



Partitioning and transformation of organic and inorganic phosphorus among dissolved, colloidal and particulate phases in a hypereutrophic freshwater estuary

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ARTICLE INFO

Article history:

Received 4 December 2020

Revised 4 March 2021

Accepted 8 March 2021

Available online 10 March 2021

Keywords:

Phosphorus cycling

Nutrients

Colloidal organic phosphorus

Chemical speciation

Fox River

Green Bay

ABSTRACT

Phosphorus (P) loadings to the Great Lakes have been regulated for decades, but re-eutrophication and seasonal hypoxia have recently been increasingly reported. It is of paramount importance to better understand the fate, transformation, and biogeochemical cycling processes of different P species across the river-lake interface. We report here results on chemical speciation of P in the seasonally hypoxic Fox River-Green Bay system and variations in sources and partitioning of P species along the aquatic continuum. During midsummer when productivity is generally high, phosphate and dissolved organic P (DOP) were the major species in river water while particulate-organic-P predominated in open bay waters, showing a dynamic change in the chemical speciation of P along the river-bay transect with active transformations between inorganic and organic P and between colloidal and particulate phases. Colloidal organic P (COP, >1 kDa) comprised 33–65% of the bulk DOP, while colloidal inorganic P was generally insignificant and undetectable especially in open bay water. Sources of COP changed from mainly allochthonous in the Fox River, having mostly smaller sized colloids (1–3 kDa) and a lower organic carbon to phosphorus (C/P) ratio, to predominantly autochthonous in open bay waters with larger sized colloids (>10 kDa) and a higher organic C/P ratio. The observed high apparent distribution coefficients (K_d) of P between dissolved and particulate phases and high-abundant autochthonous colloidal and particulate organic P in the hypereutrophic environment suggest that, in addition to phosphate, colloidal/particulate organic P may play a critical role in the biogeochemical cycling of P and the development of seasonal hypoxia.

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1. Introduction

As an essential macronutrient for living organisms, phosphorus (P) plays a critical role in regulating primary production, water quality, and biogeochemical processes in various aquatic environments (Lean et al., 1983; Karl, 2000; Orihel et al., 2017; Taylor and Lean, 2018). Previous studies have shown that excess nutrients, especially P can cause eutrophication and seasonal hypoxia not only in the Great Lakes region, such as Lake Erie and Green Bay (Scavia et al., 2014; Zhou et al., 2015; Klump et al.,

2018), but also in other river-dominated coastal marine environments (Rabalais et al., 2001; Smith, 2003; Zhang et al. 2010b).

Some P loadings to the Great Lakes have been regulated through the Great Lakes Water Quality Agreement (GLWQA) for decades, which had led to significant reductions of total P (TP) concentrations throughout the system in the Great Lakes (Chapra and Dolan, 2012; Dolan and Chapra, 2012; Finlay et al., 2013), bringing oligotrophication to some Great Lakes regions, such as Lake Michigan and Lake Ontario (Cuhel and Aguilar, 2013; Dove and Chapra, 2015; Lin and Guo, 2016a). For instance, offshore TP concentrations in Lake Ontario were in the range of 5–6 $\mu\text{g/L}$ (Dove and Chapra, 2015), well below the target concentration of 10 $\mu\text{g/L}$ from the GLWQA. On the other hand, re-eutrophication and increased hypoxia have been increasingly reported (Scavia et al., 2014; Zhou et al., 2015; Klump et al., 2018). Reconciling these

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seemingly contradictory observations and the paradox in nutrient management requires new insights in the chemical speciation, bioavailability, and biogeochemical cycling of P in aquatic environments.

Over the past few decades, a growing body of literature has examined nutrient sources and loadings to the Great Lakes as well as the fate and internal biogeochemical cycling of P in different lake systems (Hecky et al., 2004; Elsbury et al., 2009; Joosse and Baker, 2011; Lin et al., 2016; Maccoux et al., 2016; Depew et al., 2018). Phosphorus occurs in different chemical forms and physical phases in natural waters (Lean, 1973, b; van der Zee et al., 2007; Cai and Guo, 2009; Lin and Guo, 2016a), including dissolved inorganic P (DIP) or soluble reactive P (SRP), dissolved organic P (DOP), colloidal inorganic P (CIP), colloidal organic P (COP), particulate inorganic P (PIP), and particulate organic P (POP). Each form has different bioavailability, chemical reactivity, and environmental fate, which can play different roles in regulating water quality and the biogeochemical cycling of other bioactive elements in aquatic environments (Minear and Lean, 1975; Lin et al., 2012; Qin et al., 2015; Fang and Wang, 2020). Therefore, knowledge about the partitioning and transformation of P among different forms/phases is needed to better understand the environmental fate and ecological role of P in aquatic environments (Wetzel and Likens, 1991; Sylvan et al., 2006; Yoshimura et al., 2014).

A large portion of P carried through rivers to estuaries and coastal waters is associated with suspended particles (Föllmi, 1996; Jordan et al., 2008; Yuan et al., 2013). Indeed, P is a highly particle-reactive element with distribution coefficient (K_d) values as high as 10^4 – 10^7 in aquatic ecosystems (Santschi, 1995; Prastka et al., 1998; Lin et al., 2016; Lin and Guo, 2016a) and can bind preferentially with colloidal and particulate surfaces in addition to biological uptake and assimilation (Lean, 1973; Hilger et al., 1999; Cai and Guo, 2009; Lin et al., 2012). Therefore, the dynamic partitioning of P among dissolved, colloidal and particulate phases should play a critical role in controlling the abundance, transformation, bioavailability, and the overall biogeochemical cycling of P in aquatic environments. Nevertheless, many monitoring programs and loading analyses usually reported total P and did not recognize the importance of chemical speciation (Arbuckle and Downing, 2001; Dolan and Chapra, 2012; Robertson et al., 2018). There exists a knowledge gap in the understanding of chemical speciation, bioavailability, and biogeochemical cycling pathways of P in aquatic systems, especially at the river-lake interfaces.

Colloids are important intermediaries in the biogeochemical cycling of natural organic matter and particle-reactive elements due to their high specific surface areas, high biological/chemical reactivity, and short turnover times in aquatic environments (Honeyman and Santschi, 1988; Guo et al., 1997; Benner and Amon, 2015). Previous studies have shown that DOP especially the hydrophobic forms could be more bioavailable to algae (Lean and Nalewajko, 1976; Qin et al., 2015), and colloidal organic matter or high molecular weight-dissolved organic matter (HMW-DOM) contains higher hydrophobic components (Lean, 1973; Hilger et al., 1999; Cai et al., 2015), implying that a large fraction of the abundant colloidal DOP carried by river waters can be bioavailable in receiving waters. Over the past decades, colloidal P in natural waters has been described in more detail, especially colloidal organic P (Mayer and Jarrell, 1995; Haygarth et al., 1997; Cai and Guo, 2009; Chen et al., 2010; Lin and Guo, 2016a; Gottselig et al., 2017). Nevertheless, other than the abundance of bulk colloids, sources of colloidal P and the transformation of P among dissolved, colloidal and particulate phases in estuarine environments have rarely been evaluated. Systematic studies of their sources, molecular weight distributions, and transformation can provide new insights into a better understanding of the role of colloidal P in regulating dynamic cycling of different P species and its relation to

water quality and the development of eutrophication and hypoxia in estuarine and coastal ecosystems.

The lower Fox River-Green Bay, Lake Michigan, was listed as one of the Great Lakes Areas of Concern during 1980s. P inputs from wastewater treatment plants from the Fox River Basin have been regulated and their inputs have been reduced, but non-point P sources continue to contribute high P loads to the lower Fox River (Robertson et al., 2018). Hypereutrophic and seasonal hypoxic conditions in Green Bay have been frequently observed (Klump et al., 2018). Recent studies have examined the dynamics of P species in Green Bay (Lin et al., 2016, 2018). Nevertheless, the abundance and distribution of colloidal organic and inorganic P forms and their transformation among P species during transport to Green Bay have not been quantified.

