Effect of photo-biodegradation and biodegradation on the biogeochemical cycling of dissolved organic matter across diverse surface water bodies

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ABSTRACT

The objective of this research was to quantify the temporal variation of dissolved organic matter (DOM) in five distinct waterbodies in watersheds with diverse types of land use and land cover in the presence and absence of sunlight. The water bodies were an agricultural pond, a lake in a forested watershed, a man-made reservoir, an estuary, and a bay. Two sets of samples were prepared by dispensing unfiltered samples into filtered samples in 1:10 ratio (V/V). The first set was exposed to sunlight (10 hr per day for 30 days) for examining the combined effect of photo-biodegradation, while the second set was stored in dark for examining biodegradation alone. Spectroscopic measurements in tandem with multivariate statistics were used to interpret DOM lability and composition. The results suggest that the agricultural pond behaved differently compared to other study locations during degradation experiments due to the presence of higher amount of microbial humic-like and protein-like components derived from microbial/anthropogenic sources. For all samples, a larger decrease in dissolved organic carbon (DOC) concentration (10.12% ± 9.81% for photo-biodegradation and 6.65% ± 2.83% for biodegradation) and rapid transformation of DOM components (i.e., terrestrial humic-like components into microbial humic and protein-like components) were observed during photo-biodegradation experiments. Results suggest that sunlight facilitated DOM biodegradation, resulting in simpler recalcitrant molecules regardless of original composition. Overall, it was found that combined effects of light and bacteria are more efficient than bacterial effects alone in remineralizing and altering DOM, which highlights the crucial importance of sunlight in transforming aquatic DOM.

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Introduction

Dissolved organic matter (DOM) is a ubiquitous component in natural waters, composed of a complex mixture of humic acids, fulvic acids, low molecular weight organic acids, carbohydrates and bacterial-derived proteins (Coble et al., 1998; Mcknight et al., 2001; Her et al., 2003). DOM represents a significant part of global carbon cycle, influences water...
quality, color, and transparency, and plays a key role in the biogeochemical cycling of nutrients and trace metals (Cai et al., 2011; McKnight et al., 1992; Medeiros et al., 2015; Mladenov et al., 2008; Sharma et al., 2010; Stedmon et al., 2006; Sunda and Cai, 2012). Dissolved organic carbon (DOC) concentration is a measure of organic carbon content in DOM. DOM also includes other nutrients such as dissolved organic nitrogen (DON), dissolved organic phosphorus (DOP), and dissolved organic sulfur (DOS) (Cleveland et al., 2004). DOM present in aquatic environments can be autochthonous (in situ phytoplankton production and decomposition or bacterial cell lysis) or allochthonous (transported decomposed terrestrial plant and organic materials) or can originate from synthetic organic or anthropogenic sources (Shang et al., 2018; Singh et al., 2017; Mostofa et al., 2013; Stedmon and Markager, 2005; Nagata, 2000). Natural reprocessing of DOM through photodegradation (sunlight induced decomposition) and biodegradation (microbial decomposition) or their combined effect can control the quantity and quality of DOM (Hansen et al., 2016; Medeiros et al., 2015; Mopper et al., 1991; Moran et al., 2000, Moran and Zepp, 1997; Singh et al., 2017; Stedmon et al., 2006; Stedmon and Markager, 2005). Photodegradation reactions can lead to the transformation of the complex DOM into much simpler labile bioavailable forms like carbonyl compounds, carbon dioxide gas, nitrogen and phosphate compounds that can stimulate bacterial growth (Gonsior et al., 2014; Moran et al., 2000; Moran and Zepp, 1997; Timko et al., 2015). Fluorescence studies show that the terrestrial derived DOM is much more photo-reactive than the bacterial-derived DOM compounds (Kieber and Zhou, 1990; Timko et al., 2015; Lu et al., 2013).

Studies using Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) and Nuclear Magnetic Resonance (NMR) have revealed that photodegradation can decrease the molecular diversity of DOM species compared to biodegradation and also reported that the photo-labile DOM species were characterized by more aromatic, high molecular weight, sulfur-phosphorus or sulfur-nitrogen containing, or aliphatic and peptide compounds (Stubbins and Dittmar, 2015; Gonsior et al., 2014; Lu et al., 2013). However, there are conflicting views about the nature and composition of DOM produced as a result of photo and biodegradation. Some studies have shown that photodegradation can lead to the production of smaller labile bio-available byproducts and dissolved inorganic carbon (DIC) while some other studies have reported that photodegradation can form higher molecular weight refractory DOM compounds (Hansen et al., 2016; Moran and Zepp, 1997; Obernosterer et al., 1999; Biddanda and Benner, 1998). Meanwhile, the less condensed and labile low molecular weight materials like proteins, carbohydrates and organic acids can be preferentially removed during biodegradation (Hansen et al., 2016; Hur et al., 2011).

Studies have also indicated that microbial degradation of DOM can lead to the formation and enrichment of higher molecular weight fulvic and humic acids with higher aromatic content (Hansen et al., 2016; Hur et al., 2011; Kalbitz et al., 2003; Stimler et al., 2006). Bacterially derived carbon, which is lighter and recalcitrant (low molecular weight and resistant to degradation) is more resistant to microbial degradation and hence can cause an increase in their presence in water bodies (Kawasaki et al., 2013). Predominant type of vegetation in the watershed and the microbial community existing within the waterbody can also influence its DOM composition and hence the reactivity to photochemical and biological degradation (Kothawala et al., 2014; Lu et al., 2015, 2013, 2014; Singh et al., 2017, 2014; Williams et al., 2010; Shang et al., 2018). Allochthonous fulvic acids are high molecular weight heterogeneous compounds, which are photo- and biorefractory but refractory to microbial degradation (Mcknight et al., 1988; Shank et al., 2009). In contrast, autochthonous fulvic acids are less aromatic with low molecular weight and are less bioreactive and phoretactive than allochthonous DOM (Williams et al., 2010).

