Spatiotemporal variations in the abundance and composition of bulk and chromophoric dissolved organic matter in seasonally hypoxia-influenced Green Bay, Lake Michigan USA

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Abstract

Green Bay, Lake Michigan USA, is the largest freshwater estuary in the Laurentian Great Lakes and receives disproportional terrestrial inputs as a result of a high watershed to bay surface area ratio. While seasonal hypoxia and the formation of "dead zones" in Green Bay have received increasing attention, there are no systematic studies on the dynamics of dissolved organic matter (DOM) and its linkage to the development of hypoxia. During summer 2014, bulk dissolved organic carbon (DOC) analysis, UV-vis spectroscopy, and fluorescence excitation-emission matrices (EEMs) coupled with PARAFAC analysis were used to quantify the abundance, composition and source of DOM and their spatiotemporal variations in Green Bay, Lake Michigan. Concentrations of DOC ranged from 202 to 571 μM-C (average = 361±73 μM-C) in June and from 279 to 610 μM-C (average = 349±64 μM-C) in August. In both months, absorption coefficient at 254 nm (a_{254}) was strongly correlated to bulk DOC and was most abundant in the Fox River, attesting a dominant terrestrial input. Non-chromophoric DOC comprised, on average, ~32% of bulk DOC in June with higher terrestrial DOM and ~47% in August with higher aquagenic DOM, indicating that autochthonous and more degraded DOM is of lower optical activity. PARAFAC modeling on EEM data resulted in four major fluorescent DOM components, including two terrestrial humic-like, one aquagenic humic-like, and one protein-like component. Variations in the abundance of DOM components further supported changes in DOM sources. Mixing behavior of DOM components also indicated that while bulk DOM behaved quasi-conservatively, significant compositional changes occurred during transport from the Fox River to the open bay.

Keywords: Dissolved organic matter, CDOM, hypoxia, Green Bay, Lake Michigan

1. Introduction

Freshwater bays and estuaries comprise some of the most biologically productive, yet anthropogenically stressed ecosystems in the Laurentian Great Lakes (Smith et al. 1988; Herdendorf 1990). Terrestrial inputs of nutrients and organic carbon are major components driving estuary biogeochemical cycling and have implications ranging from degraded ecosystem health to food web perturbations (Klump et al. 1997). Southern Green Bay, Lake Michigan USA has historically been classified as a hypereutrophic system as a result of high nutrient and organic carbon loading from the Lower Fox River, which drains some of the most intensely farmed land in Wisconsin (Ankley et al. 1992; Smith et al. 1988). Despite decades of nutrient regulation, Green Bay has continued to experience reeutrophication, recurring bouts of hypoxic conditions, and the formation of dead zones over the past years (Klump et al. 2009; Egen 2014).

Carbon dynamics can be closely related to dissolved oxygen (DO) and the development of hypoxia (Bianchi et al. 2010), as photochemical and heterotrophic oxidation of dissolved organic matter (DOM) consumes DO in both surface and bottom waters (Diaz and Rosenberg 2008; Green et al. 2006). Additionally, DOM plays an important role in the fate and transport of many organic contaminants and heavy metals (McCarthy and Zachara 1989; Santschi et al., 1999). Therefore, knowledge of sources, abundance, chemical composition, and cycling pathways of DOM is important to better understanding of the overall ecosystem health in aquatic environments. There have been several studies on dissolved and particulate organic matter in Green Bay since the 1980s (Eadie et al. 1992; Achman 1993; O'loughlin and Chin 2009). However, the Green Bay ecosystem and Lake Michigan as a whole have experienced significant ecological and environmental change over the last two decades including increasing anthropogenic influence, the introduction of invasive species (Qualls et al. 2007; De Stasio et al.

2014), and increases in the extent of hypoxia (Egen 2014). Baseline data on the abundance, distribution, and composition of DOM in Green Bay are still lacking, but are indispensable for trend analysis and understanding the response of carbon dynamics to climate and environmental changes, especially the formation of hypoxia.

Dissolved organic matter is a heterogeneous mixture of reduced carbon compounds and comprises the largest pool of active organic matter in aquatic environments (Leenheer and Croué 2003; Guo et al. 2003). Aquatic systems receive DOM inputs from allochthonous and autochthonous sources such as riverine discharge and *in situ* production, respectively (Bauer and Bianchi 2011). Chromophoric dissolved organic matter (CDOM) is operationally defined as the fraction of bulk DOM that absorbs light over a broad range of visible and UV wavelengths (Coble 2007; Rochelle-Newall and Fisher 2002; Zhang et al. 2009). As such, CDOM plays many important roles in aquatic systems such as influencing biological production by regulating the penetration of photosynthetically active radiation, protecting organisms from UV radiation, and contributing to the organic carbon cycle due to high photoreactivity (Belzile et al. 2002; Williamson et al. 1999). In addition to providing many important roles in aquatic systems, CDOM is also useful in tracing riverine inputs and biogeochemical cycling of DOM (Helms et al. 2008; Matsuoka et al. 2012). A sub-fraction of CDOM with fluorescent properties (fluorescent-DOM, or FDOM) has received increasing interest in recent years due to its usefulness as a proxy for the bulk DOC pool (Chen et al., 2003; Cory and McKnight, 2011). Fluorescence excitation-emission matrices (EEMs) combined with parallel factor analysis (PARAFAC) have been successfully used to identify allochthonous and autochthonous fluorescent DOM components in a diverse range of natural environments (Stedmon and Markager 2005; Wang et al. 2007; Chari et al. 2012; Xu et al. 2013; Zhou et al. 2016). So far,

there are no systematical studies on bulk DOC and chromophoric DOM in Green Bay using fluorescence EEMs coupled with PARAFAC modeling to track changes in DOM composition across the trophic gradient along the terrestrial-aquatic continuum in Green Bay.

The objectives of this study were to 1) examine the abundance, composition, spatial distribution, and temporal variation of DOC, CDOM, and FDOM in Green Bay; 2) evaluate the relative importance of non-chromophoric DOM and allochthonous and autochthonous DOM sources in different sampling months; 3) identify cycling processes and transformation pathways of DOM in the water column under different thermal stratification conditions and the linkage between DOM dynamics and hypoxic conditions; and 4) establish baseline data for trend analysis and to provide new insights into better understanding the impact of organic carbon loading to the health of Green Bay.

2. *Materials and methods*

2.1. Study Site

Green Bay, located in northwestern Lake Michigan, USA is the largest freshwater estuary in the Laurentian Great Lakes (Fig. 1). The average depth of the bay is 20 m, ranging from ~3 m in the far southern end to 53 m in the north. Extending ~190 km from south to north and ~22 km from west to east, Green Bay has a volume of ~67 km^3. Green Bay's watershed drains about 40,000 km^2, which encompasses almost half of the drainage basin of Lake Michigan and resultantly is highly impacted by surrounding landscapes (Harris and Christie 1987; Klump et al. 1997). Of the 11 rivers and streams that drain into Green Bay, the Fox River is the largest input with a mean discharge of 118 $m^3 s^{-1}$ (Mortimer 1978). The Lower Fox River watershed is 50.2% agriculture, 34.6% urban, 14.7% natural forests and wetlands, and the remaining 0.6% under construction (WDNR, 2012). Additionally, the lower Fox River used to contain the highest

density of pulp and paper mills in the world resulting in large amounts of nutrients and contaminants being flushed into the southern portion of the bay (Harris and Christie 1987), consequently causing high productivity, eutrophic conditions and the formation of dead zones, especially in southern Green Bay (Valenta, 2013; Lin et al., 2016).