Our hypothesis is that colloidal organic P has high biological/chemical reactivity and/or high degradation rates in aquatic environments, which may increase the rate of eutrophication and seasonal hypoxia in estuarine and coastal environments. Thus, studies on the details of chemical speciation of P and the estuarine mixing of different P species at the river-lake interface is expected to provide a better understanding of biogeochemical cycling of P and improve scientific bases for environmental, ecological, and water resource management in the Fox River-Green Bay ecosystem. Our major objectives were to (1) quantify the abundance of inorganic and organic P species in dissolved, colloidal, and particulate phases along a transect from the Fox River to open Green Bay during midsummer when P typically limits productivity; (2) identify the estuarine mixing and transport of different molecular sized colloidal organic P across the river-lake interface; and (3) examine sources of different P species and their transformation between different phases as well as the role of colloidal organic P in the biogeochemical cycling of P along the river-bay continuum.

2. Materials and methods

2.1. Study area

Green Bay is the largest freshwater estuary in the Laurentian Great Lakes (Fig. 1) and accounts for ~9% of surface area of Lake Michigan; however, Green Bay receives 15–30% of the total P loading to Lake Michigan from its primary tributary, the Fox River (Klump et al., 2009; Robertson et al., 2019). Among all rivers and streams discharging into Green Bay, the Fox River has the largest input with an average discharge of $128 \text{ m}^3/\text{s}$ (Robertson and

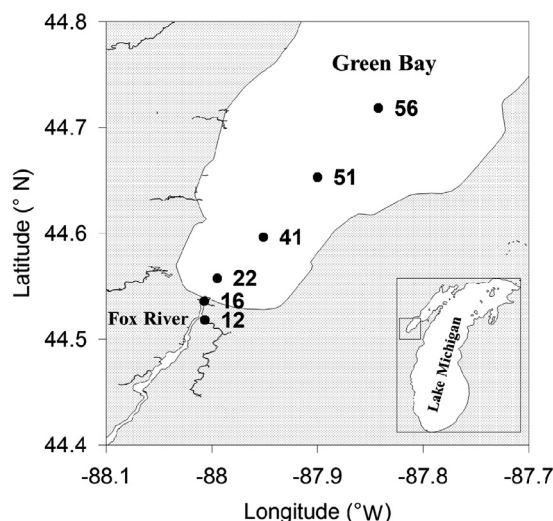


Fig. 1. Sampling stations in the lower Fox River-Green Bay, a hypereutrophic ecosystem, in northwestern Lake Michigan.

Table 1

Sampling locations, distance from the river mouth and hydrographic data including water depth, water temperature (T), specific conductivity (Sp. Cond), pH, dissolved oxygen (DO%), suspended particulate matter (SPM), turbidity, and chlorophyll-*a* (Chl-*a*) levels for surface waters from the lower Fox River to open Green Bay during July 2018.

Station ID	Latitude (°N)	Longitude (°W)	Depth (m)	T (°C)	Sp. Cond (μS/cm)	pH	DO (%)	SPM (mg/L)	Turbidity (NTU)	Chl- <i>a</i> (μg/L)
12	44.52	88.01	4.0	26.4	463	8.49	100	28.7	38.4	55.2
16	44.54	88.01	9.0	26.5	382	8.55	141	33.8	38.5	76.9
22	44.56	88.00	8.4	25.5	376	8.33	106	26.3	30.5	65.8
41	44.60	87.95	8.7	24.6	343	8.39	116	12.2	10.5	27.2
51	44.65	87.90	9.2	24.3	332	8.24	114	5.7	1.7	8.5
56	44.72	87.84	10.0	23.4	322	8.05	109	4.0	3.6	10.7

Saad, 2019), and contributes approximately 70% of the nutrient and suspended sediment load (Klump et al., 1997). Even though the point sources throughout the Fox River system have been regulated and extensive clean up and restoration in southern Green Bay has occurred over past decades, total P loads are at times higher than the Total Maximum Daily Load (TMDL) target (WI-DNR, 2019). Deteriorated water quality, eutrophic conditions, and formation of “dead zones” remain frequent in southern Green Bay largely due to excessive P loading from the Fox River (Klump et al., 2018).

2.2. Sampling

Samples were collected from a series of sampling stations along a transection from the lower Fox River to the open Green Bay on July 10th, 2018 (Fig. 1). The average discharge from the Fox River on this date was 126 m³/s (<http://waterdata.usgs.gov/usa/nwis/uv?040851385>). Basic water-quality data, including water temperature, specific conductivity, pH, dissolved oxygen (DO), and chlorophyll-*a* fluorescence (Chl-*a*) were measured by *in situ* sensors (YSI Sonde) and are given in Table 1 along with the specific sampling locations.

Surface-water samples (~1 m below surface) were collected in pre-cleaned HDPE bottles and stored in a cooler with ice. Samples were filtered in the laboratory within 6 h of sampling through pre-combusted (450°C for 4 h) glass-fiber filters (GF/F, Whatman, with a pore-size of 0.7 μm). Thus, dissolved P species here are operationally defined as those in the < 0.7 μm filtrate, which may contain living biomass and thus overestimate DOP compared to those in the < 0.45 μm or < 0.2 μm filtrates. Both the filtrate and filter samples were kept frozen for the measurements of total dissolved P (TDP), dissolved inorganic P (DIP) or soluble reactive P (SRP), total particulate P (TPP), particulate inorganic P (PIP), total dissolved nitrogen (TDN), dissolved inorganic nitrogen (DIN), and dissolved organic carbon (DOC). In addition, aliquots of whole water samples were also filtered through pre-weighed GF/F filters (Whatman) for the measurements of suspended particulate matter (SPM), particulate organic carbon (POC) and particulate nitrogen (PN) and their stable isotope compositions (δ¹³C and δ¹⁵N). Additional filtrate from each sample was collected for ultrafiltration to determine the abundance of colloidal inorganic P (CIP) and colloidal organic P (COP). Due to labor-intensive nature in using ultrafiltration, only six sampling stations were occupied, which may miss possible small variations between sampling stations.

2.3. Ultrafiltration

In addition to dissolved (< 0.7 μm) and particulate (> 0.7 μm) P, different size-fractions of colloidal P were further isolated using stirred cell ultrafiltration units equipped with membrane discs with different nominal molecular weight cutoffs (MWCOs), including 1 kDa (YM1, Millipore-Sigma), 3 kDa (YM3), and 10 kDa (YM10) membranes. The bulk DIP and dissolved organic phosphorus (DOP) were then size fractionated into the < 1 kDa low-molecular-weight (LMW) and the 1–3 kDa, 3–10 kDa and > 10 kDa high-molecular-weight (HMW) or colloidal fractions.

Before ultrafiltration, all stirred cell units were acid cleaned and the membrane discs were pre-cleaned with 0.05 M NaOH solution and ultrapure water until the blank ultrafiltrate had an undistinguishable UV-absorbance signature from the ultrapure water (Xu and Guo, 2017). To avoid LMW retention and thus over-estimation of colloids, colloidal abundance had to be determined from the ultrafiltration permeation model (Guo et al., 2000). Previous studies and our initial ultrafiltration results showed that when the concentration factor was ≥ 30, the difference in the colloidal P abundance was within 5% between those derived from the ultrafiltration permeation model and by concentration difference between the integrated permeate and initial solution (Cai and Guo, 2009). Thus, the same concentration factor (30 here) was employed for all samples ultrafiltration to reduce the analytical workload and for easy comparisons between samples. Both the initial water and integrated permeate solution were sampled for the measurements of DIP and TDP to calculate the abundances of CIP and COP for each sample (Cai and Guo, 2009; Lin et al., 2012).

2.4. Measurements of inorganic and organic phosphorus species

Predetermined volumes of filtrate samples were transferred into a Teflon vial with a Teflon screw cap and were digested with K₂S₂O₈ solution (50 g/L, pH = 1) in an oven at 140°C for 4 h (Lin et al., 2012). Concentrations of TDP were then measured using the standard phosphomolybdenum blue method on an Agilent 8453 UV-visible spectrophotometer in 5-cm cuvettes at 882 nm (Hansen and Koroleff, 1999). Concentrations of DIP or phosphate were directly measured without digestion. The concentration of DOP was calculated from the difference between TDP and DIP (i.e., DOP = TDP – DIP). The detection limit was 8–10 nM based on replicate blank sample measurements using 5 cm cuvettes, with a precision of < 2% for both DIP and TDP at 0.1 μM level (Cai and Guo, 2009).