In the present study, we have evaluated the reactivity of DOM with respect to its sources using a series of laboratory-based photo and biodegradation experiments and fluorescence and absorption techniques. We have modeled our data using parallel factor analysis (PARAFAC) and multivariate statistics to understand the reactivity of DOM over time based on its source. Our main objectives were to (1) evaluate the differences in the reactivity of DOM generated from diverse sources in the presence and absence of sunlight, and (2) understand the changes in the nature and composition of DOM due to photo-transformation and biodegradation.

1. Methods

1.1. Study sites and experimental set-up

The study sites include a forested lake (Bluff Lake, L, Mississippi), an agricultural pond (Brooksville, P, Mississippi), a man-made reservoir (Ross Barnett, R, Mississippi), and an estuary (Lower Pearl River, E, Louisiana) and a bay (Weeks Bay, B, Alabama). These water bodies were selected based on the distinct land use and hinterland vegetative cover to encompass a broad range of variability in DOM chemistry in the southern states of USA (Fig. 1).

The land use land cover classification of the study areas was evaluated from the geospatial data that was collected from United States Department of Agriculture (USDA) data gateway and later processed in the ArcGIS® 10.3.1 software platform (Table 1). The seven major land cover classes surrounding the water bodies were urbanized area, barren land, forest, pasture, agriculture and woody/herbaceous wetland. Among the waterbodies, the estuary (E) was the biggest (170.56 km², 3.60%) and pond (P) was the smallest (4.32 km², 3.58%). The lake (L) was surrounded by forest (67.28 km², 33.79%) and woody/herbaceous wetland (75.71 km², 38.02%). The pond (P) was surrounded by pasture (35.09 km², 29.08%) and agricultural area (30.50 km², 25.28%). Predominant land use land cover surrounding the reservoir (R) was forest (178.28 km², 36.35%) and woody/herbaceous wetland (943.81 km², 19.94%). The immediate surrounding of the bay (B) is also covered by forest (45.18 km², 18.69%) and woody/herbaceous wetland (42.88 km², 17.66%).

Three liters of surface water samples was collected in clean, acid-washed high density polyethylene (HDPE) bottles from each of the five different water bodies. A total of seven samples were collected following the protocol of Dash et al. (2015); one each from the agricultural pond, forested lake, estuary and two samples each from reservoir and bay (Fig 1). The pond (P) and
lake (L) samples were collected from the central location and estuary (E) samples were collected from the river thalweg. The two of the bay samples were collected near the mouth of the two rivers draining into the bay, one was from the northern end (B1) and the other from the southeastern side (B2). One reservoir sample was collected near the spillway (R1), while the other was from the central part of the reservoir (R6). Immediately after the collection, samples were stored in an airtight cooler filled with ice and were transported back to the lab for filtration. Initially, the water samples were filtered using 0.7-μm Whatman® GFF filter to remove larger particles. Afterwards, the filtrate was re-filtered through 0.2-μm Whatman® nucleopore track-etch membrane filters.

1.2. Photo-biodegradation and biodegradation experiments

For each 0.2-μm filtrate sample, two sets of samples were prepared for photo-biodegradation and biodegradation...
experiments, respectively. For samples E and B2, each set consisted of six samples and six duplicates, whereas for the rest of the sites, each set consisted of seven samples and seven duplicates. The above sets were prepared by dispensing 20 mL of unfiltered fresh water sample (inoculum) in 180 mL of the filtered sample in the ratio of 1:10 (V/V) in an acid washed pre-combusted 250-mL Fisherbrand® French square transparent glass bottles using a pipette. The inoculum was added to disperse the actual bacterial community back into the experimental system. Sufficient headspace was maintained by not filling the mixture to the mouth of the bottle. The resultant solution was mixed well via manual shaking. The mouth of the bottle was covered by parafilm® to avoid contamination but to allow gas (oxygen) exchange during the experiment. The transparent glass and parafilm provide maximum visibility with no chemical inference with the samples. However, some ultraviolet (UV) wavelengths (<290 nm) were blocked by the glass (Overway, 2017). The photo-biodegradation experiments involved exposing one set of samples and the duplicates to a minimum of 10 hr of sunlight per day in an unshaded area on the campus of Mississippi State University, and at the end of day samples were stored back in a dark room at 25°C. The mean daily temperature during the period (June–September 2016) of the photo-biodegradation experiment at Starkville, MS was 26.55°C with a minimum of 25°C and maximum of 28°C.

Similarly, the biodegradation experiment involved incubating the subsamples and duplicates in a dark room at 25°C. Before biodegradation incubations, the sub-samples and duplicates were wrapped in aluminum foil to prevent light exposure. Both experiments were continued for 30 days. After the experiments, both sub-samples and duplicates were re-filtered using 0.2-μm Whatman® nuclepore track-etch membrane filter into pre-washed preheated clean Fisherbrand® amber glass bottles and were refrigerated at 4°C for further analysis.

### 1.3. Sample analysis

The DOC and total nitrogen (TN) concentration of the experimental solutions (samples and duplicates) were measured using a SHIMADZU® TOCv-TNM1 total organic carbon-total nitrogen analyzer equipped with an ASI-V autosampler following the method described by Shang et al. (2018).