2.2. Sampling

Water samples were collected from Green Bay on board the RV Neeskay on the 4th and 5th of June 2014 and on the 24th, 25th, and 26th of August 2014 at 30 and 43 sampling locations, respectively (Fig. 1). Surface samples were taken with a submersible pump and stored in acid washed, triple-rinsed, 2 L HPDE bottles (Nalgene). Samples were kept on ice in the dark until further processing (within 2 days). Hydrographic data, including dissolved oxygen (DO), specific conductivity, pH, chlorophyll-a fluorescence (Chl-*a*) and water temperature, were measured using a CTD (Seabird) and two YSI sondes. Water samples were filtered through pre-combusted 0.7 μm GF/F (Whatman) and stored in pre-combusted (550°C) glass vials for DOC analysis and in acid washed, triple-rinsed, HPDE bottles (Nalgene) for CDOM and EEM measurements. DOC samples were acidified with concentrated HCl to a pH≤2. Both DOC and CDOM samples were stored at 4°C until analysis (within two weeks).

2.3. Measurements of DOC and UV-vis absorbance

Concentrations of DOC were measured with a Shimadzu TOC-L analyzer equipped with an ASI-L autosampler using the high temperature combustion method (Guo and Santschi 1997). Prior to analysis, samples were extensively sparged with zero air for >5 minutes to remove inorganic carbon. Three to five replicate measurements of 150 μL each were made for each sample. Blanks, including water blanks and instrument blanks, were usually <2 μM-C and compensated from the original measurements accordingly. Detection limit was ≤1 μM and

precision was ≤2% in terms of coefficient of variance. Calibration curves were generated before analysis and ultra-pure water, internal standards, and certified DOC standards (University of Miami) were measured every eight samples for quality assurance (Zhou and Guo 2012).

UV-visible absorption spectra were measured with a spectrophotometer (Agilent 8453) using a 1 cm path-length quartz cuvette over a wavelength range of 190-1,100 nm with 1 nm increments. Samples were diluted with ultrapure water to an absorbance value of ≤ 0.02 at 260 nm to minimize inner-filtering effects (Ohno, 2002). Ultrapure water was scanned as a blank before samples analysis daily. The water blank was subtracted and the refractive index effect was corrected by subtracting the averaged absorbance between 650 and 800 nm (Stedmon et al. 2000; Zhou and Guo 2012). Absorption coefficients at a specific wavelength λ ($a_{(\lambda)}$, in m^{-1}) were calculated as $a_{(\lambda)} = 2.303A(\lambda)/L$, where $A(\lambda)$ is the absorbance at wavelength λ (nm) and L is the cuvette path-length (in m). Specific UV absorbance at 254 nm ($SUVA_{254}$) was calculated as A_{254}/DOC concentration resulting in a dimension of m^{-1}/mg-C/L or L/mg-C/m. Spectral slope through linear fit of the logarithm of absorption coefficients over the wavelength interval of 275-295 nm ($S_{275-295}$) was calculated to provide information on DOM molecular weight and the influence of terrigenous DOM inputs (Helms et al. 2008). Spectral slope has been found to negatively correlate with DOM molecular weight, or the higher molecular weight DOM has a lower spectral slope value.

2.4. Measurements of fluorescence EEMs and PARAFAC Modeling

Fluorescence excitation emission matrices (EEMs) were measured using a Horiba Fluoromax-4 spectrofluorometer. Before analysis, samples were diluted with ultra-pure water to an absorbance value of ≤0.02 at 260 nm to minimize inner-filtering effects (Ohno 2002). Each sample was scanned from excitation wavelength 220-480 nm with 5 nm increments and emission

wavelength 240-600 nm with 2 nm increments in a 1 cm path-length quartz cuvette. The bandpass width was 5 nm for both excitation and emission. A water blank was subtracted from each sample and areas affected by Rayleigh and Raman scattering peaks were eliminated by setting data to zero. The fluorescence index (FIX), an indicator of DOM quality, was calculated as the ratio of fluorescence intensities between emission wavelengths 450 and 500 nm at excitation wavelength 370 nm (McKnight et al. 2001). The biological index (BIX), a DOM source indicator, was calculated as the ratio of emission intensity at 380 nm divided by the emission intensity maximum observed between 420 and 435 nm, obtained at excitation wavelength 310 nm (Parlanti et al. 2000). The humification index (HIX), which provides insight into the degree of DOM humification, was calculated as the area under the emission spectra 435-480 nm divided by the peak area under the emission spectra 300-345 + 435-480 nm, at excitation wavelength 254 nm (Ohno 2002).

PARAFAC modeling was done with MATLAB software using the DOMFlour Toolbox (Stedmon and Bro 2008). Sample matrices were calibrated, corrected, and normalized to maximum fluorescence intensity (excluding water scattering peaks) before analysis. A non-negativity outlier test was performed and no samples were identified and subsequently removed as outliers. A split-half analysis was performed for model validation. PARAFAC analysis was also run separately for June and August to examine differences in DOM components between months.

2.5. Statistical and spatial analyses

Statistical analyses (e.g. ANOVA, T-Test, significance) were performed with Sigmaplot software (version 12.5). Contour maps were generated using Surfer 12 (Golden Software) using the kriging method.

3. Results and discussion

3.1. Hydrological features of Green Bay

During June 2014, Green Bay had yet to fully thermally stratify and water temperature ranged from 11.1 to 22.1°C with an average of 15.7±2.7°C. In contrast, by August the bay was fully stratified and the average water temperature was 22.0±0.7°C, which was significantly warmer than June (p <0.0001, Table 1). As a result, DO concentrations were highly variable between the two sampling months. In June, DO was regulated predominantly by temperature in both surface and subsurface water, as indicated by a significant negative correlation (r^2 = 0.7532; p <0.0001, Table 1). Conversely, a much less significant, positive relationship existed between water temperature and DO in August (r^2 = 0.0829; p = 0.0314, Table 1). Further, bottom water DO level was as low as 2.6 mg L^{-1} and an apparent hypoxic zone had developed in the colder bottom waters of the bay (Fig. 2), accompanied by an increase in phosphate concentration in deeper waters in central Green Bay (Lin et al., 2016). This is likely related to increased sediment oxygen demand and reduced mixing of aerated surface water resulting from thermal stratification of the water column as well as the degradation of DOM in the water column (Klump et al. 2009; also see discussion below). In contrast, bottom water DO in June averaged 12.6±1.5 mg L^{-1}, and the lowest concentration was 8.6 mg L^{-1}, which is higher than criteria for hypoxia.

Surface distributions of specific conductivity (Figs 3a and 3e), which can be used as a tracer of river water inputs due to its conservative mixing behavior, highlight significant differences in hydrology between June and August (Modlin and Beeton, 1970). Surface distributions of specific

conductivity in June were consistent with previous observations indicating the presence of the Fox River plume (Lathrop et al. 1990). The Fox River discharges into Green Bay at the southernmost end and is the predominant river input into the bay (Mortimer 1978). The river flow deflects east due to the Coriolis force as it moves north and mixes with open bay water. Additionally, the combination of the Coriolis force and prevailing winds have also been shown to induce a compensating southward flow of open Lake Michigan water along the west coast of the bay, which was evident due to a strong specific conductivity gradient along both the north-south and east-west axes of the bay (Martin et al. 1995). Conversely, specific conductivity was elevated along the western coast of the bay in August, suggesting temporal variations in Green Bay's surface hydrology.

Although Chl-*a* concentrations were not significantly different between the two sampling months ($p = 0.261$), spatial distributions were somewhat variant (Figs. 3d and 3h). While Chl-*a* was highest at the southernmost sample stations for both months, Chl-*a* was elevated west of Sturgeon Bay in June, but was highly concentrated in the southeastern portion of the bay in August in the same region as the surface water DO minimum (Fig. 3e), suggesting DO consumption from organic matter degradation. However, no such trend was observed in June (Fig. 3b).