The TPP concentration was determined after high temperature combustion and acid hydrolysis (HCl) of filter samples as described in Solórzano and Sharp (1980) with some modifications (Zhang et al., 2010a). Briefly, the filter samples were wetted using 0.5 M MgCl₂ solution and heated in an oven at 95°C until dry, followed by ashing in a muffle furnace at 550°C for 2 h. The residue was extracted with 1 M HCl solution at room temperature in the dark for 24 h. Particulate inorganic P (PIP) was extracted directly from filter samples with 1 M HCl solution for 24 h (Aspila et al., 1976). Both extractions of TPP and PIP were quantified as DIP after neutralization and dilution. Similar to DOP, the POP concentration was calculated from the difference between TPP and PIP (i.e., POP = TPP – PIP).

2.5. Measurements of organic carbon, dissolved nitrogen, stable isotopes and SPM

The concentrations of DOC and TDN were measured using the high temperature combustion method on a Shimadzu TOC-L analyzer, with a precision < 2% for both DOC and TDN (Guo and Mac-

donald, 2006). Filtrate samples were acidified with concentrated HCl to a pH < 2 before analysis. Concentrations of dissolved inorganic nitrogen (DIN), including NO_3^- , NO_2^- , and NH_4^+ , were measured using colorimetry on a Technicon AutoAnalyzer. Concentrations of dissolved organic nitrogen (DON) were calculated from the difference between TDN and DIN concentrations (i.e., $\text{DON} = \text{TDN} - \text{DIN}$) (Guo et al., 2004; Guo and Macdonald, 2006). GF/F filter samples were treated with HCl acid fumes overnight for the measurements of POC and PN contents and stable carbon ($\delta^{13}\text{C}$) and nitrogen isotope ($\delta^{15}\text{N}$) compositions on an elemental analyzer interfaced with a Thermo-Fisher Scientific isotopic ratio-mass spectrometer (Delta V). The precision and accuracy of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were $\pm 0.1\text{‰}$ and $\pm 0.2\text{‰}$ respectively, as determined by replicate analysis of standards (Yang et al., 2015). The suspended particulate matter (SPM) concentrations were determined using the weight difference between dried filter samples and blank filters. Filter samples were weighed after freeze drying until achieving a constant weight.

2.6. Determination of distribution coefficient of P between dissolved and particulate phases

The partitioning of P species between dissolved and particulate phases and their particle reactivity could be quantitatively evaluated using the apparent distribution coefficient or partition coefficient (K_d) approach. This approach has been used in previous studies to examine the partitioning and adsorption behavior of P in aquatic environments (e.g., Santschi 1995; Prastka et al. 1998; Lin et al. 2016). The apparent K_d values were calculated based on the following equation:

$$K_d = \frac{C_p}{C_d \times [\text{SPM}]}$$

where C_p is the concentration of particulate P in the inorganic, organic, or total P pool (in μM); C_d is the concentration of inorganic, organic, or total P (in μM) in the dissolved phase; SPM is the concentration of suspended particulate matter (kg/L or g/mL). Accordingly, K_d has a unit of mL/g or L/kg and its values are reported here in $\log K_d$.

3. Results

3.1. Water-quality data

Surface water temperatures varied from 23.4°C to 26.5°C, with an average of $25.1 \pm 1.2^\circ\text{C}$ (Table 1). Specific conductivity decreased from 463 $\mu\text{S}/\text{cm}$ in the Fox River to 322 $\mu\text{S}/\text{cm}$ in open Green Bay (Table 1) showing a typical feature of negative estuaries (Xu et al., 2018a). Surface water pH ranged from 8.05 to 8.55, with an average of 8.34 ± 0.18 (Table 1), exhibiting the effect of limestone in the basin and the characteristic of carbonate water chemistry (Lin and Guo, 2016b). Concentrations of DO (in terms of % saturation) were $\geq 100\%$ at all stations (Table 1). SPM concentrations decreased rapidly from the Fox River ($29.6 \pm 3.8 \text{ mg/L}$) to open Green Bay ($7.3 \pm 4.3 \text{ mg/L}$, Table 1), indicating that the Fox River was the main source of suspended sediment and turbidity to the water column. Levels of Chl-*a* in surface waters were somewhat variable, ranging from 8.5 $\mu\text{g/L}$ to 76.9 $\mu\text{g/L}$, with an average of $40.7 \pm 29.2 \mu\text{g/L}$ (Table 1). Elevated Chl-*a* was observed at the river-bay interface, and then decreased in general from estuary to open water, showing a strong trophic gradient driven by the Fox River with high nutrients.

3.2. Variations in dissolved inorganic and organic phosphorus

Concentrations of DIP monotonically decreased with decreasing specific conductivity from 4.15 μM at the Fox River end member

station (St-12) to 0.11 μM at the open station (St-56), with an average of $1.47 \pm 1.57 \mu\text{M}$ and showing quasi-conservative mixing between river water and bay water (Table 2; Fig. 2a). Concentrations of DOP ranged from 0.24 to 2.61 μM with an average of $1.18 \pm 1.00 \mu\text{M}$ and a little addition or production of DOP at stations 16 and 22 compared to the theoretical mixing line between river water and bay water (Fig. 2a). Both DIP and DOP decreased rapidly from the Fox River to open Green Bay, showing a dominant source from the river basin for both dissolved P species (Fig. 2a). Within the total dissolved P pool, DIP contributed up to $48 \pm 10\%$, varying from 61% in the Fox River to 31% in open bay water, while DOP comprised $52 \pm 10\%$ of the total dissolved P, ranging from 39% in Fox River water to 69% in open bay water. While DIP was the dominant dissolved P species in river waters, DOP became the major dissolved P species in the open Green Bay (Fig. 2e), with an active transformation between the dissolved P species along the river-bay transect. Similar to DOP, concentrations of DOC and DON also decreased from the Fox River (869 $\mu\text{M-C}$ and 100 $\mu\text{M-N}$, respectively) to the open Green Bay (441 $\mu\text{M-C}$ and 19 $\mu\text{M-N}$, respectively) (Table 2), showing again a strong trophic gradient along the river-bay transect.

3.3. Variations in particulate inorganic and organic phosphorus

The concentration of TPP (PIP + POP) decreased from 3.39 μM in the Fox River to 2.75 μM in open bay waters with an average of $3.16 \pm 0.35 \mu\text{M}$ (Table 3). Within the total particulate P pool, PIP (average of $1.08 \pm 0.62 \mu\text{M}$) generally decreased from river waters (stations 12 and 16) to open bay, while POP (average of $2.08 \pm 0.31 \mu\text{M}$) generally increased from river water to bay water or increased with decreasing specific conductivity (Fig. 2b). The decrease in PIP and increase in POP concentrations from river water to bay water are consistent with the dynamic transformation between DIP and DOP in the water column and point to a rapid change in sources of particulate P species along the river-bay transect.

While TPP concentrations (in μM) decreased from the river to bay waters, both SPM-normalized PIP and POP concentrations (in mg-P/g-particles), in contrast, increased, especially POP (Table 3, Fig. 2c). In other words, the amount of P in the particles increased from river to bay waters.

3.4. Partitioning of P between dissolved, colloidal, and particulate phases

The TDP/TP ratio was 0.57 ± 0.09 in river/estuarine region, indicating that P exported from the Fox River during summer was, on average, slightly higher in the dissolved phase. Nevertheless, the TDP/TP ratio sharply decreased from the estuary to open bay (0.16 ± 0.05), and particulate P became the predominant P species making up > 80% of the total P pool in open bay waters (Fig. 2f), showing a considerable transformation between dissolved and particulate P along the river-bay transect.

In terms of phase partitioning of inorganic P, the LMW-DIP comprised 67% of the total inorganic P (TIP) in river waters and decreased dramatically to as low as 23% in open bay waters, while CIP only made up 0–4% of the TIP (Fig. 3); PIP comprised, on average, $55 \pm 18\%$ of the TIP, increasing from 30% in river waters to 71% in open bay waters (Fig. 3). In contrast to the phase partitioning of inorganic P, the LMW-DOP and COP made up $17 \pm 14\%$ and $15 \pm 11\%$ of the TOP, respectively, while POP comprised $68 \pm 23\%$ of TOP, increasing from 38% in river waters to 91% in open bay waters, showing elevated contribution from *in situ* biological (algal) production and incorporation of DIP (Fig. 3).