The absorption characteristics of the experimental solutions were analyzed using a PerkinElmer Lambda 850 double-beam spectrophotometer equipped with a 150-mm spectrolon coated integrating sphere following the analytical procedure described by Singh et al. (2017). The absorption spectrum was collected between 200 and 750 nm wavelengths at the 2 nm interval by using a clean 4 mL quartz cuvette of 1 cm path length. The blank subtracted sample values were used to remove the effect of water and scattering. Then Napierian absorption coefficient (α) for each of the sample and duplicates was calculated from the blank corrected absorbance value (A) for each wavelength (i) in inverse meters (Green and Blough, 1994; Singh et al., 2017, 2010; Yates et al., 2016).

The fluorescence characteristics in terms of excitation-emission matrices (EEMs) of the samples (photo-biodegraded and initial samples) were analyzed in a Horiba Jobin Yvon Inc., Fluoromax-4 spectrofluorometer (Edison, NJ, USA) by following the procedure described in Singh et al. (2017). The EEM spectra for each sample were recorded with excitation wavelength range from 240 to 450 nm at 10 nm increment and with a slit width of 5 nm. Similarly, the emission spectra of the samples were collected from 300 to 550 nm wavelengths at every 2 nm increment with a slit width of 5 nm over 0.25 sec of integration time. The company specified instrumental corrections were also applied while collecting the blank and sample EEMs. The sample EEMs were corrected for blanks, Raman normalization, and inner filter effects prior to the calculation of fluorescence indices and PARAFAC modeling (Kothawala et al., 2013; Lawaetz and Stedmon, 2008; Murphy et al., 2010; Singh et al., 2017).

### 1.4. Absorption and fluorescence indices and PARAFAC modeling

A total of 87 samples from the photo-biodegradation and biodegradation experiments with 126 emissions and 43 excitation wavelengths were used to create a PARAFAC model. The total 87 samples constitute 40 samples from the photo-biodegradation experiment, 40 samples from biodegradation experiment and 7 initial samples (time = 0 hr). The duplicates were not used for the PARAFAC modeling because they can artificially increase the sample size and hence the power of statistical validation test. Prior to the PARAFAC modeling, the emission wavelengths were interpolated at 4 nm increments and excitation wavelengths were interpolated at 5 nm increments for all EEMs. Next, the EEMs were normalized to the daily determined water Raman integrated area under maximum fluorescence intensity (350 ex/397 em, 5-nm bandpass) to normalize the EEM data to comparable Raman units (R.U.). After the outlier analysis (removing exceptionally different samples), the sample size was reduced to 85. The model validation was carried out using split-half analysis and random initialization, and a four-component PARAFAC model was created. The model explained 99% of the variation among the total EEM data. The scores of four PARAFAC components were expressed in Raman units (R.U) as their fluorescence intensity maximum (Fmax). The DOMFluor toolbox in MATLAB® computing environment was used for PARAFAC analysis (Stedmon and Bro, 2008). The liability-related statement
of each PARAFAC component was directly assessed via the degradation experiments in the present study. The absorption and fluorescence spectra were also used to generate several proxies that are used to identify DOM sources and quality. Specific UV absorbance at 254 nm (SUVA254) was calculated by dividing a254 by the DOC concentration. Slope ratio (SR) is the ratio of the average absorption coefficient from 275 to 295 nm and from 350 to 400 nm. Fluorescence index (FI) was calculated by taking the ratio of absorption coefficients at 254 nm and 520 nm acquired at excitation wavelength 370 nm. Humification index (HIX) was calculated by dividing peak integrated area of emission spectra between 300 and 345 nm with the sum of peak integrated area of emission spectra between 300–345 nm and 435–480 nm at 254 nm excitation. Biological index or freshness index (BIX) was the ratio of emission intensity at 380 nm divided by the emission intensity maximum observed between 420 and 435 nm, obtained at ex 310 nm. These proxies are well-accepted indicators for the origin and lability of DOM. The details of the calculations and its uses were described in Hansen et al. (2016). The values of the indices were expressed by taking the mean and standard deviation of the duplicate measurement of each sample.

1.5. Rate of degradation of DOM and aromaticity decline

The rate of DOC degradation and aromaticity decline during photo-bio and biodegradation experiments were calculated separately for all samples and duplicates by fitting a single, two, or three parameters exponential decay equation (Eq. (1)) along their DOC concentration and absorption coefficients (a254) as:

$$A = A_0 e^{-kt}$$  \hspace{1cm} (1)

where $A$ is the DOC concentration expressed in terms of a254 (m$^{-1}$) at the time $t$; $A_0$ is the DOC concentration expressed in terms of a254 (m$^{-1}$) when time $t$ is zero, $k$ (day$^{-1}$) is the rate, and $t$ (hr) is the length of the exposure/incubation time. The half-life of both DOC degradation and aromaticity decline (days) in all study areas were also calculated (Lu et al., 2013; Shang et al., 2018; Shank et al., 2009) as:

$$A = A_0 2^{-t/h}$$  \hspace{1cm} (2)

Fig. 2 – Temporal change in concentrations of dissolved organic carbon (DOC) and total nitrogen (TN) during photo-biodegradation (P + B) and biodegradation (B).
where \( h \) is the half-life in days. The rate and half-life, as well as their mean and standard deviation between the sample and duplicate were calculated separately for each sample.