3.2. DOC abundance and distribution

DOC concentrations ranged from 202 to 561 μM-C with an average of 361±73 μM-C in June, and from 279 to 610 μM-C with an average of 349±64 μM-C in August. No significant difference of DOC abundance between June and August was observed ($p = 0.343$) (Table 2). Throughout the summer, the highest DOC concentrations were observed in the lower Fox River (546 - 610 μM) and the lowest concentrations were found at the northernmost sampling stations

influenced with waters from Lake Michigan (Figs. 4a and 4e). Although DOC concentration was slightly higher in the Fox River in August than June (610 vs. 546 μM), potentially due to dilution by high river discharge in June, DOC concentrations were elevated farther north in the bay in June than August, likely also a result of ~2.5 times higher Fox River discharge in June than August (251 m^3 sec^{-1} vs. 92 m^3 sec^{-1}; Data from USGS gauging station 040851385). This highlights the importance of river inputs on temporal DOC dynamics in Green Bay. Also apparent in Green Bay were low DOC concentrations along the northwestern coast of the bay, concurrent with low specific conductivity suggesting the influence of open Lake Michigan water, which is significantly lower in DOC (average of 154 μM-C in June and July, 2013; Zhou et al., 2013). This supports previous observations and indicates the importance of the surface water hydrology and water mixing with open Lake Michigan water on DOC dynamics in Green Bay (Martin et al. 1995; Mortimer 1978; Modlin and Beeton, 1970).

3.3 Variations in CDOM characteristics

The absorption coefficient at 254 nm (a_{254}) ranged from 12.4 to 58.5 m^{-1} (average = 27.9±9.3 m^{-1}) in June and from 16.8 to 59.8 m^{-1} (average = 24.7±8.1 m^{-1}) in August. Values of a_{254} were significantly greater in June than those in August (p = 0.028), showing higher chromophoric DOM components early in the summer. Significant correlation was observed between a_{254} and DOC, both in June (r^2 = 0.9408; p <0.0001) and August (r^2 = 0.8968; p <0.0001), indicting similar chromophoric DOM sources in Green Bay throughout the summer. In addition, spatial distributions of a_{254} resembled those of bulk DOC in both months (Figs. 4b and 4f). The correlation coefficient between a_{254} and DOC was slightly higher in June than August, further attesting to the dominance of riverine inputs to DOC dynamics in June and suggesting alternative DOM sources, for example, from *in situ* production in August (Table 2).

240 Throughout the summer, the Fox River was a source of highly aromatic DOM, as indicated
241 by elevated $SUVA_{254}$ values ranging from 2.22 to 3.77 L mg-C^{-1} m^{-1} (average = 3.01±0.0.29 L
242 mg-C^{-1} m^{-1}) in June, and from 2.02 to 3.55 L mg-C^{-1} m^{-1} (average = 2.58±0.34 L mg-C^{-1} m^{-1}) in
243 August. Higher $SUVA_{254}$ values were found in June possibly associated with a higher influx of
244 allochthonous DOM from terrestrial and/or anthropogenic sources. A distinct south to north
245 gradient was also apparent in both months (Figs. 4c and 4g) suggesting rapid mixing of river and
246 bay waters.
247 Bulk DOM molecular weight in southern Green Bay in August appeared to be affected by
248 processes other than river-bay mixing, as no distinct south-to-north gradient was observed for
249 $S_{275\text{-}295}$ as was seen in June (Figs 4d and 4h, respectively). Elevated $S_{275\text{-}295}$ values in August
250 were located in the same region where Chl-*a* was high and DO was lowest suggesting DO
251 consumption from organic matter degradation during an algal bloom decline (Figs 3h and 3e,
252 respectively). Given that DOC concentration does not follow this trend, it is likely that biological
253 activity plays a more important role in altering the composition, and therefore DOM molecular
254 weight in southeastern Green Bay later in the summer (Moran et al., 2000; Amon and Benner,
255 1996). In both sampling months, however, $S_{275\text{-}295}$ showed irregular distributions, possibly as a
256 result of spatial heterogeneity and *in situ* processes such as production and microbial and
257 photochemical degradation that were non-uniformly affecting DOM molecular weight (Miller
258 and Moran 1997). Values of $S_{275\text{-}295}$ ranged from 0.0173 to 0.0222 nm^{-1} (average =
259 0.0197±0.0012 nm^{-1}) in June and from 0.0202 to 0.0292 nm^{-1} (average = 0.0233±0.002 nm^{-1}) in
260 August. Higher $S_{275\text{-}295}$ values in August indicate an overall lower molecular weight DOM pool.
261 This could result from a variety of factors including decreased terrestrial DOM inputs, increased
262 photodegradation, and additional *in situ* DOM processing. In both months, lower $S_{275\text{-}295}$ values

263 indicate the Fox River was also a source of higher molecular weight (HMW) DOM (Fig. 4d). On
264 the other hand, it seems that both autochthonous and degraded DOM gave rise to an overall
265 higher spectral slope value and thus lower molecular weight DOM pool.

266 Although DOC concentrations were not significantly different between June and August, all
267 derived optical properties, including a_{254}, $SUVA_{254}$, and $S_{275\text{-}295}$, showed great differences. This
268 indicates that, while the abundance or quantity of DOM did not vary significantly, its quality and
269 composition did change over the summer. In general, the bulk DOC pool was less
270 chromophoric, less aromatic, and lower in molecular weight in August than in June. Non-
271 chromophoric DOC comprised, on average, ~33% of bulk DOC in June while it comprised up to
272 47% in August. Lower non-chromophoric DOC abundance in the early summer is consistent
273 with higher riverine or terrestrial DOM inputs which in general have a higher aromaticity
274 compared to open lake waters (Table 2) and higher average $SUVA_{254}$ values in June compared to
275 August (3.01±0.29 L mg-C^{-1} m^{-1} vs. 2.59±0.34 L mg-C^{-1} m^{-1}). This is in agreement with higher
276 a_{254} values in June and may be potentially attributed to increased photochemical degradation and
277 *in situ* DOM sources as indicated by higher Chl-*a* abundance in August than in June (2.96 ± 1.40
278 μg L^{-1} vs. 2.38±0.89 μg L^{-1}, and Table 1).

279 **3.4. Sources of dissolved organic matter**

280 By using specific conductivity as a mixing index between Fox River and bay waters and
281 Chl-*a* as a proxy for primary production, it was possible to infer the relative importance of each
282 source on DOC dynamics throughout the summer. In June, DOC was more significantly
283 correlated to specific conductivity ($r^2 = 0.6443$, $p < 0.0001$) than Chl-*a* ($r^2 = 0.3030$, $p = 0.0097$).
284 In contrast, DOC in August was more significantly correlated to Chl-*a* ($r^2 = 0.4098$, $p < 0.0001$)
285 than specific conductivity ($r^2 = 0.1260$, $p = 0.0246$), indicating a shift from predominant

terrestrial DOM sources in June to more *in situ* DOM sources and/or photochemically degraded and microbially modified DOM in August.

There were, however, two distinct relationships between specific conductivity and bulk DOC in August (Fig. 5a). A highly linear trend (r^2 = 0.6443, p <0.0001) existed and spatially corresponded to what has previously been identified as the Fox River plume along the eastern coast of the bay (Fig. 5b). A separate cluster with no significant linear relationship (r^2 = 0.1313, p = 0.2237) also existed and was spatially located along the western coast of the bay as well as near Sturgeon Bay. Given that specific conductivity is elevated along the western coast of the bay, one possible explanation is that river discharge along the western shore more strongly influenced DOM dynamics in August. For example, in June the Oconto, Peshtigo, and Menominee rivers along Green Bay's west shore collectively comprised ~58% of Fox River discharge. However, in August the same rivers comprised upwards of 80% of Fox River discharge (Data from USGS gauging stations). Therefore, not only was primary production more influential in August, discharge from rivers other than the Fox River also played a significant role in regulating DOM dynamics in August.