As shown in Fig. 4, the percentage of DIP and DOP in the TP pool decreased from the Fox River to open Green Bay, resulting

Table 2

Concentrations of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), dissolved inorganic phosphorus (DIP) and dissolved organic phosphorus (DOP), and the abundances (%) of DIP and DOP in total dissolved phosphorus (TDP), and molar ratios of DOC/DON and DOC/DOP in surface waters from the lower Fox River to open Green Bay.

Station ID	DOC (μM)	DON (μM)	DIP (μM)	DOP (μM)	DIP/TDP (%)	DOP/TDP (%)	DOC/DON	DOC/DOP
12	869	100	4.15	2.61	61	39	9	333
16	790	37	1.94	1.76	52	48	21	449
22	676	36	2.00	1.78	53	47	19	380
41	536	23	0.38	0.40	49	51	23	1340
51	487	20	0.22	0.31	42	58	24	1571
56	441	19	0.11	0.24	31	69	23	1838

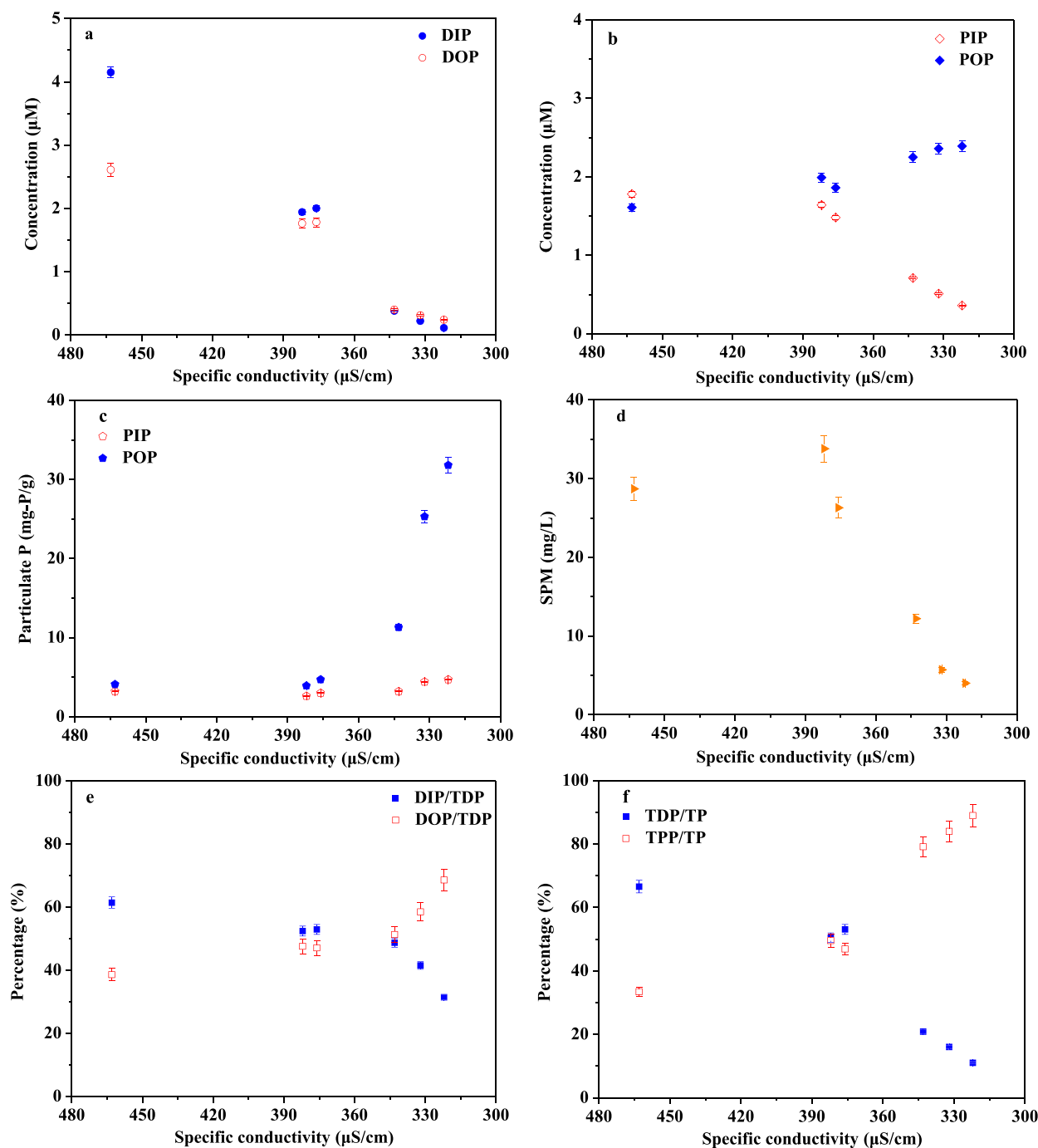


Fig. 2. Variations in (a) dissolved inorganic P (DIP) and dissolved organic P (DOP), (b) particulate inorganic P (PIP) and particulate organic P (POP), (c) PIP and POP in mg-P/g-particles, (d) suspended particulate matter (SPM), (e) the percentage of DIP and DOP in the total dissolved P (TDP) pool, and (f) the percentage of TDP and TPP in the total P (TP) pool along the lower Fox River - Green Bay ecosystem, where the specific conductivity decreased from river water to open bay water (Table 1), showing characteristics of a negative estuary (error bars are mean $\pm$ SD).

Table 3

Concentrations of POC, PN, PIP, and POP and the abundances (in %) of PIP and POP in the total particulate phosphorus (TPP), as well as the POC/PN and POC/POP molar ratios and stable carbon ($\delta^{13}\text{C}$ -POC) and nitrogen ($\delta^{15}\text{N}$ -PN) isotope composition. Both PIP and POP concentrations are given in μM and $\mu\text{mol/g}$ -particles, with errors $\leq 5\%$.

Station	POC (μM)	PN (μM)	PIP (μM)	POP (μM)	PIP/TPP (%)	POP/TPP (%)	POC/PN	POC/POP	$\delta^{13}\text{C}$ -POC (‰)	$\delta^{15}\text{N}$ -PN (‰)	PIP ($\mu\text{mol/g}$)	POP ($\mu\text{mol/g}$)
12	227	24.4	1.78	1.61	53	47	9.3	141	-27.47	2.58	103	132
16	286	35.2	1.64	1.99	45	55	8.1	144	-28.76	3.19	84	126
22	231	27.6	1.48	1.86	44	56	8.4	124	-29.00	6.86	97	152
41	240	36.5	0.71	2.25	24	76	6.6	107	-29.38	6.99	103	365
51	245	37.7	0.51	2.36	18	82	6.5	104	-30.90	7.27	142	816
56	249	38.9	0.36	2.39	13	87	6.4	104	-32.97	12.41	152	1026

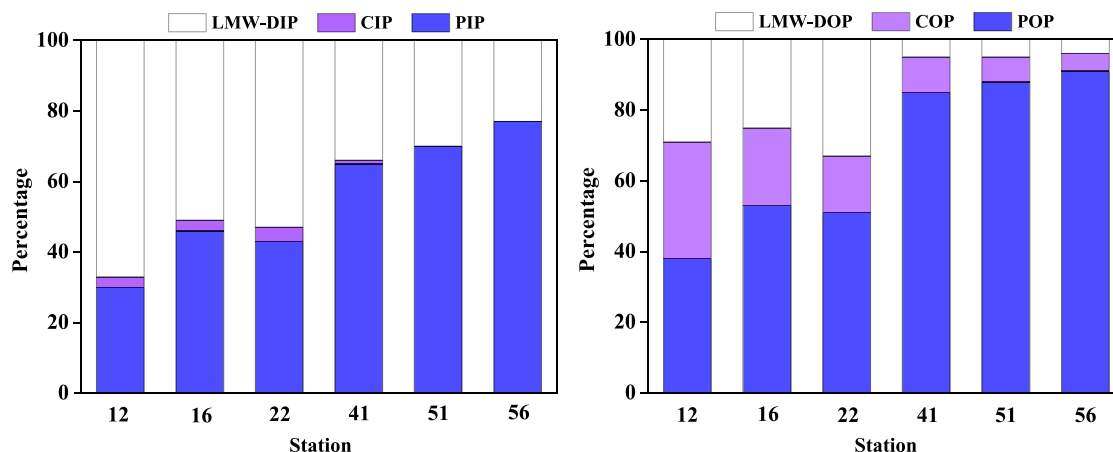


Fig. 3. Variations in the abundances (or percentage) of LMW-dissolved, colloidal and particulate inorganic (LMW-DIP, CIP, and PIP) and organic P (LMW-DOP, COP, and POP) in the total inorganic P (left panel) and total organic P (right panel) pools along the river-bay transect in the Fox River - Green Bay system.

from physical dilution and biological uptake. Conversely, the percentage of POP was higher in open Green Bay than that in the Fox River. On average, POP had a significantly higher contribution ($46 \pm 26\%$) to the TP pool compared to PIP ($18 \pm 4\%$).