1.6. Statistical analyses

Principal component analysis (PCA) was performed among the PARAFAC components, fluorescence indices, absorption indices, DOC-TN concentration, and exposure times to understand relationships among these parameters and to classify samples based on their DOM source. A Mann–Whitney-U test (\( \alpha = 0.05 \)) was performed to test if the photo-biodegradation was significantly faster or slower than the biodegradation experiment. The t-test of regression (\( \alpha = 0.05 \)) was performed to calculate the \( p \) value to estimate the significance of slope for absorption and fluorescence indices in predicting the extent or rate of photo-bio and biodegradation.

![Fig. 3](image-url)

**Fig. 3** – Excitation (blue curve) and emission (red curve) loadings for four dissolved organic matter (DOM) components derived using a PARAFAC model that was validated using split-half analysis as well as random initialization. Contour plots corresponding to the components identified in the PARAFAC model are shown for each of the components.
2. Results

2.1. Temporal variation of DOC and TN during photo and biodegradation experiments

There is an overall decrease in DOC concentration, for most of the samples during both photo-biodegradation (10.12% ± 9.81%) and biodegradation (6.65% ± 2.83%) experiments (Fig. 2). The only exception was B2 (southern part of the bay) sample that showed a slight increase (2% ± 1.24%) in DOC concentration during the photo-biodegradation experiment. The TN concentration did not follow any regular patterns during the photo-biodegradation experiment. By comparison, there was an overall increase in TN for all samples except for B2 (5.27% ± 8.61% decrease) during the biodegradation experiment. Certain local fluctuations (increase or decrease) in DOC and TN concentrations were observed over the course of both experiments.

2.2. PARAFAC components and DOM indices

A total of four different PARAFAC components (C1, C2, C3, C4) were determined. Among the four components, two were microbial or tryptophan-like or tyrosine-like (C1 and C4) autochthonous DOM components, and the other two (C2 and C3) were of humic-like or fulvic acid-like DOM of terrestrial origin (Fig. 3). All four PARAFAC components have been previously reported (Table 2). Over the course of both photo-bio and biodegradation experiments, the $F_{\text{max}}$ (in R.U.) for the component C1 was the highest for all the samples (Figs. 4 and 5). In all the samples, the changes in component C1 and C4 displayed trends opposite to those of components C2 and C3. The component C4 in the bay (B1, B2) and agricultural pond (P) showed greater fluctuations during the biodegradation experiments (Fig. 5).

2.3. Absorption and fluorescence indices and their temporal variation

During photo-biodegradation, both $a_{254}$ and SUVA$_{254}$ measures of aromaticity showed decreasing trends while the SR, which is inversely related to molecular weight, showed an increasing trend (Fig. 6). The estuary samples had the greatest decrease in $a_{254}$ values ($p = 0.20; 48.08\% ± 1.76\%$) and the agricultural pond samples showed the lowest decrease ($p = 0.001; 18.4\% ± 0.13\%$). For SUVA$_{254}$, the forested lake samples showed the lowest decrease ($p = 0.39; 15.65\% ± 3.37\%$), while estuary samples showed the greatest reduction ($p = 0.24; 40.03\% ± 0.47\%$). The greatest increase in SR values was observed for the estuary samples ($p = 0.19; 166.84\% ± 1.74\%$), while the minimum increase was observed for samples from the central part of the reservoir, R6 ($p = 0.60; 40.23\% ± 3.65\%$).

<table>
<thead>
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<th>Table 2 – Parallel factor (PARAFAC) analysis components.</th>
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<td>Components (Ex/Em) (nm)</td>
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<tr>
<td>C1 (&lt;250–300/402)</td>
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<tr>
<td>C2 (&lt;250–350/436)</td>
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<tr>
<td>C3 (260–380/504)</td>
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<tr>
<td>C4 (280/328)</td>
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The change in trends of \( a_{254} \), SUVA_{254}, and SR values of the samples during biodegradation was different from that during photo-biodegradation (Fig. 7). Like photo-biodegradation (34.26% ± 10.64% decrease), all the samples showed a decrease in \( a_{254} \) values during the biodegradation experiment, but the magnitude of decrease was much smaller (9.8% ± 6.57%). For the SUVA_{254} values, the samples from the bay, B1 (\( p = 0.07; \) 6.2% ± 0%), B2 (\( p = 0.90; \) 8% ± 18.16%), and central part of the reservoir (R6) showed a slight increase (\( p = 0.95; \) 0.7% ± 0.73%), while the other samples showed a decrease in SUVA_{254} values. An increase in SR was observed for B2 (\( p = 0.82; \) 3.5% ± 1.2%), pond (\( p = 0.55; \) 3.9% ± 16.5%), estuary (\( p = 0.69; \) 0.5% ± 0.81%), R1 (\( p = 0.01; \) 37.5% ± 2.66%) and R6 (\( p = 0.82; \) 13.1% ± 1.81%) samples, while the samples B1 (\( p = 0.94; \) 0.4% ± 0%) and lake (\( p = 0.27; \) 10.8% ± 3.69%) showed a decrease in SR values.

During photo-biodegradation, decreased values for both HIX (81.11% ± 12.76%) and FI (8.10% ± 3.04%) were observed, while the BIX (15.53% ± 7.19%) values showed an increasing trend for all the samples (Fig. 8). The greatest decrease in HIX values was observed for lake (L) samples (\( p = 0.002; \) 54.90% ± 3.91%). The pond samples showed a minimum decrease in FI (\( p = 0.04; \) 5.37% ± 0.93%), while a maximum decrease was observed for southern part of bay (B2) samples (\( p = 0.03; \) 14.38% ± 2.46%). The greatest increase in BIX values was observed for northern part of bay (B1) sample (\( p = 0.008; \) 25% ± 0%), while the minimum increase was observed for pond samples (\( p = 0.001; \) 5.29% ± 0.32%) during the photo-biodegradation experiment.