3.5. Variations in excitation-emission matrix characteristics and fluorescence indices

Fluorescence EEMs in Green Bay waters resembled typical spectra for natural DOM samples with distinct terrestrial humic-like peaks A and C and protein-like peaks T and B (Coble 2007) (Fig. 6). In June, peak A was significantly more abundant than August (p = 0.049) while peak T was significantly less abundant (p = 0.0161), indicating the increased importance of *in situ* produced DOM as summer progresses, which is in agreement with increased Chl-*a* concentrations in August (Fig. 6). However, peaks C and B were not significantly different month to month suggesting the moieties producing these peaks did not vary temporally (Table

309 3). Further, in June both terrestrial and *in situ* DOM peaks were most abundant in the Fox River.
310 In August only terrestrial humic-like peaks were most abundant in the Fox River, suggesting a
311 temporal change in riverine DOM composition. Protein-like peaks were most abundant at station
312 GB6E, located in the southeastern portion of the bay in close proximity to the Chl-*a* maximum.

313 Indices derived from fluorescence EEMs all indicate that although DOM from the Fox River
314 predominated in the bay, as the summer progressed freshly produced DOM became more
315 dominant in the bulk DOM pool. The HIX, an indicator of the degree of DOM humification
316 (Ohno 2002), was significantly higher in June (Tables 3 and 4) suggesting that terrestrial DOM
317 was more abundant early in the summer before hypoxia development, which is in agreement
318 with higher a_{254} and discharge from the Fox River discussed above. The BIX, which indicates the
319 contribution of autochthonous or freshly produced DOM, was significantly higher in August
320 (Tables 3 and 4) indicating increased importance of *in situ* produced DOM as the summer
321 progressed and hypoxia began to develop. The FIX is also an indicator of DOM source, with
322 higher values (~1.8) indicating DOM from the extracellular release and leachate from bacteria
323 and algae and lower values (~1.2) related to terrestrial DOM sources (McKnight et al. 2001). In
324 both months, the average FIX value was low (Tables 3 and 4) indicating a dominant terrestrial
325 source in Green Bay regardless of month. Yet, the FIX was still significantly higher in August
326 than June (p <0.0001) suggesting that although terrestrial DOM was predominant, autochthonous
327 DOM was more influential to the bulk DOM pool in August and may affect DO dynamics more
328 than allochthonous DOM.

329 In comparison, the contribution of freshly produced DOM in Green Bay was lower than
330 those reported for the Yangtze River Estuary, another anthropogenically stressed system, but
331 DOM humification was greater (Sun et al., 2014 and Table 4). In relation to the rest of the

Laurentian Great Lakes, DOM humification in Green Bay was consistently higher than open Lakes Superior, Lake Michigan, and Lake Huron (Zhou et al., 2016 and Table 4). However, it was less than those in the lower Great Lakes, including Lake Erie and Lake Ontario in August (Table 4). On the other hand, freshly produced DOM in Green Bay, indicated by BIX values, was consistently lower than the Great Lakes as a whole (Table 4). Average FIX values were also lower in Green Bay than all other Great Lakes (Table 4). Overall, DOM in Green Bay seemed to contain more degraded components than open lake waters where *in situ* production could be more dominant.

3.6. Variations in fluorescent DOM components derived from PARAFAC analysis

Four PARAFAC-derived fluorescent-DOM components were identified each sampling month, with slight differences in Ex/Em wavelength (Table 5 and Fig. 7). It should be noted that, because PARAFAC was run separately for June and August, the assignment of components was not the same. In both sampling months, C1 and C2 were identified as terrestrial humic-like and aquagenic humic-like, respectively (Guéguen et al. 2011; Coble 2007; Stedmon and Markager 2005; Yamashita et al. 2008; Cory and McKnight 2005). In June, C3 was identified as terrestrial-humic like while C4 was identified as protein-like. However, in August, the protein-like component was identified as C3 while C4 was identified as terrestrial humic-like (C3 and C4 are reversed in June and August) (Yamashita et al. 2008; Cory and McKnight 2005). These differences are outlined in Table 5, and Fig. 7 represents the average PARAFAC components for both months, which is why C3 and C4 in June and August are reversed in Table 5.

Humic like DOM was more abundant early in the summer while protein –like DOM became more dominant in August indicating a shift from a predominant terrestrial source in June to a more biologically controlled system in August. In June, both terrestrial humic-like DOM

components were highly correlated with each other (r^2 = 964, p <0.0001) (Figs. 8a and 8c) and with bulk DOC (r^2 = 0.772, p <0.0001 and r^2 = 0.764, p <0.0001) suggesting their source was from Fox River discharge. Elevated abundance of aquagenic humic-like DOM north of Sturgeon Bay along the eastern shore suggests the presence of an alternative source other than the Fox River (Fig. 8b). Conversely, the aquagenic humic-like component was not elevated north of sturgeon bay in August, suggesting temporal variations in its source and composition (Figs. 8e, 8f, and 8h). Differences in the spatial distribution of protein-like DOM were evident between June and August (Figs. 8d and 8g) and may be linked to nutrient availability regulated by Fox River discharge. When Fox River discharge was high early in the summer and the plume migrated farther north, protein-like DOM was abundant farther north. Later in the summer when discharge decreased, protein-like DOM was most abundant in the southern bay in close proximity to the Chl-*a* maxima attesting its biological origin.

3.7. Behavior of DOM during river-bay water mixing

Previous studies have reported rapid mixing of Fox River water with open Green Bay water in the southern portion of the bay (Klump et al. 2009). As a result, high sedimentation rates occur in the southern bay, removing riverine constituents from the water column at an elevated rate (Klump et al. 1997). Similar patterns were observed in Green Bay in June and August, where two distinct mixing zones were evident (Fig. 9). In June, DOC decreased 27% from ~545 to ~400 μM-C within zone I, which extended 29 km from the Fox River mouth (Fig. 9a). In August, however, DOC decreased 47% from ~610 to ~325 μM-C within zone I which only extended 19 km from the Fox River mouth, likely as a result of lower discharge indicating temporal variations in mixing behavior (Fig. 9c), stemming from reduced river discharge as summer progresses. In both months, DOC concentration remained fairly constant in zone II.

However, greater fluctuation was seen in zone II in June indicating a highly dynamic system while DOC appeared more conservative in zone II in August.

Chromophoric DOM (a_{254}) displayed similar trends with DOC during the summer with two distinct mixing zones, but decreases in zone I were much greater. In June, a_{254} decreased 47% from ~57 m^{-1} to ~30 m^{-1} in zone I (Fig. 9b). In August, a_{254} decreased almost 75% from ~60 m^{-1} to ~19 m^{-1} in Zone I suggesting a highly dynamic and reactive nature of CDOM during early mixing of riverine and bay waters (Fig. 9d). Similar to DOC, a_{254} values remained fairly constant in zone II in both months with higher variations in CDOM abundance in the early summer.

Mixing plots based on the relationship between DOC or a_{254} and specific conductivity were used to estimate the apparent removal/addition of DOM and CDOM in Green Bay (Loder and Reichard 1981). The hydrological cycle of Green Bay is complex and is influenced by multiple riverine sources, mixing with Lake Michigan waters, and variable wind patterns (Mortimer 1978; Martin et al. 1995; Waples and Klump 2002). As a result, simple two end-member mixing of constituents may be difficult to model. With an average addition of less than 10%, bulk DOM and a_{254} mixing plots suggest a conservative mixing behavior of DOM although there were evident DOM additions at most stations in June, likely due to light shielding by high CDOM and thus lower photodegradation and *in situ* production (Figs. 10a and 10b). In August, DOM as a whole appeared less conservative than in June with a removal of ~13%. However, when sample stations were divided into the Fox River plume and the western coast as determined by specific conductivity in Fig. 5, a quasi-conservative mixing behavior of bulk DOC and CDOM was observed with both addition and apparent removal at some individual stations (Figs. 10c and 10d). In August, CDOM removal appears to be much higher than DOC in both the Fox River plume and along the western coast indicating again a higher reactivity of optically active DOM

components, which is further buttressed by higher non-chromophoric DOC relative to June. However, when an averaged endpoint which was more representative of other open Green Bay stations was used to generate the theoretical mixing line, CDOM appeared highly conservative (Fig. 10).