Large variations in dissolved and particulate P concentrations were evident along the river-bay transect (Fig. 2), with concentrations decreasing from river water to bay water for all P species except for POP, which, in contrast, increased. There was a significant inverse correlation between POP and DIP or DOP ($p < 0.001$) (Supplementary Fig. S1), suggesting a dynamic transformation of P between dissolved and particulate phases during estuarine mixing and biological production. Overall, dissolved P slightly dominated the total P pool in the river/estuarine region, while particulate P became predominant in the total P pool in open Green Bay (Fig. 4).

To quantify the apparent partitioning of P between dissolved and particulate phases, values of $\log K_d$ were compared between different P species. As shown in Supplementary Table S1, values of $\log K_d$ varied from 4.2 to 6.4 for inorganic, organic or total P. The differences in $\log K_d$ values between P species at a specific station are quite small (Fig. 5). However, $\log K_d$ values increased in general from river to bay waters, showing the effects of biological productivity and SPM concentration or “particle concentration effect”. Indeed, there is a significant negative correlation between $\log K_d$ values and $\log \text{SPM}$ for inorganic, organic and total P pools in the Fox River plume (Fig. 5), as has been observed in other estuarine systems (e.g., Caraco et al., 1990; Santschi, 1995; Turner and Tyler, 1997; Lin et al., 2012; Lin and Guo, 2016a).

3.5. Abundances and molecular weight distributions of colloidal phosphorus

The COP concentration in the 1 kDa–0.7 μm colloidal fraction varied from 0.14 to 1.37 μM , corresponding to 33–65% of the bulk DOP; therefore, much of the P typically described as dissolved P is actually colloidal organic P. CIP concentrations, however, ranged from below the detection limit to 0.15 μM , making up 0–7.5% of

the bulk DIP (Table 4). As shown in Fig. 6, the CIP concentration remained somewhat similar (0.12–0.15 μM) in the river/estuarine region (stations 12, 16, and 22) and then decreased rapidly and became undetectable in open bay waters. This indicated that CIP is mainly derived from river input and DIP is exclusively present in ionic forms in open Green Bay, consistent with those reported for other aquatic environments, such as the Mississippi River plume and Milwaukee River estuary (Cai and Guo, 2009; Lin et al., 2012).

The percentage of COP in the bulk DOP was relatively high in the Fox River and decreased in the inner bay, showing an evident river or terrigenous source (Fig. 6). In addition, the percentage of COP increased again from the inner bay to open bay and remained at similarly high levels in open bay waters (Fig. 6), resulting from autochthonous sources. In contrast to the abundant COP at all stations, the amount of CIP was small and was detectable only in Fox River and its estuarine waters, suggesting river input is the sole CIP source.

The abundance of COP changed significantly ($p < 0.05$) across the river-lake interface (Fig. 7), shifting from fairly high bulk COP in river water (52%, sum of COPs) to rather low COP (33 $\pm$ 8%) in the estuarine mixing zone to even higher COP again in open Green Bay (59 $\pm$ 9% COP). Large variations in the bulk COP abundance with a bi-mode distribution along the river-bay transect indicated a dynamic transformation between LMW- and HMW-DOP pools during estuarine mixing. Based on the detailed size distributions of DOP among the < 1 kDa, 1–3 kDa, 3–10 kDa and 10 kDa–0.7 μm size-ranges, the major colloidal component within the bulk COP pool was the 1–3 kDa small COP (48%) in river water, the 3–10 kDa medium COP (54%) in the estuarine mixing zone, and the 10 kDa–0.7 μm large COP (48–79%) in open bay water, respectively, showing a progressive increase towards the higher molecular weight or large sized colloids from the Fox River to open Green Bay (Fig. 7). Dynamic changes in the molecular weight distribution of COP observed here clearly indicate the unique colloidal molecular size characteristics between terrestrial and autochthonous sources and a rapid turnover of COP across the river-bay interface.

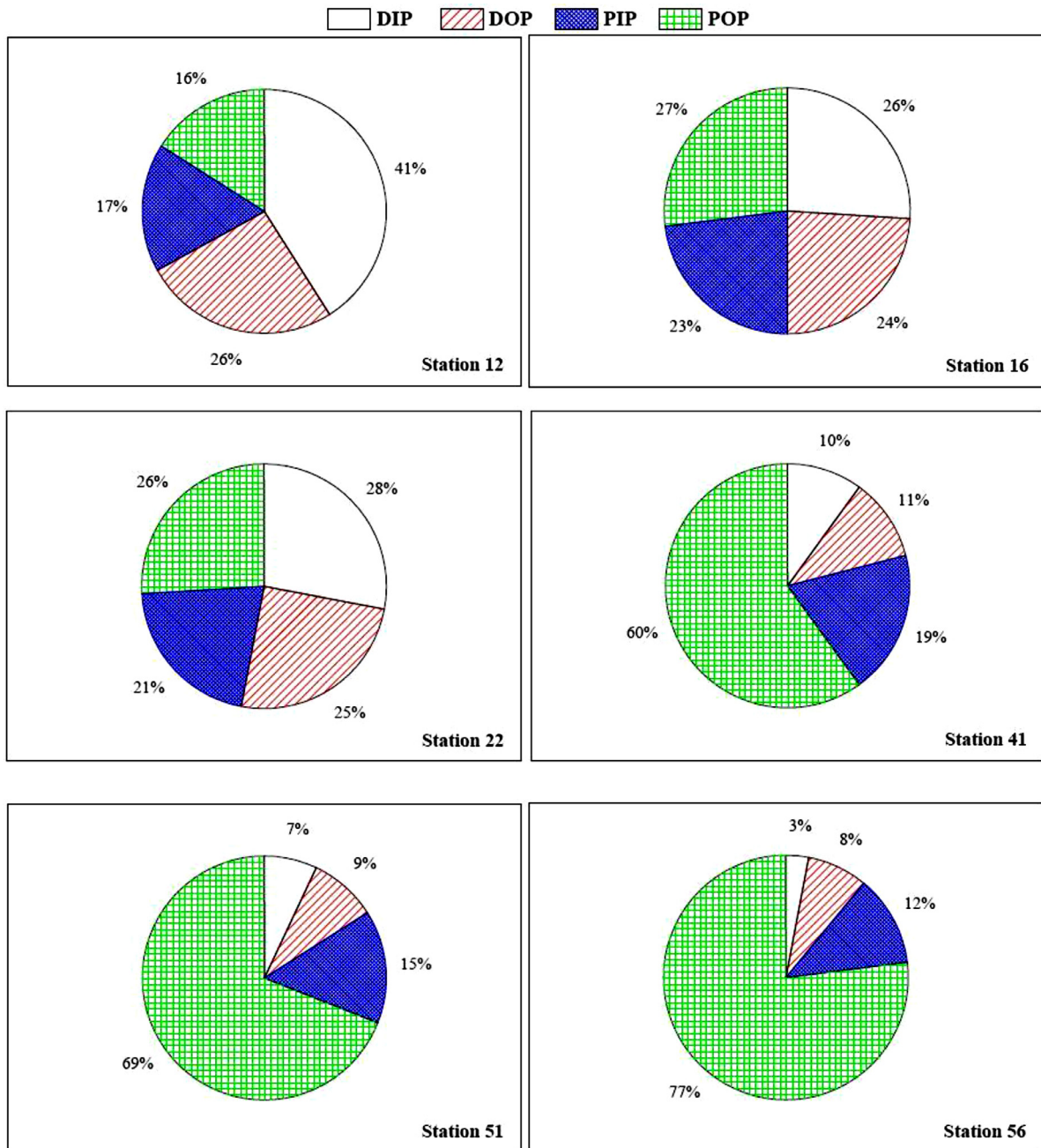


Fig. 4. Partitioning of P among dissolved inorganic P (DIP), dissolved organic P (DOP), particulate inorganic P (PIP), and particulate organic P (POP) along the river-bay transect from the lower Fox River (Stations 12 and 16) to open Green Bay (Station 56).