During biodegradation, the HIX decreased for most of the samples, with exception of the pond (\( p = 0.005; \) 32.5% ± 19.74%) and lake (\( p = 0.61; \) 1.7% ± 6.42%). In both pond and lake, an increase in HIX was observed. Similarly, FI values increased for pond (\( p = 0.92; \) 0.1% ± 0.08%), lake (\( p = 0.08; \) 0.57% ± 0.28%), and estuary (\( p = 0.06; \) 0.69% ± 0.64%) samples, while rest of the samples showed a decrease in FI values (Fig. 9). For all the samples, the BIX values increased (2.23% ± 2.24%), with a minimum increase for estuary (\( p = 0.04; \) 0.69% ± 0.64%) and maximum increase for southern part of the bay, B2 (\( p = 0.003; \) 6.80% ± 3.59%).

The variability of samples from the same location was also observed for the bay (B1 and B2) and reservoir (R1 and R6). The
samples were collected from the bay on two separates dates, whereas for reservoir they were collected from separate locations. Prior to the date of sample collection in B1 (06.19.2016), there was a heavy rainfall (38.1–25.4 mm) from 06.01.2016 to 06.20.2016. After 06.20.2016, there was no rain in the area until the date of sampling from B2 (06.26.2016). Also, Weeks Bay covers a small area of 7 km² and it empties its water (residence time ~5 days) to the Mobile Bay (Caffrey et al., 2014), suggesting a difference in DOM input during two separate sampling dates.

2.4. Rate of DOC degradation, aromaticity decline, and half-life

There was a considerable difference in the rate of disintegration and half-life of DOC concentration and a254 decline between the photo-biodegradation and biodegradation experiments (Table 3). Higher degradation rates and shorter half-lives were observed for all the samples during photo-biodegradation versus biodegradation experiments (Fig. 10). DOC disintegrated faster in the lake (L) sample during photo-biodegradation (27.84 ± 7.59 days) and the rate was 0.0228 ± 0.00509 day⁻¹. While the pond (P) sample showed low DOC degradation during photo-biodegradation and its corresponding half-life and rate were 120.66 ± 25.39 days and 0.0024 ± 0.00339 day⁻¹) and slower in R6 sample (543.09 ± 63.83 days and 0.00115 ± 0.000346 day⁻¹). During photo-biodegradation experiments, the estuarine (E) samples showed the highest rate of decline in a254 (1.71 ± 0.441 day⁻¹, which is an outlier, that is greater than 3 times inter quartile range above the third quartile) and lowest half-life (13.67 ± 0.71 days), while samples from agricultural pond had the highest half-life (39.65 ± 0.30 days) and the corresponding rate of a254 decline was 0.27 ± 0.0526 day⁻¹. On the other hand, all samples showed a slow rate of decline in a254 values and an increase in half-life during the biodegradation experiments (Table 3). Among them, R1 had the shortest half-life (89.64 ± 2.67 days) and B2 had the longest half-life (818.88 ± 440.29 days).

The Mann Whitney U-test performed on the half-life and rate data set of both DOC degradation and a254 decline. The tests confirmed that the half-life of photo-biodegradation was significantly lower ($U = 49, p = 0.0005; U = 49, p = 0.0006$) and the rate was significantly higher ($U = 8.5, p = 0.04; U = 6, p = 0.02$) than that of biodegradation.

2.5. Principal component analysis

PCA was performed by combining the data from both photo-biodegradation and biodegradation experiments (Fig. 11). The variables used in PCA loading included PARAFAC components.
C1, C2, C3, and C4, HIX, FI, BIX, a254, SR, SUVA254, DOC, TN, and exposure times (exposure to sunlight for photo-biodegradation in hours and incubation in the darkness for biodegradation in hours). The initial samples (t = 0 hr) were also included in the PCA. Principal component-1 (PC1, 29.54%) and principal component-2 (PC2, 24.50%) together explained 54.04% of the variation in data. The PC1 is controlled more by bulk fluorescence and absorbance indices, whereas the PC2 is controlled more by PARAFAC components. The negative PC1 loadings for C2, C3, HIX, a254, and SUVA254 indicate terrestrially derived humic-like or fulvic-like DOC were structurally complex, refractory, or aromatic compounds derived from higher plants. C1, C4, SR, FI and BIX were having positive PC1 loadings, which indicates structurally simpler and more labile DOM (tryptophan-like or tyrosine like), which was derived from microbial sources. The exposure time is strongly related to the components C1 and C4, indicating their production over the course of experiments. Although not all, but most of the photo-biodegradation data is distributed along the positive side of the PC1 axis, while most of the biodegradation data is distributed along the negative side the PC1. PCA also suggest that during the course of photo-biodegradation, there is an increase in SR and BIX as indicated by the distribution of photo-biodegradation data (represented by completely filled symbols) along the positive PC1 axis. Indices (BIX and FI) on PC1 indicate that photo-biodegradation leads to higher microbial activity in aquatic system. PC2 indicates that C2 and C3 decrease but C1 and C4 increase with incubation time, and this pattern is more evident for biodegradation experiments. The distribution of biodegradation data (represented by unfilled symbols) mostly along the positive PC2 axis and with higher HIX, a254, and SUVA254 mean they are of higher molecular weight and have not degraded as much as photo-biodegradation samples.