In June, $SUVA_{254}$ values decreased sharply while $S_{275\text{-}295}$ increased sharply with distance from the Fox River mouth indicating a rapid decrease in both aromaticity and mean molecular weight of the bulk DOM pool (Figs. 4c and 4d). While $SUVA_{254}$ showed a similar trend in August, no such trend was observed for $S_{275\text{-}295}$ indicating other controlling factors besides mixing/dilution were affecting DOM molecular weight such as biological and photochemical degradation of DOM (Figs. 4g and 4h). Regardless, DOM composition appears to be altered the most significantly during early mixing in the southern portion of the bay.

The mixing behavior of individual DOM components relative to specific conductivity also suggests a highly dynamic nature of the DOM pool. In June, the terrestrial humic-like DOM components behaved conservatively with an average removal of only 7%, the aquagenic humic-like component displayed an average removal of 17%, while the protein-like component displayed an addition of 15%. Bulk FDOM, however, behaved conservatively with a calculated removal of <4% between all four components. This, in conjunction with the mixing behavior of DOC, suggests that although bulk DOC appeared to behave conservatively, DOM composition was significantly altered with different extents highlighting the dynamic nature of DOM mixing and cycling in Green Bay in June. In August, all humic-like DOM components underwent significant removal with an average removal of 24%, consistent with higher CDOM removal and higher abundance of non-chromophoric DOC. Contrary to June, the protein-like component C3

423 behaved conservatively with addition of only 5% suggesting the rate of microbial degradation
424 was greater than the rate of *in situ* production of DOM.

425 The conservative nature of terrestrial humic-like components in June may be explained by
426 their refractory nature. The percent contribution of both C1 and C3 increased in the southern bay
427 with distance from the Fox River (Figs. 11a and 11c). Because the Fox River appears to be their
428 source, this observation can potentially be explained by the removal of other DOM components
429 relative to C1 and C3. Further, C1 and C3 normalized to DOC concentration (C1/DOC and
430 C3/DOC, respectively) both remained relatively constant until as far north as Sturgeon Bay
431 (Figs. 12a and 12c). This suggests that although DOC concentration decreased rapidly with
432 distance from the Fox River, the relative abundance of C1 and C3 did not, further buttressing
433 their similar source and refractory nature (Ishii and Boyer, 2012; Diffey, 2002). No such trend
434 was observed for the other DOM components (Figs. 11 and 12).

435 ## *4. Conclusions*

436 Dissolved organic carbon dynamics in Green Bay displayed both spatial and temporal
437 variability. Although bulk DOC concentration was not significantly different between June and
438 August, optical characteristics (i.e. a_{254}, $SUVA_{254}$, and $S_{275\text{-}295}$) were, indicating the chemical
439 composition or quality of DOM was different. A significant difference in the percent of non-
440 chromophoric DOC (33% in June and 47% in August) further supports this observation and
441 indicates a less aromatic and lower molecular weight DOM pool in August. Four similar
442 fluorescent DOM components were identified with PARAFAC analysis in June and August: two
443 terrestrial humic-like, one aquagenic humic-like, and one protein-like DOM. While Green Bay
444 was dominated by allochthonous DOM, *in situ* production was more influential in late summer
445 with more protein-like DOM presence, resulting in changes in chemical composition of the bulk

446 DOM pool. Further, *in situ* production may have influenced dissolved oxygen dynamics and
447 hypoxia development in the southern portion of the bay as a result of heterotrophic oxidation of
448 DOM in the water column. The humification index was significantly higher in June while the
449 biological and fluorescence indices were significantly higher in August. This indicates that the
450 bulk DOM pool was more humified in June and comprised of fresher DOM in August,
451 concurrent with higher concentration of Chl-*a* in August.

452 Hydrology was also a significant factor influencing both the spatial and temporal variation
453 of DOM. The dominant circulation in the bay (Hamidi et al. 2015) significantly influenced the
454 spatial distribution of DOM, deflecting DOC-rich Fox River water along the eastern coast with
455 DOC-poor open Lake Michigan waters being transported south along the western coast of the
456 bay. While bulk DOM was generally conservative during estuarine mixing, August displayed
457 unique mixing behaviors between the southern portion of the bay and the western coast,
458 influenced by a significant increase in the discharge of small rivers along the western coast
459 relative to the Fox River. Specifically, each fluorescent DOM component displayed two separate,
460 linear relationships with specific conductivity indicating the increased complexity of DOM
461 sources and sinks in August relative to June. DOM dynamics in Green Bay have unique temporal
462 variation controlled by both allochthonous organic matter loading as well as *in situ* production,
463 and are linked to riverine discharge, biological activity, hydrological condition, and dissolved
464 oxygen dynamics in the bay.

465

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Figure Captions

Fig. 1. A map of the study area and sample locations in Green Bay, Lake Michigan in June and August 2014.

Fig. 2. Distributions of bottom water dissolved oxygen (DO, mg L^{-1}) in June (a) and August 2014 (b) highlighting the hypoxic zone in Green Bay that developed in August but was not present in June

Fig. 3. Spatial distributions of dissolved oxygen (DO, mg L^{-1}), specific conductivity (μS cm^{-1}), surface water temperature (°C), and chlorophyll-*a* (Chl-*a*, μg L^{-1}) in Green Bay during June (upper panels, a-d) and August (lower panels, e-h) 2014.

Fig. 4. Spatial distributions of dissolved organic carbon (DOC, μM-C), absorption coefficient at 254 nm (a_{254}, m^{-1}), Specific UV absorbance at 254 nm ($SUVA_{254}$, L mg-C^{-1} m^{-1}), and spectral slope between 275 and 295 nm ($S_{275-295}$, nm^{-1}) in Green Bay during June (upper panels) and August (lower panels) 2014.

Fig 5. Linear regression between dissolved organic carbon (DOC) and specific conductivity in August (a). White circles represent sample locations in the Fox River plume while red circles represent sample locations along the western coast and near Sturgeon Bay and are spatially depicted in (b). Fox River Plume – y=2.0956x-281.1078, r^2=0.6443, p<0.0001. Western Coast – y=1.6178x-223.6927, r^2=0.1313, p=0.2237.

Fig. 6. Examples of excitation-emission matrices (EEMs) from a) the Fox River mouth, b) the Chl-*a* maxima, c) bottom water (30 m) at Station GB-48, and d) surface water (1 m) at Station GB-17.

Fig. 7. Excitation-emission plots of fluorescent-DOM components identified by PARAFAC analysis in both June and August (See Table 5 for specific Ex/Em data of each component). In June, components 1 and 3 were terrestrial humic-like, component 2 was aquagenic humic-

like, and component 4 was protein-like. In August, components 1 and 4 were terrestrial humic-like, component 2 was aquagenic humic-like, and component 3 was protein-like.

Fig. 8. Spatial distributions of PARAFAC-derived fluorescent-DOM components (in ppb-QSE) in June (a-d) and August 2014 (e-h). A more detailed description of each component can be found from Table 5.

Fig. 9. Relationship between distance from the Fox River mouth and DOC concentration in June (a), distance from the Fox River mouth and a_{254} in June (b), distance from the Fox River mouth and DOC concentration in August (c), and distance from the Fox River mouth and a_{254} in August (d) in Green Bay showing two distinct mixing zones. Black ovals represent Zone 1 and red ovals represent Zone 2.