Table 4

Concentrations of inorganic and organic P in different operationally defined size-fractions, including the < 1 kDa low-molecular-weight and the 1–3 kDa, 3–10 kDa, and 10 kDa–0.7 μm colloidal or high-molecular-weight phases, as well as their abundances (in %) in the traditional dissolved phase (<0.7 μm) in the lower Fox River–Green Bay system (B.D. denotes below detection limit).

Station ID	Dissolved inorganic phosphorus				Dissolved organic phosphorus			
	< 1 kDa μM (%)	1–3 kDa μM (%)	3–10 kDa μM (%)	10 kDa–0.7 μm μM (%)	< 1 kDa μM (%)	1–3 kDa μM (%)	3–10 kDa μM (%)	10 kDa–0.7 μm μM (%)
12	4.00 (96.4%)	0.08 (1.9%)	0.04 (1.0%)	0.03 (0.7%)	1.24 (48%)	0.66 (25%)	0.40 (15%)	0.31 (12%)
16	1.82 (93.8%)	0.02 (1.0%)	0.05 (2.6%)	0.05 (2.6%)	0.95 (54%)	0.28 (16%)	0.44 (25%)	0.09 (5%)
22	1.85 (92.5%)	0.06 (3.0%)	0.05 (2.5%)	0.04 (2.0%)	1.20 (67%)	0.05 (3%)	0.24 (14%)	0.29 (16%)
41	0.37 (97.4%)	0.01 (2.6%)	B.D.	B.D.	0.14 (35%)	0.03 (8%)	0.07 (17%)	0.16 (40%)
51	0.22 (100%)	B.D.	B.D.	B.D.	0.14 (45%)	0.02 (7%)	0.06 (19%)	0.09 (29%)
56	0.11 (100%)	B.D.	B.D.	B.D.	0.10 (42%)	0.01 (4%)	0.02 (8%)	0.11 (46%)

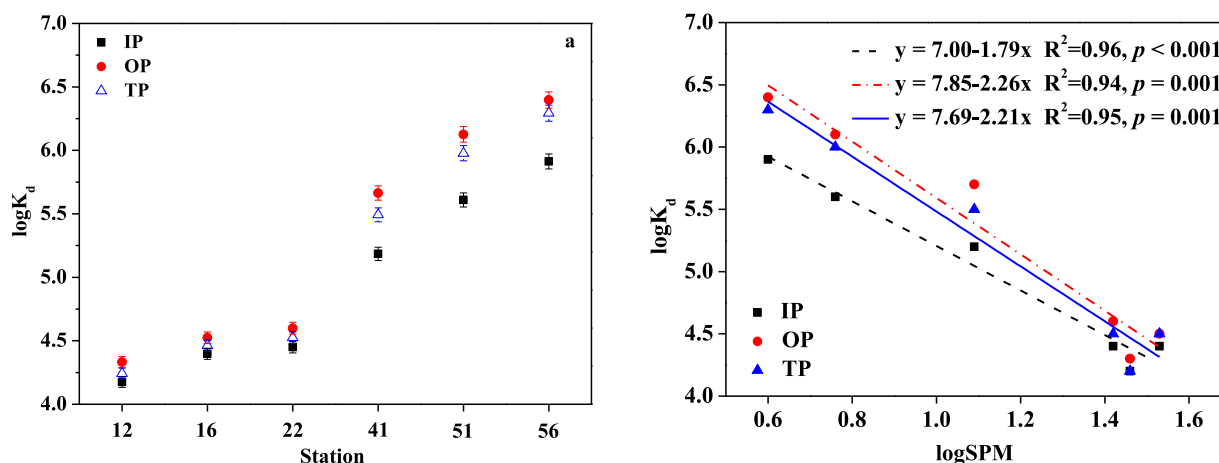


Fig. 5. Variations in the apparent distribution coefficient (K_d) of inorganic P (IP), organic P (OP) and total P (TP) between dissolved and particulate phases along the river-bay transect from the lower Fox River (Stations 12 and 16) to open Green Bay (Station 56) (left panel, error bars are mean $\pm$ SD) and the relationship between $\log K_d$ and suspended particulate matter concentration (in $\log SPM$) (right panel) in the lower Fox River-Green Bay ecosystem.

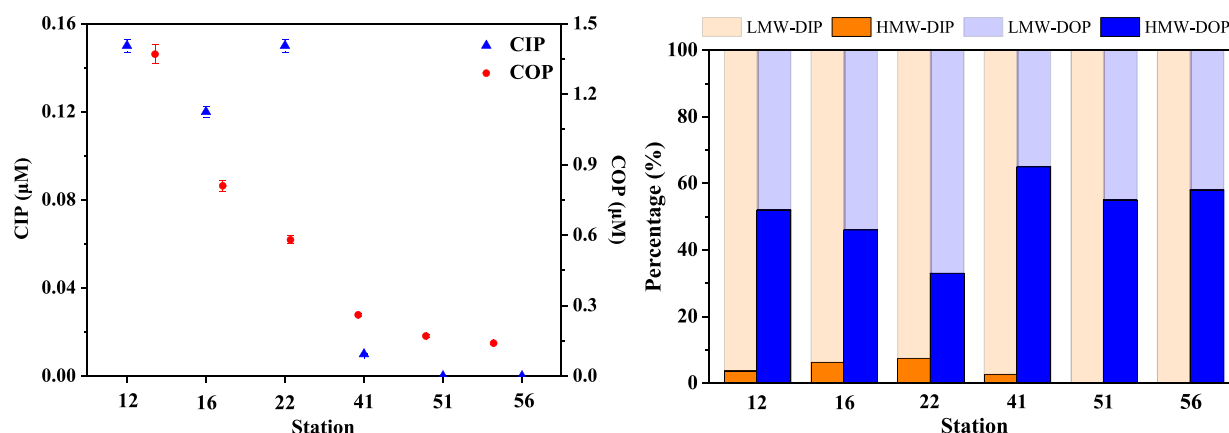


Fig. 6. Variations in the concentrations of colloidal inorganic P (CIP) and colloidal organic P (COP) (left panel, error bars are mean $\pm$ SD) and changes in the distribution of P between the <1 kDa (or LMW) and the >1 kDa (or HMW) phases in the traditional DIP and DOP pools (right panel) along the river-bay transect from the Fox River plume (stations 12, 16, 22) to open Green Bay (station 56).

4. Discussion

4.1. Comparisons in chemical speciation of phosphorus with other aquatic environments

Unlike rivers heavily affected by anthropogenic activities and having elevated DIP/TDP ratios (up to 0.9), such as the Maumee River (Baker et al., 2014) and the Mississippi River (Cai and Guo, 2009) that affected development of hypoxia in Lake Erie and the northern Gulf of Mexico, respectively, the Fox River had an intermediate DIP/TDP ratio (0.55 ± 0.05) and DOP/TDP ratio (0.45 ± 0.05) during the summer sampling period, which agrees well with previous observations (Table 5). These differences may be due to Lake Winnebago located upstream of the Fox River that converts much of the DIP to DOP prior to reaching the Fox River mouth or differences between sampling seasons. Relatively higher DOP/TDP ratios in the lower Fox River may exert a critical role in the development of eutrophication and seasonal hypoxia in Green Bay enhanced by the consumption of dissolved oxygen and the release of DIP during DOP and POP degradation P in the water column and sediment (Lin et al., 2016). Direct utilization of DOP by algae is also possible (Qin et al., 2015; Thompson and Cotner, 2018). The TDP/TP ratio was slightly higher than the TPP/TP in the Fox River, similar to those observed in the Milwaukee River (Table 5). The partitioning of P between organic and inorganic phases (DIP vs.