3. Discussion

3.1. Initial DOM character versus watershed land use and land cover

We have related four predominant land use and land cover classes (forest, woody/herbaceous wetland, agriculture, and pasture) in the watershed controlling the DOM input (source) to the five different waterbodies used in this study. The FI values and the distribution of PARAFAC components in the PCA space confirmed that the initial DOM character of these waterbodies was influenced by their respective land use and land cover in the watersheds (Fig. 11). In particular, the FI values of reservoir, estuary, bay and lake were less than 1.5 and initial samples of these waterbodies were distributed on the negative side of the PC1, indicating terrestrial humic-like DOM components derived from the higher plants in their catchments. On the other hand, the FI of the initial agricultural pond sample was greater than 1.6 and the pond samples
were distributed along the positive side of the PC1 axis, suggesting agricultural input.

3.2. Photo-biodegradation versus biodegradation and spatial differences in DOM degradation

3.2.1. Temporal change in DOC and TN concentration
We observed higher reduction in the amount of DOC (10.12% ± 9.81%) during photo-biodegradation relative to biodegradation (6.65% ± 2.83%). This pattern suggests that the sunlight and the composition of DOM present in the water are the major factors affecting the reduction of DOC concentration (Fig. 2), which is consistent with the findings of Kieber and Zhou (1990), Lu et al. (2013), Timko et al. (2015) and Cory and McKnight (2005). The low amount of change in DOC concentration of agricultural pond during photo-biodegradation as it contains a higher amount of microbial humic-like components. Our results suggest that sunlight may not be very effective in further breaking down such smaller microbial-like or tryptophan-like or tyrosine-like DOM components present in the agricultural pond compared to other study locations. It needs to be acknowledged that the outdoor incubations temperature (median T = 27.10°C, with a maximum and minimum of 28°C and 25.50°C) was slightly higher than the laboratory incubations (T = 25°C), and a higher temperature could enhance the photo-biodegradation rate of DOM. However, our results on DOM compositions (i.e., reduction of aromaticity and transformation of humic components) suggest that sunlight is primarily responsible for the greater degradation rate of DOC and a greater decline in aromaticity in the photo-biodegradation experiments. Raymond and Bauer (2000) have empirically determined Q10, the factor by which the rate of a biodegradation reaction increases for every 10°C rise in the temperature, as 2.1. Following Raymond and Bauer (2000) and Reyes et al. (2008), we calculated the increase in biodegradation reaction rate as 1.17 times for a temperature increase of 2.1°C. A comparison of our rates for the decline in aromaticity and DOC degradation rates reveal that in our photo-biodegradation experiments, the reaction rate was much higher than 1.17 × the biodegradation rates, which suggests that the temperature had a minor role and the sunlight was the major factor in degradation of DOM.

3.2.2. Absorption indices, half-life, and fluorescence indices
A larger reduction in both a254 and SUVA254, and an associated greater increase in SR values was observed during photo-biodegradation relative to biodegradation (Figs. 6 and 7). The difference in intensity of variation in a254, SUVA254, and SR between the photo-bio and biodegradation experiments implies that in the complete absence of sunlight, bacterial action alone cannot completely disintegrate larger reactive terrestrial humic-like or fulvic-like DOM components into smaller labile microbial humic-like components. The higher decreasing trend of a254 and SUVA254 and corresponding higher increase of SR values suggest greater production of low molecular weight, less aromatic DOM
molecules during photo-biodegradation than biodegradation. The $a_{254}$ and SUVA$_{254}$ values in this study indicate that the locations which receive more terrestrial humic input (B, E, R and L) are more reactive and sensitive to sunlight, hence higher reduction of aromaticity was observed in those water bodies than the agricultural pond. The high amount of less aromatic and low molecular weight microbial components in the pond was not able to undergo further photo-biodegradation. The higher increase in SR values during photo-biodegradation was observed for the water bodies which receive more terrestrial input (B 107.8% ± 1.5%; L 65.2% ± 4.5%; E 166.8% ± 1.7% and R 58.6% ± 26%) than the agricultural input (P 45% ± 1.8%). Increases in SR values indicate a decrease in the mean molecular weight of the DOM compounds in the water sample for all locations. Similar results showing the gradual increase of SR values during photo-biodegradation experiments in similar settings (lake, estuarine, and wetland water samples) were reported previously (Helms et al., 2008; Zhang et al., 2013). The SR results obtained from our photo-biodegradation experiments also suggest that the locations receiving the higher amount of terrestrial humic input (R, B, L and E) are undergoing a more substantial reduction in molecular weight of DOM than the agricultural pond samples. While comparing the difference among the forest dominated watersheds, the estuary samples showed the maximum amount of change of absorption index ($a_{254}$) during the photo-biodegradation experiment (Fig. 6). The larger change in estuary samples could be due to the presence of higher amount of reactive terrestrial humic-like or fulvic-like DOM components derived from forest cover in the watershed compared to other regions (Table 1). However, the changes in SR during biodegradation do not follow such a trend. Our data suggest that such low molecular weight DOM compounds present in the agricultural pond are very difficult to disintegrate by photo-biodegradation or by biodegradation and can remain in the water column for a longer period. Similar observations were reported from Lake Kasumigaura in Japan (Kawasaki et al., 2013).