Fig. 10. Relationship between dissolved organic carbon (DOC) concentration (μM-C) and specific conductivity (μS cm^{-1}) in Green Bay in June (a), absorption coefficient at 254 nm (a_{254}, m^{-1}) and specific conductivity in June (b) DOC and specific conductivity in August (c), and a_{254} and specific conductivity in August (d). F.R. = Fox River, W.C. = west coast. Mixing lines were derived from the equation of line between the points of lowest and highest specific conductivity. In August, the station with the lowest specific conductivity had higher than usual concentrations of DOC, so an averaged endpoint was also plotted for comparison.

Fig. 11. Spatial distributions of the percent contribution of each fluorescent-DOM component to bulk fluorescent-DOM (FDOM) in Green Bay during June (a-d) and August (e-h).

Fig. 12. Spatial distributions of PARAFAC-derived fluorescent-DOM components normalized to DOC concentration (C_i/DOC, ppb-QSE/μM-C) in Green Bay during June (a-d) and August (e-h).

Table 1. Sampling locations and their hydrographic parameters, including water depth, water temperature (Temp), specific conductivity (Sp. Cond), dissolved oxygen (DO), and chlorophyll-a (Chl-*a*) in surface waters of Green Bay during June and August 2014.

Station ID	Latitude (N)	Longitude (W)	Temp (°C)	Sp. Cond ($\mu S\ cm^{-1}$)	DO ($mg\ L^{-1}$)	Chl-*a* ($\mu g\ L^{-1}$)	Water Depth (m)
June 2014							
DePere Dam	-	-	-	-	-	-	6.7
Fox Mouth	44°32'15.72''	88°00'08.28''	22.1	410	7.25	-	7.8
GB-6	44°39'26.81''	87°53'00.74''	18.3	330	11.04	5.4	5.9
GB-12 (Sta-F)	44°45'28.20''	87°45'03.00''	18.8	327	10.11	3.3	9.9
Sta-B	44°47'15.55''	87°47'32.49''	16.9	302	10.57	2.2	11.6
GB-38 B	44°57'58.21''	87°23'48.83''	18.9	305	-	-	19.4
GB-17	44°47'37.11''	87°45'36.74''	16.5	295	10.56	2.0	13.2
GB-42	45°01'14.83''	87°22'58.99''	18.7	301	11.23	3.1	30.7
GB-20 (Sta-G)	44°50'26.64''	87°41'39.00''	17.9	298	10.14	3.2	15.3
Sta-D	44°40'18.25''	87°52'40.04''	17.6	309	10.35	1.9	6.5
GB-25 (Sta-H)	44°54'25.66''	87°32'04.37''	18.3	308	10.57	4.4	23.0
GB-30	44°55'39.98''	87°26'40.98''	17.3	298	10.72	3.7	18.0
Sta-C	44°43'42.95''	87°51'13.39''	16.9	324	10.35	-	8.8
Sta-A	44°49'48.72''	87°40'51.95''	18.1	307	10.02	1.9	14.0
GB-9 (Sta-E)	44°42'22.03''	87°49'11.65''	15.9	298	10.56	2.4	8.1
GB-26	44°53'01.22''	87°37'57.88''	14.0	293	11.32	2.1	21.4
Condos	44°56'30.28''	87°50'09.26''	12.1	300	11.14	1.2	10.2
GB-SB	44°53'42.32''	87°24'50.33''	17.3	309	-	-	13.0
GB-CI	45°10'01.60''	87°16'56.14''	14.9	300	11.07	-	18.6
GB-39	44°58'39.76''	87°30'40.16''	12.4	295	12.07	-	27.5
GB-47	45°04'00.24''	87°23'00.26''	13.8	296	12.45	3.5	29.7
GB-44 (Sta-I)	45°01'32.44''	87°28'41.06''	12.7	287	12.00	2.5	32.8
GB-64 B	45°14'21.47''	87°19'26.94''	14.6	295	11.38	-	11.3
GB-52	45°06'37.96''	87°23'02.65''	13.2	286	11.70	1.8	25.1
GB-59 (Sta-J)	45°10'17.77''	87°26'48.71''	14.0	290	10.56	1.3	29.9
GB-67	45°11'58.05''	87°26'50.67''	13.7	296	11.81	1.7	29.3
GB-48	45°04'16.12''	87°26'47.12''	12.2	281	12.40	2.3	31.2
GB-64B (Sta K)	45°12'00.91''	87°15'27.76''	14.7	-	-	-	23.8
GB-53	45°06'39.47''	87°26'42.43''	12.3	284	12.31	1.1	30.0
GB-73	45°14'38.36''	87°23'02.61	11.1	289	13.60	1.9	31.2
Average			15.7	304	11.0	2.5	18.8
±Stdev			±2.72	±24	±1.2	±1.1	±9.1
August 2014							
Fox River	44°32'15.72''	88°00'08.28''	-	-	-	-	-
GB-6E	44°39'27.53''	87°49'49.44''	22.1	318	10.8	3.9	4.6
GB-SC	44°34'29.03''	87°59'06.75''	23.4	361	14.2	5.6	7.6
GB-2E	44°36'14.58''	87°56'05.11''	22.8	332	5.6	6.1	4.3
GB-2S	44°36'10.51''	87°56'49.88''	22.2	340	11.4	3.7	5.0
GB-2W	44°35'57.63''	87°57'56.29''	-	-	-	-	3.3
GB-5	44°39'27.53''	87°49'49.44''	22.6	311	7.1	5.3	6.2
GB-8	44°42'25.68''	87°45'28.09''	21.9	322	10.4	3.2	7.3
GB-1B	44°38'31.81''	87°53'17.70''	22.9	316	6.5	6.6	5.3
GB-9	44°42'19.91''	87°49'05.62''	21.9	315	12.1	3.2	8.2
GB-10B	44°41'32.09''	87°50'20.96''	22.0	312	11.2	3.7	7.5
GB-16B	44°47'44.72''	87°41'44.87''	21.7	312	11.1	2.8	11.2

GB-13	44°45’02.52’’	87°48’28.75’’	21.7	314	11.0	3.4	-
MR-Mouth	45°05’15.34’’	87°32’49.95’’	22.1	336	9.3	1.2	17.2
Condos	44°46’28.25’’	87°50’09.79’’	21.9	308	12.8	2.4	10.1
GB-18	44°47’40.25’’	87°49’14.56’’	22.4	340	9.6	2.7	11.2
GB-30B	44°54’59.19’’	87°27’28.51’’	20.7	-	9.1	-	-
GB-12	44°44’58.52’’	87°45’49.63’’	21.6	309	11.5	3.4	9.6
GB-18B	44°48’26.76’’	87°48’06.56’’	22.3	340	10.0	5.4	11.1
GB-7	44°39’30.29’’	87°56’46.46’’	23.4	342	9.3	2.8	4.6
GB-17	44°47’40.13’’	87°45’46.16’’	21.5	308	11.6	2.2	12.2
GB-11	44°42’11.06’’	87°56’15.27’’	23.3	339	9.3	3.3	-
GB-25	44°52’30.46’’	87°33’35.36’’	20.8	296	9.3	3.1	-
GB-20	44°50’50.68’’	87°37’51.79’’	21.3	300	9.4	2.7	14.4
GB-31	44°55’17.99’’	87°30’23.36’’	21.0	339	9.5	2.5	23.7
GB-14B	44°45’25.88’’	87°52’44.90’’	22.7	340	9.6	4.0	6.1
GB-19	44°47’24.49’’	87°52’15.44’’	23.0	330	9.6	4.0	6.1
GB-32	44°55’39.63’’	87°34’14.43’’	21.1	304	10.8	1.7	-
GB-26S	44°49’24.32’’	87°40’14.24’’	21.0	305	11.3	2.4	13.2
GB-45	45°01’21.51’’	87°34’20.81’’	21.7	337	8.7	1.5	14.8
GB-22	44°50’18.35’’	87°45’33.47’’	22.0	339	9.7	3.8	13.3
GB-39	44°58’30.38’’	87°30’27.45’’	21.3	301	11.8	1.6	27.4
GB-44	45°01’12.50’’	87°30’29.90’’	21.5	300	11.4	1.5	28.0
GB-48	45°04’02.23’’	87°26’38.18’’	22.0	335	9.5	1.8	30.7
GB-48W	45°06’14.15’’	87°31’12.02’’	22.2	318	9.4	1.4	21.0
GB-30E	44°52’58.85’’	87°24’52.17’’	22.3	339	10.5	3.8	7.1
GB-30	44°55’37.91’’	87°26’45.72’’	21.4	301	13.4	2.5	17.9
GB-39-Pump	44°58’30.39’’	87°30’27.46’’	21.3	301	11.8	1.6	27.4
GB-38	44°58’28.02’’	87°26’43.57’’	23.3	318	7.3	1.3	27.4
GB-54	45°06’40.74’’	87°30’18.73’’	22.0	334	9.5	1.7	22.5
GB-43	45°01’14.88’’	87°26’51.71’’	21.2	299	11.6	1.5	31.2
GB-47	45°04’00.81’’	87°22’59.11’’	21.3	298	11.8	1.5	29.4
GB-42	45°01’09.16’’	87°23’09.90’’	21.6	300	11.7	1.6	30.7
Average			22.0	320	10.3	3.0	14.6
±Stdev			±0.7	±17	±1.7	±1.4	±9.2