DOP or PIP vs. POP) and between dissolved and particulate phases (TDP vs. TPP) seems highly variable among rivers (Table 5), showing distinct features in the phase distribution of P.

The average concentrations of both TDP and TPP in the southern Green Bay were much higher than those in Lake Michigan (Table 5), indicating the effects of the Fox River on Green Bay (Robertson et al., 2018). Compared to other Laurentian Great Lakes, both southern Green Bay and Lake Erie had elevated TDP and TPP concentrations and similar partitioning between dissolved and particulate phases (Table 5), consistent with their environmental settings both with intensive anthropogenic influence, non-point sources, and seasonal hypoxia (Wynne et al., 2010; Kane et al., 2014; Zhou et al., 2015). It is likely that high TPP abundance especially POP and oxides/mineral-associate P, which may be converted into bioavailable or potentially bioavailable P species during their transport and sedimentary diagenesis (Paytan et al., 2017; Lin et al., 2018; Depew et al., 2018; Liu et al., 2020), may help contribute to the development of eutrophication and hypoxia in Green Bay and Lake Erie.

4.2. Sources of P species and dissolved/particulate organic matter

In general, high LMW-DIP and LMW-DOP concentrations were observed in river waters but decreased sharply in the estuarine area and remained low in open bay waters (Supplementary Ta-

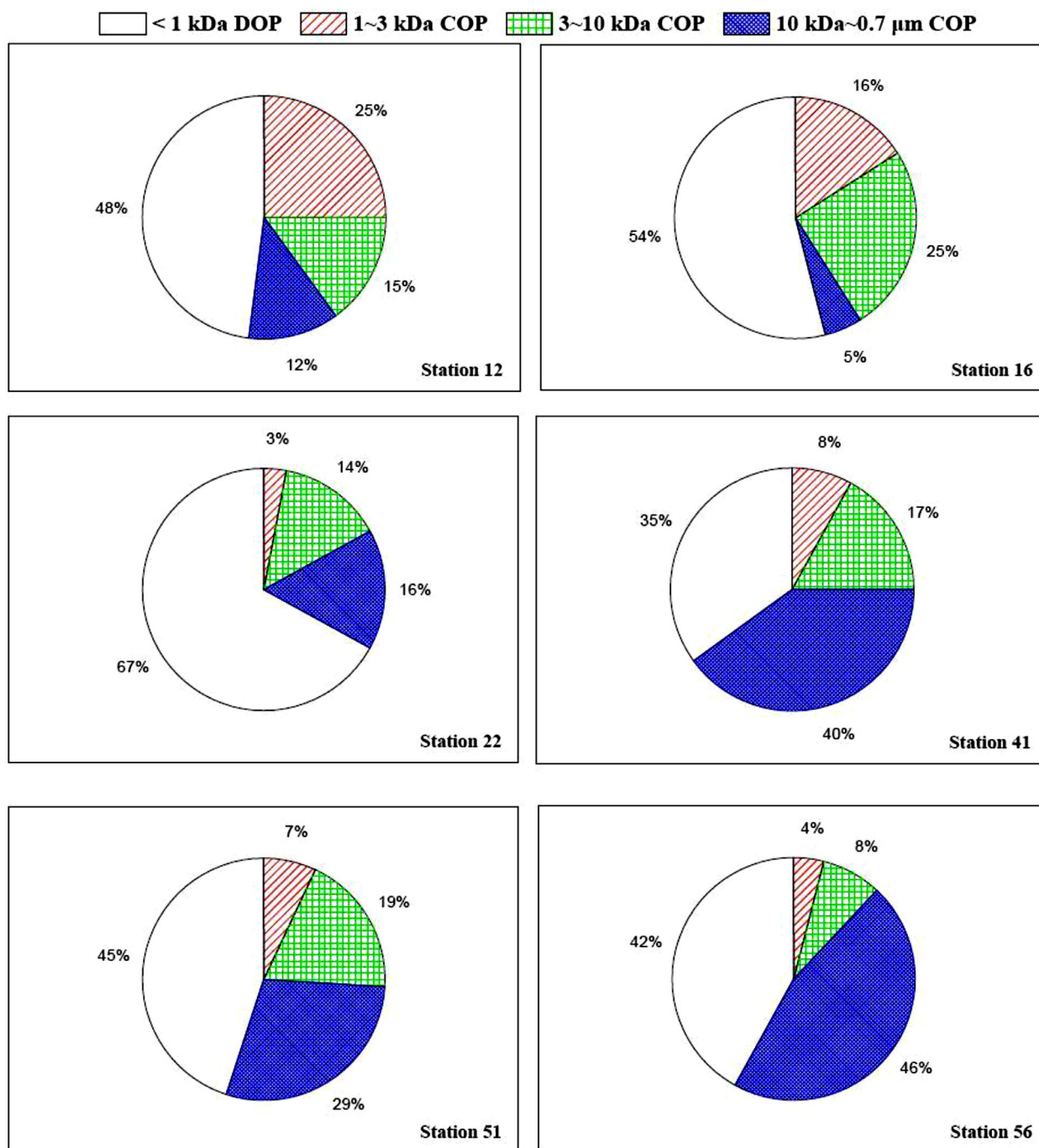


Fig. 7. Changes in the partitioning of organic P among the <1 kDa, 1–3 kDa, 3–10 kDa, and 10 kDa–0.7 μm molecular size-fractions in the traditional DOP pool along the river-bay transect from the lower Fox River (Stations 12 and 16) to open Green Bay (Station 56).

ble S2), indicating a strong riverine source for both LMW-DIP and LMW-DOP. Although the concentrations of each P species changed differently, environmental consistency exists in the size distributions of both inorganic and organic P pools along the river-bay transect regulated by physicochemical and biological processes (Figs. 2 and 3).

The molar ratios of DOC/DOP (or organic C/P ratio) ranged from 333 in the river end-member water to 1838 in open bay water (Table 2), which are considerably higher than the Redfield ratio of 106 (Redfield et al., 1963), suggesting either an overall P-depleted DOM pool or DOM in the study area containing mostly highly degraded organic components. Due to the prefer-

ential degradation of P-containing organic compounds, highly degraded organic matter is expected to have a higher C/P ratio in comparison with the freshly produced organic matter in aquatic systems (Sannigrahi et al., 2006; Cai and Guo, 2009; Yates et al., 2019). On the other hand, highly degraded soil organic matter in a semi-closed environment has a lower organic C/P ratio due to the loss of OC in the form of CO₂ and the re-assimilation of phosphate into soil organic matter pool (Dou et al., 2017; Gao et al., 2018). Therefore, DOM with lower C/P ratio in river water suggests sources from eroded soil organic matter, while autochthonous DOM with higher C/P ratio in open bay waters was indicative of rapid recycling and preferential regeneration of P relative to C.

Table 5

Comparisons in the concentrations of DIP, DOP, PIP, and POP and their partitioning in the total P (TP) pool in surface waters of different rivers and lakes (when available, the average concentrations or percentages and their standard deviations are given).