Local variability within the waterbody was observed for the reservoir and the bay samples. Both reservoir samples (R1 and R6) were collected from two different sites and exposed to the same period of sunlight (280 hr) during photo-biodegradation. The R1 sample, which was collected near the spillway on the southwestern side of the reservoir showed a substantial reduction of $a_{254}$ and SUVA$_{254}$ values, and the greatest increase in SR values compared to R6, which was collected from the upstream side of the reservoir. In general, it was noted that higher DOM was concentrated near the spillway compared to other parts of the reservoir. The higher amount of DOM was mainly from the accumulation of organic-rich

Fig. 8 – Temporal variation of humification index (HIX), fluorescence index (FI), and biological index (BIX) during photo-biodegradation (P + B) experiment. The mean and standard deviation were calculated from duplicate measurements of each sample.
suspended sediments near the spillway. Comparable observations were reported in different aquatic settings by Shank et al. (2011) and Singh et al. (2017). Similarly, the differences in the progressive variation of absorbance indices for the bay (both B1 and B2) during both photo-biodegradation and biodegradation could be related to the difference in the initial...

**Table 3** - Rate of degradation and half-life of dissolved organic matter (DOM). Degradation and aromaticity decline were calculated based on the change in DOC concentration and a254 values.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Photo-biodegradation</th>
<th>Biodegradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate (day(^{-1}))</td>
<td>Half-life (days)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate of decline in Aromaticity calculated based on decline in a254</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>0.276 ± 0.00</td>
<td>20.2 ± 0.00</td>
</tr>
<tr>
<td>B2</td>
<td>0.113 ± 0.115</td>
<td>18.84 ± 1.31</td>
</tr>
<tr>
<td>P</td>
<td>0.27 ± 0.0526</td>
<td>39.65 ± 0.30</td>
</tr>
<tr>
<td>L</td>
<td>0.262 ± 0.0305</td>
<td>17.19 ± 1.54</td>
</tr>
<tr>
<td>E</td>
<td>1.71 ± 0.441</td>
<td>13.67 ± 0.71</td>
</tr>
<tr>
<td>R1</td>
<td>0.042 ± 0.0017</td>
<td>14.37 ± 0.18</td>
</tr>
<tr>
<td>R6</td>
<td>0.0192 ± 0.00339</td>
<td>28.77 ± 3.03</td>
</tr>
</tbody>
</table>

Rate of DOC degradation calculated based on decline in DOC concentration

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Rate (day(^{-1}))</th>
<th>Half-life (days)</th>
<th>Rate (day(^{-1}))</th>
<th>Half-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>0.0096 ± 0.00</td>
<td>112.11 ± 0.00</td>
<td>0.0048 ± 0.00</td>
<td>160.33 ± 0.00</td>
</tr>
<tr>
<td>B2</td>
<td>0.0048 ± 0.00339</td>
<td>80.70 ± 40.25</td>
<td>0.0024 ± 0.00339</td>
<td>121.29 ± 11.36</td>
</tr>
<tr>
<td>P</td>
<td>0.000578 ± 0.000817</td>
<td>120.66 ± 25.39</td>
<td>0.00179 ± 0.000602</td>
<td>216.84 ± 16.99</td>
</tr>
<tr>
<td>L</td>
<td>0.0228 ± 0.000509</td>
<td>27.84 ± 7.59</td>
<td>0.006 ± 0.0017</td>
<td>224.67 ± 29.61</td>
</tr>
<tr>
<td>E</td>
<td>0.012 ± 0.00339</td>
<td>63.06 ± 11.40</td>
<td>0.00181 ± 0.000461</td>
<td>396.09 ± 44.61</td>
</tr>
<tr>
<td>R1</td>
<td>0.0204 ± 0.0017</td>
<td>40.92 ± 3.49</td>
<td>0.00181 ± 0.000775</td>
<td>405.75 ± 26.42</td>
</tr>
<tr>
<td>R6</td>
<td>0.0072 ± 0.00</td>
<td>102.39 ± 20.37</td>
<td>0.00115 ± 0.000346</td>
<td>543.09 ± 63.83</td>
</tr>
</tbody>
</table>

Fig. 9 – Temporal variation of humification index (HIX), fluorescence index (FI), and biological index (BIX) during biodegradation (B) experiment. The mean and standard deviation were calculated from duplicate measurements of each sample.
composition of DOM input during the two separate sampling dates (at $t = 0$, FI for B1—1.46 and B2—1.56; a254 for B1—31.68 and B2—28.95).

Our data also suggests statistically significant faster rates and shorter half-lives for both DOC disintegration ($p < 0.04$, $p < 0.0006$) and reduction in aromaticity ($p < 0.02$, $p < 0.0006$) during photo-biodegradation relative to biodegradation (Fig. 10). From the DOC degradation and aromaticity decline data, we observed a shorter half-life for the samples containing DOM derived from forested watersheds (E, R, B, L) than the one having DOM derived from an agricultural land dominated watershed (pond) during photo-biodegradation (Table 3). This again confirms that the DOM derived from forest cover is much more aromatic and photosensitive than the low molecular weight bacterially derived DOM compounds; thus, reduces aromaticity and DOC concentration at a faster rate in the presence of sunlight. The estuary sample showing the highest reduction in aromaticity (outlier) corresponds to the highest forest cover (55.56%) in the watershed (Fig. 10). Additionally, the half-life of all samples increased to many folds during the biodegradation experiments. This again confirms the fact that sunlight is a major factor which influences the disintegration of DOC and lowering the aromaticity of DOM present in the samples.