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Table 2. Dissolved organic carbon (DOC) and optical properties of dissolved organic matter, including absorption coefficient at 254 nm (a_{25}), specific UV absorbance at 254 nm ($SUVA_{254}$), and spectral slope at 275-295 nm ($S_{275-295}$) in Green Bay in June and August 2014.

Station ID	DOC (μM-C)	a_{254} (m^{-1})	$SUVA_{254}$ (L mg-C^{-1} m^{-1})	$S_{275-295}$ (nm^{-1})
		June 2014		
DePere Dam	561	58.4	3.77	0.0173
Fox Mouth	546	56.5	3.74	0.0173
GB-6	442	35.0	2.86	0.0202
GB-12 (Sta-F)	414	33.4	2.92	0.0213
Sta-B	396	31.7	2.90	0.0194
GB-38 B	396	33.9	3.10	0.0186
GB-17	394	32.6	2.99	0.0201
GB-42	385	34.1	3.21	0.0190
GB-20 (Sta-G)	384	31.7	2.99	0.0203
Sta-D	383	31.1	2.94	0.0199
GB-25 (Sta-H)	378	32.0	3.06	0.0191
GB-30	375	32.3	3.12	0.0190
Sta-C	375	32.7	3.15	0.0192
Sta-A	374	31.1	3.01	0.0197
GB-9 (Sta-E)	374	30.4	2.94	0.0201
GB-26	371	32.1	3.13	0.0193
Condos	360	29.1	2.93	0.0197
GB-SB	354	31.2	3.19	0.0195
GB-CI	354	29.4	3.01	0.0203
GB-39	336	27.7	2.98	0.0194
GB-47	321	25.6	2.88	0.0216
GB-44 (Sta-I)	320	27.2	3.07	0.0188
GB-64 B	310	22.1	2.58	0.0213
GB-52	309	26.2	3.06	0.0185
GB-59 (Sta-J)	304	27.9	3.33	0.0188
GB-67	291	24.5	3.05	0.0207
GB-48	291	23.6	2.94	0.0189
GB-64B (Sta K)	288	22.1	2.78	0.0203
GB-53	250	17.8	2.58	0.0214
GB-73	202	12.4	2.22	0.0222
Average	361	30.5	3.01	0.0197
±Stdev	73	9.3	0.40	0.0012
		August 2014		
Fox River	610	59.8	3.55	0.0202
GB-6E	495	40.3	2.95	0.0237
GB-SC	488	41.5	3.08	0.0247
GB-2E	458	29.8	2.35	0.0257

GB-2S	436	37.8	3.14	0.0224
GB-2W	426	38.2	3.25	0.0215
GB-5	390	27.9	2.59	0.0220
GB-8	385	28.5	2.68	0.0212
GB-1B	365	31.0	3.08	0.0212
GB-9	358	25.7	2.59	0.0217
GB-10B	356	24.2	2.46	0.0226
GB-16B	350	26.8	2.77	0.0213
GB-13	349	24.7	2.57	0.0217
MR-Mouth	349	21.2	2.20	0.0225
Condos	345	23.8	2.49	0.0225
GB-18	344	25.9	2.73	0.0211
GB-30B	342	21.5	2.28	0.0215
GB-12	339	27.4	2.92	0.0215
GB-18B	336	23.4	2.52	0.0282
GB-7	335	26.9	2.91	0.0220
GB-17	333	24.6	2.67	0.0210
GB-11	325	24.5	2.73	0.0224
GB-25	324	21.5	2.41	0.0238
GB-20	322	25.6	2.88	0.0214
GB-31	321	18.7	2.11	0.0266
GB-14B	321	25.0	2.82	0.0226
GB-19	319	24.4	2.76	0.0228
GB-32	317	21.7	2.48	0.0226
GB-26S	316	20.9	2.40	0.0257
GB-45	313	17.9	2.08	0.0261
GB-22	312	24.5	2.84	0.0222
GB-39	312	20.8	2.41	0.0230
GB-44	311	17.6	2.05	0.0266
GB-48	311	21.4	2.49	0.0221
GB-48W	310	21.1	2.46	0.0263
GB-30E	310	21.3	2.49	0.0221
GB-30	308	22.2	2.60	0.0211
GB-39-Pump	306	20.3	2.41	0.0223
GB-38	302	16.8	2.02	0.0292
GB-54	300	18.3	2.21	0.0256
GB-43	293	19.7	2.43	0.0230
GB-47	289	16.9	2.11	0.0268
GB-42	279	17.3	2.24	0.0254
Average	349	25.3	2.59	0.0233
±Stdev	64	8.1	0.34	0.0020

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Table 3. Fluorescence indices and fluorescent-DOM component abundance derived from PARAFAC modeling based on fluorescence excitation-emission matrices for samples collected from Green Bay during June and August 2014. FIX=fluorescence index, BIX=biological index, and HIX=humification index. C1, C2, C3 and C4 are fluorescent-DOM component-1, 2, 3, and 4, respectively.

