M. Spencer, C. A. Stedmon, J. R. Boehme, and G. R. Aiken. 2010. Measurement of dissolved organic matter fluorescence in aquatic environments: An interlaboratory comparison. Environ. Sci. Technol. 44: 9405–9412. doi:10.1021/es102362t

Murphy, K. R., A. Hambly, S. Singh, R. K. Henderson, A. Baker, R. Stuezt, and S. J. Khan. 2011. Organic matter fluorescence in municipal water recycling schemes: Toward a unified PARAFAC model. Environ. Sci. Technol. 45: 2909–2916. doi:10.1021/es103015e

Obernosterer, I., B. Reitner, and G. J. Herndl. 1999. Contrasting effects of solar radiation on dissolved organic matter and its bioavailability to marine bacterioplankton. Limnol. Oceanogr. 44: 1645–1654. doi:10.4319/lo.1999.44.7.1645

Obernosterer, I., and R. Benner. 2004. Competition between biological and photochemical processes in the mineralization of dissolved organic carbon. Limnol. Oceanogr. 49: 117–124. doi:10.4319/lo.2004.49.1.0117

Ohno, T. 2002. Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. Environ. Sci. Technol. 36: 742–746. doi:10.1021/es015527e

Olefeldt, D., M. R. Turetsky, and C. Blodau. 2013. Altered composition and microbial versus UV-mediated degradation of dissolved organic matter in boreal soils following wildfire. Ecosystems 16: 1396–1412. doi:10.1007/s10021-013-9691-y

Opsahl, S., and R. Benner. 1998. Photochemical reactivity of dissolved lignin in river and ocean waters. Limnol. Oceanogr. 43: 1297–1304. doi:10.4319/lo.1998.43.6.1297

Para, J., P. G. Coble, B. Charrriere, M. Tedetti, C. Fontana, and R. Sempere. 2010. Fluorescence and absorption properties of chromophoric dissolved organic matter (CDOM) in coastal surface waters of the northwestern Mediterranean Sea, influence of the Rhone River. Biogeosciences 7: 4083–4103. doi:10.5194/bg-7-4083-2010

Parlanti, E., K. Worz, L. Geoffroy, and M. Lamotte. 2000. Dissolved organic matter fluorescence spectroscopy as a tool to estimate biological activity in a coastal zone submitted to anthropogenic inputs. Org. Geochem. 31: 1765–1781. doi:10.1016/S0146-6380(00)00124-8

Pellerin, B. A., P. J. Hernes, J. Saraceno, R. G. Spencer, and B. A. Bergamaschi. 2010. Microbial degradation of plant leachate alters lignin phenols and trihalomethane precursors. J. Environ. Qual. 39: 946–954. doi:10.2134/jeq2009.0487

Repeta, D. J., T. M. Quan, L. I. Aluwihare, and A. M. Accardi. 2002. Chemical characterization of high molecular weight dissolved organic matter in fresh and marine waters. Geochim. Cosmochim. Acta 66: 955–962. doi:10.1016/S0016-7037(01)00830-4

Spencer, R. G. M., A. Baker, J. M. E. Ahad, G. L. Cowie, R. Ganeshram, R. C. Upstill-Goddard, and G. Uher. 2007. Discriminatory classification of natural and anthropogenic waters in two U.K. estuaries. Sci. Total Environ. 373: 305–323. doi:10.1016/j.scitotenv.2006.10.052

Spencer, R. G. M., and others. 2009. Photochemical degradation of dissolved organic matter and dissolved lignin phenols from the Congo River. J. Geophys. Res. Biogeosci. 114: G03010. doi:10.1029/2009JG000968

Spencer, R. G. M., K. D. Butler, and G. R. Aiken. 2012. Dissolved organic carbon and chromophoric dissolved organic matter properties of rivers in the USA. J. Geophys. Res. 117: G03001. doi:10.1029/2011JG001928

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., and S. Markager. 2005. Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. Limnol. Oceanogr. 50: 686–697. doi:10.4319/lo.2005.50.2.0686

Stedmon, C. A., and R. M. Cory. 2014. Biological origins and fate of fluorescent dissolved organic matter in aquatic environments, p. 278–300. In P. Coble, J. Lead, A. Baker, D. Reynolds, and R. G. M. Spencer [eds.], Aquatic organic matter fluorescence. Cambridge Univ. Press.