The low FI values of the initial samples ($t = 0$ hr) of B1 (1.46), B2 (1.56), R1 (1.47), R6 (1.47), E (1.43) and L (1.49) indicate terrestrial plant and soil organic matter as the source of DOM, whereas the high FI value of the initial pond sample (1.63) indicates extracellular release or leachate from bacteria and algae as the DOM source (Figs. 8 and 9). Like the variations in the absorption indices, larger variation of fluorescence indices was also observed during photo-biodegradation relative to biodegradation. The areas receiving more humic terrestrial input (bay, reservoir, estuary and lake) showed the largest decrease in both HIX and FI values and the greatest increase in BIX values, relative to waterbodies having agricultural or microbial DOM components (pond). These decreases in HIX and FI values during photo-biodegradation suggest a greater reduction in the amount of humified organic matter and moderate bacterial activity. The corresponding increase in BIX values during photo-biodegradation indicates the production of fresh or more recently derived organic matter. This means that large aromatic terrestrial organic matter was broken down to smaller molecules in the presence of sunlight to form...
fresh smaller organic matter. This size reduction process can increase the bacterial activity in the system. These results also match with the results obtained from the changes of $a_{254}$, $SUVA_{254}$ and $SR$ during photo-biodegradation. It is also important to note that the changes in $HIX$, $FI$, and $BIX$ during biodegradation were minor compared to the photo-biodegradation experiments (Figs. 8 and 9). However, the greater increase in $HIX$, the smallest increase in $FI$, and the modest increase in $BIX$ values of pond samples compared to the other locations indicate the presence of higher amount of hydrophilic fractions and low bacterial activity as the result of low biodegradation, which is consistent with findings of Kalbitz et al. (2003).

3.2.3. Statistical analysis integrating spatiotemporal variation in DOM

The PCA clearly shows that upon exposures to sunlight, the aromatic, humic-like or fulvic-like terrestrial components ($C_2$ and $C_3$) were transformed into fresh, low-molecular-weight, protein-like components ($C_1$ and $C_4$). The greater spread of the photo-biodegradation data (scores) in the bottom right quadrant of PC space (compared to the biodegradation data in the central and upper left quadrant) indicate greater production of fresh low molecular weight recalcitrant protein-like DOM components and greater reduction of humified materials during exposure to sunlight. On the other hand, biodegraded DOM samples showed aromatic and terrestrial humic composition indicating less alteration during the degradation reactions. Additionally, the agricultural pond (P) samples position along the positive side of the PC1 axis showed the lack of alteration during both experiments (Fig. 11). The gradual spread of photo-biodegradation data of the waterbodies having predominant forest cover from the positive side of the PC2 axis to its negative side indicates a decrease in aromaticity, molecular weight, and humification because of exposure to sunlight. These results all confirm that the DOM present in the water bodies dominated by the forest land cover type in their watershed (estuary, bay, lake and reservoir) were more photolabile than the waterbody (agricultural pond), which was agriculturally dominated.

4. Conclusions

Reactivity of DOM is very much dependent upon bacterial activity as well as sunlight induced decomposition. During this study, we have investigated the difference in reactivity of DOM present in diverse water bodies in the southeastern states of USA, surrounded by forest and woody/herbaceous wetland as well as agriculture and pastoral coverage. The composition of the DOM was evaluated by PARAFAC analysis.
The reactivity of DOM was evaluated by applying decay equations to absorbance data (a254) and DOC concentration. Later, PCA was applied to spectroscopic data, PARAFAC components, DOM concentrations, and exposure times to evaluate the change in DOM composition during photo-biodegradation and biodegradation experiments in different water bodies. Our study clearly shows that DOC degradation and reduction of aromaticity of DOM were faster during photo-biodegradation than during biodegradation; thus, bacterial action alone cannot be very effective in changing the DOM composition. While comparing the different watersheds, humic-like or fulvic like, aromatic, high molecular weight terrestrial DOM (forest and woody/herbaceous wetland source) is much more photo reactive than the fresh low molecular weight, simpler bacterial DOM (agricultural source). Our results suggest that during both photo-biodegradation and biodegradation, the DOM present in the water samples undergoes size reduction processes (increase in SR values and corresponding decrease in the a254 and SUVA254 values), though SR was much less during biodegradation. The size reduction process can generate simpler DOM molecules as well as can change the composition of DOM to be more recalcitrant. The simpler DOM can act as the food source for bacteria as indicated by an increase in BIX and a corresponding decrease in HIX values during both experiments.

DOM source for coastal and inland water bodies of the southern United States are a wide variety of land use and land cover. DOM, being in all surface waters, is an integral part, which controls various water quality parameters like pH, light penetration, and nutrient and toxic metal cycling. DOM chemistry is very sensitive to natural (sunlight) and biological causes (bacterial). Recent climate change reports predict that southern states, (particularly Mississippi, Alabama, and Louisiana) will experience inclement climate conditions in the near future (Hsiang et al., 2017). Thus, results of this study are important to understand the reactivity of DOM in terms of its source (LUIC) and the presence or absence of sunlight. The present study highlights the importance of DOM reactivity based on its origins and also emphasizes the influence of anthropogenic land-use on DOM reactivity. Overall, photo and biodegradation are more efficient mechanism than biodegradation alone in remineralizing and altering DOM in diverse water bodies, implying canopy coverage and light penetration may play a crucial role in the fate and metabolism of aquatic DOM. As such, riparian forest conservation and restoration should consider DOM chemistry and its role in water quality and water-air CO2 fluxes.

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REFERENCES