Station ID	FIX	BIX	HIX	C1	C2	C3	C4
				(ppb-QSE)			
June 2014							
DePere Dam	1.22	0.66	2.58	9.49	6.49	3.79	4.37
Fox Mouth	1.23	0.65	2.17	8.66	6.07	3.32	4.69
GB-6	1.19	0.69	2.08	5.95	3.61	2.31	4.05
GB-12 (Sta-F)	1.17	0.67	2.39	6.10	3.60	2.43	2.86
Sta-B	1.15	0.71	2.59	3.64	3.74	1.72	2.06
GB-38 B	1.11	0.67	2.94	4.57	3.37	2.10	2.11
GB-17	1.15	0.63	1.71	5.26	3.02	2.15	3.78
GB-42	1.15	0.69	2.96	4.48	3.61	1.82	1.98
GB-20 (Sta-G)	1.16	0.65	1.76	5.42	3.19	2.27	3.98
Sta-D	1.13	0.67	2.85	4.90	3.04	2.20	2.38
GB-25 (Sta-H)	1.14	0.68	3.11	5.46	2.68	2.20	2.01
GB-30	1.15	0.64	0.93	3.07	2.18	1.31	3.95
Sta-C	1.16	0.77	2.23	3.48	4.14	1.54	2.32
Sta-A	1.14	0.69	2.79	5.62	2.81	2.36	2.81
GB-9 (Sta-E)	1.13	0.66	2.76	5.44	2.87	2.20	2.56
GB-26	1.23	0.65	2.31	5.46	2.94	2.23	2.88
Condos	1.13	0.67	3.13	4.49	2.94	2.09	2.05
GB-SB	1.12	0.67	2.71	4.91	3.12	2.28	2.62
GB-CI	1.16	0.72	2.73	3.51	3.59	1.68	1.91
GB-39	1.13	0.65	1.30	5.19	2.85	2.06	4.57
GB-47	1.14	0.65	1.47	4.41	2.20	1.81	3.22
GB-44 (Sta-I)	1.15	0.68	1.55	4.20	2.50	1.73	3.59
GB-64 B	1.14	0.69	1.09	3.64	3.74	1.72	2.06
GB-52	1.11	0.65	3.05	4.07	3.30	1.93	1.95
GB-59 (Sta-J)	1.10	0.64	2.56	3.81	2.69	1.84	2.19
GB-67	1.12	0.72	2.31	2.46	2.58	1.26	1.55
GB-48	1.13	0.70	2.76	4.02	3.10	1.78	1.89
GB-64B (Sta K)	1.12	0.70	2.97	3.26	2.90	1.53	1.57
GB-53	1.09	0.66	1.38	3.03	1.87	1.33	2.68
GB-73	1.11	0.67	2.85	1.85	1.24	0.77	0.90
Average	1.14	0.68	2.33	4.67	3.20	2.00	2.71
±Stdev	0.04	0.03	0.65	1.60	1.03	0.58	0.99
August 2014							
Fox River	1.24	0.66	2.78	9.39	7.87	4.28	4.49
GB-6E	1.23	0.69	1.12	5.72	5.19	7.81	2.70

GB-SC	1.21	0.68	2.41	6.29	5.23	4.22	2.97
GB-2E	1.22	0.69	1.54	5.11	4.59	4.90	2.45
GB-2S	1.22	0.70	1.65	5.77	4.40	5.74	2.70
GB-2W	1.22	0.69	1.57	5.94	5.32	5.47	2.80
GB-5	1.18	0.73	0.97	3.47	3.28	5.78	1.67
GB-8	1.19	0.71	1.41	3.83	3.57	4.18	1.87
GB-1B	1.16	0.74	2.00	4.02	4.16	3.56	1.99
GB-9	1.17	0.75	2.25	3.39	3.44	2.59	1.69
GB-10B	1.18	0.73	1.93	4.53	3.95	3.22	2.19
GB-16B	1.17	0.72	1.44	3.51	3.32	3.78	1.75
GB-13	1.19	0.74	1.59	4.53	3.88	4.57	2.18
MR-Mouth	1.13	0.72	0.85	2.26	1.93	3.99	1.06
Condos	1.16	0.75	1.56	3.26	3.01	3.61	1.57
GB-18	1.17	0.75	1.71	3.31	3.29	3.56	1.63
GB-30B	1.16	0.73	2.58	3.05	2.66	2.07	1.49
GB-12	1.18	0.75	2.02	3.31	3.43	3.03	1.63
GB-18B	1.19	0.75	1.29	3.41	3.22	4.00	1.65
GB-7	1.18	0.74	1.77	3.46	3.20	2.77	1.68
GB-17	1.18	0.73	2.28	3.46	3.08	2.75	1.66
GB-11	1.20	0.76	3.26	3.49	3.30	1.74	1.71
GB-25	1.16	0.72	1.14	2.94	2.68	4.03	1.43
GB-20	1.18	0.73	2.61	2.54	2.84	1.63	1.28
GB-31	1.17	0.72	1.54	3.28	3.39	3.58	1.63
GB-14B	1.18	0.73	2.35	3.48	3.17	2.42	1.65
GB-19	1.17	0.74	1.70	3.60	3.25	3.01	1.76
GB-32	1.15	0.72	1.45	3.55	3.03	3.82	1.69
GB-26S	1.18	0.75	2.45	3.55	3.40	2.63	1.79
GB-45	1.16	0.73	1.53	2.55	2.75	3.07	1.32
GB-22	1.17	0.72	1.39	3.52	3.26	3.52	1.70
GB-39	1.17	0.76	1.06	1.73	1.93	3.24	0.86
GB-44	1.14	0.74	1.64	2.71	2.73	2.83	1.37
GB-48	1.17	0.70	2.04	2.87	2.66	2.61	1.44
GB-48W	1.15	0.72	1.15	2.81	2.54	3.70	1.36
GB-30E	1.16	0.77	1.58	2.70	2.84	3.14	1.36
GB-30	1.15	0.77	1.54	2.69	3.04	3.33	1.41
GB-39-Pump	1.15	0.73	1.42	2.84	2.61	3.29	1.36
GB-38	1.15	0.72	0.67	1.56	1.40	3.75	0.73
GB-54	1.16	0.72	1.05	2.91	2.75	4.23	1.46
GB-43	1.15	0.72	0.42	0.71	0.89	2.95	0.37
GB-47	1.14	0.72	1.39	2.67	2.74	3.38	1.33
GB-42	1.13	0.73	2.10	2.40	2.56	1.95	1.23
Average	1.17	0.73	1.68	3.54	3.30	3.57	1.72
±Stdev	0.03	0.02	0.59	1.45	1.14	1.16	0.67

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714 **Table 4.** Comparison of the values of the humification index (HIX), the biological index (BIX),
715 and the fluorescence index (FIX) between Green Bay, the Laurentian Great Lakes, Lake Taihu,
716 and the Yangtze River estuary. Values are averages ± 1 standard deviation.

Site	HIX	BIX	FIX	Reference
Green Bay (June)	2.33 ± 0.65	0.68 ± 0.03	1.14 ± 0.03	This study
Green Bay (August)	1.68 ± 0.59	0.73 ± 0.02	1.17 ± 0.02	"
Lake Superior	1.13 ± 0.02	0.91 ± 0.06	1.14 ± 0.02	Zhou et al. (2016)
Lake Michigan	1.28 ± 0.25	0.89 ± 0.03	1.19 ± 0.01	"
Lake Huron	1.23 ± 0.28	0.90 ± 0.06	1.21 ± 0.02	"
Lake Erie	1.98 ± 0.59	0.91 ± 0.02	1.22 ± 0.02	"
Lake Ontario	1.91 ± 0.68	0.92 ± 0.03	1.22 ± 0.02	"
Lake Taihu	1.7-3.3	0.71-0.89	1.55-1.79	Yang et al. (2014)
Yangtze River Estuary	1.10 ± 0.39	1.16 ± 0.23	No data	Sun et al. (2014)

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719 **Table 5**. Description of PARAFAC-derived fluorescent-DOM components in Green Bay during
720 June and August 2014. Note that the order of C3 and C4 are reversed between sampling months.
721 The assignment of component plots in Figure 7 corresponds to the order in August in this table.

June Ex/Em (nm)	August Ex/Em (nm)	Component Description
C1 – 255 (310)/440	C1 – 250 (315)/458	Similar to Peak A - UVC humic like. Dominates export from natural catchments and exported from agricultural catchments. Allochthonous.
C2 – 240 (290)/354	C2 – 240 (300)/392	Similar to Peak M – marine humic-like. May be allochthonous, autochthonous, or a result of microbial activity.
C3 – 285/508	C4 – 280/508	UVA humic like. High molecular weight and aromatic. Fluorescence resembles fulvic acid. Widespread.
C4 – 265/312	C3 – 270/316	Similar to Peak B – Tyrosine-like. May indicate amino acids, free or bound proteins, and may indicate more degraded peptide material.

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