Stepanauskas, R., M. A. Moran, B. A. Bergamaschi, and J. T. Hollibaugh. 2005. Sources, bioavailability, and photoactivity of dissolved organic carbon in the Sacramento-San Joaquin River Delta. Biogeochemistry 74: 131–149. doi:10.1007/s10533-004-3361-2

Stubbins, A., and others. 2010. Illuminated darkness: Molecular signatures of Congo River dissolved organic matter and its photochemical alteration as revealed by ultrahigh precision mass spectrometry. Limnol. Oceanogr. 55: 1467–1477. doi:10.4319/lo.2010.55.4.1467

Stubbins, A., J. Niggemann, and T. Dittmar. 2012. Photoactivity of deep ocean dissolved black carbon. Biogeosciences 9: 1661–1670. doi:10.5194/bg-9-1661-2012

Tranvik, L. J., and S. Bertilsson. 2001. Contrasting effects of solar UV radiation on dissolved organic sources for detritus production in boreal lakes. J. Geophys. Res. Biogeosci. 106: 131–149. doi:10.1029/2000JG000740

1031
bacterial growth. Ecol. Lett. 4: 458–463. doi:10.1046/j.1461-0248.2001.00245.x

U.S. Environmental Protection Agency. 2005. Method 415.3 determination of total organic carbon and specific UV absorbance at 254 nm in source water and drinking water, EPA Document #: EPA/600/R-05/055. http://www.epa.gov/microbes/Method%20415_3_Rev1_2_Final.pdf

Weishaar, J. L., G. R. Aiken, B. A. Bergamaschi, M. S. Fram, 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

Wetzel, R. G., P. G. Hatcher, and T. S. Bianchi. 1995. Natural photolysis by ultraviolet irradiance of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism. Limnol. Oceanogr. 40: 1369–1380. doi:10.4319/lo.1995.40.8.1369

Wilson, H. F., and M. A. Xenopoulos. 2009. Effects of agricultural land use on the composition of fluvial dissolved organic matter. Nat. Geosci. 2: 37–41. doi:10.1038/ngeo391

Yang, L., and J. Hur. 2014. Critical evaluation of spectroscopic indices for organic matter source tracing via end member mixing analysis based on two contrasting sources. Water Res. 59: 80–89. doi:10.1016/j.watres.2014.04.018

Zhang, Y., M. A. Van Dijk, M. Liu, G. Zhu, and B. Qin. 2009. The contribution of phytoplankton degradation to chromophoric dissolved organic matter (CDOM) in eutrophic shallow lakes: Field and experimental evidence. Water Res. 43: 4685–4697. doi:10.1016/j.watres.2009.07.024

Zsolnay, A., E. Baigar, M. Jimenez, B. Steinweg, and F. Saccomandi. 1999. Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. Chemosphere 38: 45–50. doi:10.1016/S0045-6535(98)00166-0

Acknowledgments

We thank Elizabeth Stumpner and Laurel Moll for laboratory analyses and Travis von Dessonneck for support with data processing. We thank Aron Stubbins for his guidance in constructing a photosimulator. We also thank Matt Miller and two anonymous reviewers whose valuable comments and suggestions improved the quality of this manuscript. This work was funded by the California Water Science Center Research Program and the California Department of Water Resources. The use of brand names in this manuscript is for identification purposes only and does not imply endorsement by the US Geological Survey.

Submitted 30 July 2015
Revised 17 December 2015
Accepted 6 January 2016

Associate editor: Anna Romani