Study area	DIP (μM)	DOP (μM)	DIP/TP (%)	DOP/TP (%)	PIP (μM)	POP (μM)	PIP/TP (%)	POP/TP (%)	References
<i>Rivers</i>									
Scheldt estuary	3.3	0.7	36	8	4	1.2	43	13	van der Zee et al. (2007)
Chena River	0.149 $\pm$ 0.066	0.060 $\pm$ 0.063	19 $\pm$ 9	7 $\pm$ 4	0.6 $\pm$ 0.3		74 $\pm$ 10		Cai et al. (2008)
Changjiang River	0.2–1.0	0.18–0.49	6	8	0.62–6.5		86		Duan et al. (2008)
Mississippi River	2.89 $\pm$ 1.15	0.345 $\pm$ 0.148	34	4	3.45	1.81	41	21	Cai and Guo (2009) Shim et al. (2012)
Yukon River	0.145 $\pm$ 0.042	0.113 $\pm$ 0.018	7.8 $\pm$ 6.9	4.2 $\pm$ 4.0	5.85 $\pm$ 6.36		88 $\pm$ 10		Guo et al. (2012)
Jourdon River	0.03	0.16	3	14	0.07	0.9	6	77	Lin et al. (2012)
Jiulong River	2.14	0.55	27	7	3.95	1.16	51	15	Lin et al. (2013)
Maumee River	2.74	0.07	16	0.3	14.26		84		Baker et al. (2014)
Milwaukee River	0.69–3.05 (1.81 $\pm$ 1.19)	0.11–0.91 (0.54 $\pm$ 0.38)	28–76 (47 $\pm$ 21)	3–25 (16 $\pm$ 9)	0.40–0.71 (0.58 $\pm$ 0.15)	0.34–1.03 (0.68 $\pm$ 0.31)	13–22 (17 $\pm$ 5)	9–27 (20 $\pm$ 8)	Lin and Guo. (2016a)
Dafengjiang River	0.22–1.59 (1.06 $\pm$ 0.46)	0.10–0.91 (0.45 $\pm$ 0.26)	58	27	0.10–0.32 (0.23 $\pm$ 0.06)		15		Yang et al. (2018)
Warnow River	1.1 $\pm$ 0.69	0.3 $\pm$ 0.34	34	9.4	0.5 $\pm$ 0.29	1.3 $\pm$ 0.57	16	41	Rönspeiß et al. (2020)
Fox River	1.94–4.15 (2.70 $\pm$ 1.26)	1.76–2.61 (2.05 $\pm$ 0.49)	26–41 (32 $\pm$ 8)	24–26 (25 $\pm$ 1)	1.48–1.78 (1.63 $\pm$ 0.15)	1.61–1.99 (1.82 $\pm$ 0.19)	17–23 (20 $\pm$ 3)	16–27 (23 $\pm$ 6)	This study
<i>Green Bay and Laurentian Great Lakes</i>									
Green Bay	0.24 $\pm$ 0.14	0.32 $\pm$ 0.08	7 $\pm$ 4	9 $\pm$ 2	0.53 $\pm$ 0.18	2.33 $\pm$ 0.07	15 $\pm$ 4	69 $\pm$ 9	This study
Lake Michigan	0.011 $\pm$ 0.007	0.111 $\pm$ 0.071	7 $\pm$ 7	76 $\pm$ 14	0.008 $\pm$ 0.004	0.024 $\pm$ 0.011	3 $\pm$ 1	13 $\pm$ 8	Lin et al. (2016)
Lake Superior	0.049 $\pm$ 0.021		62 $\pm$ 9		0.029 $\pm$ 0.010		38 $\pm$ 9		Lin et al. (2016)
Lake Erie	0.173 $\pm$ 0.068		42 $\pm$ 14		0.254 $\pm$ 0.119		58 $\pm$ 14		Lin et al. (2016)

Phosphate has a molecular mass of 95 Da and is expected to occur only in the < 1 kDa LMW phase. Nevertheless, a detectable level of bulk CIP has been reported for semiarid soils surface runoff and water extracts (Sinaj et al., 1998; Turner et al., 2004) and small black water rivers, such as the Pearl River, Mississippi (Cai and Guo, 2009) and Jourdan River, Mississippi (Lin et al., 2012), but not detectable in Mississippi River waters (Cai and Guo, 2009). The detectable CIP in the lower Fox River could also be the result of DIP associated with Fe-oxyhydroxides and mineral colloids due to the particle-reactive nature of P and its high K_d values (Fig. 5; Turner et al., 2004; Lin et al., 2018; River and Richardson, 2019). As river water mixes with bay water, the abundance of CIP decreased from detectable in river waters to undetectable in open bay waters (Fig. 6). The variation in CIP concentration along the Fox River plume was similar to that of PIP, suggesting an active exchange between colloidal and particulate phases during estuarine mixing and biological uptake.

The concentration of COP was the highest in river water and decreased consistently out into the bay (Fig. 6), showing a predominant COP source from the Fox River, similar to terrestrial DOM (DeVilbiss et al., 2016). The decrease in COP abundance and its percentage in the bulk DOP pool along the river-bay transect indicates the occurrence of disaggregation or peptization of riverine colloidal organic matter (COM) resulting in an increase in the percent of LMW-DOP but a decrease in percent of COP from river to inner bay (Fig. 6). The dynamic change in the partitioning of P between LMW and colloidal phases is consistent with the decrease in specific conductivity from river water to estuarine water in this negative estuary (Xu et al., 2018a). In the open Green Bay, however, the percentage of COP increased again showing an even higher percent of COP compared to those in the river plume (Fig. 6). This dynamic variation in COP abundance indicated new

COP from autochthonous sources via *in situ* biological production or the release of algal exudates in open waters, similar to those observed in coastal marine environments (Pakulski et al., 2000; Lin et al., 2012). The COP abundance observed in open Green Bay (59 $\pm$ 5%) was considerably higher than those in Lake Michigan (23 $\pm$ 5%, Lin and Guo, 2016a), consistent with their different trophic conditions, hypereutrophic in Green Bay vs. oligotrophic in Lake Michigan (Cuhel and Aguilar, 2013; Zhou et al., 2016; Klump et al., 2018).

Particulate P has been shown to be the dominant P species in other rivers and estuaries (van der Zee et al., 2007; Cai et al., 2008; Duan et al., 2008; Guo et al., 2012); therefore, contributing potentially to the release of DIP and DOP into the water column. Terrestrially derived particulate P typically has higher abundances of PIP compared with the autochthonous particulate P in natural waters (Yoshimura et al., 2007; Asahi et al., 2014). Thus, higher PIP/TPP (ranging from 44% to 53% with an average of 47 $\pm$ 5%) at the river/estuarine stations (stations 12, 16 and 22) is consistent with a higher contribution from terrestrially derived PIP to the particulate P budget from the Fox River, while lower PIP/TPP (18 $\pm$ 5%) but higher POP/TPP (82 $\pm$ 6%) in open bay waters (Table 3) suggests a major particulate P source from algal-derived or preexisting POP. Indeed, the POC/PN ratios of particulate organic matter (POM) at the river stations (8.1–9.3, Table 3) closely resembled those of soil organic matter (Kendall et al., 2001), attesting to a dominant terrestrial POM source. On the other hand, the average particulate C/N ratio (6.5 $\pm$ 0.1) or particulate organic C/P ratio (105 $\pm$ 2) in open bay waters is close to the Redfield ratio (C/N = 6.6, C/P = 106), indicating mostly autochthonous POM sources. Furthermore, values of $\delta^{13}\text{C}$ -POC decreased from -27.47‰ in the Fox River to -32.97‰ in open Green Bay, while $\delta^{15}\text{N}$ -PN values increased from 2.58‰ in river water to 12.41‰ in open bay water (Table 3),

showing a dynamic change in POM sources from dominantly terrigenous sources with higher $\delta^{13}\text{C}$ -POC and lower P contents (in mg-P/g) in the river/estuary region to mostly autochthonous origin with lower $\delta^{13}\text{C}$ -POC and higher P contents (in mg-P/g) in open Green Bay (Kendall et al., 2001). The increase in the particulate P content (in mg-P/g) from river to bay waters (Fig. 2c) likely reflect the net result between sinking of denser soil/mineral particles with low P concentrations, sorption of DIP onto particles, and biological uptake of P. For example, POP content (in mg-P/g) increased over three times from river to bay waters suggesting a distinct change in sources of particulate P from largely terrestrial particles (soil/mineral) with low P content to more autochthonous particles (algae) with higher P content in open bay waters. The dynamic changes in particle quantity and quality are also consistent with the increasing POP fraction in the total P pool along the trophic transect (Fig. 4). The transformation/partition between PIP and POP (Supplementary Fig. S2a) and the abundance of DOP and COP were highly related to SPM, which was mostly derived from allochthonous (at stations 12, 16 and 22) or autochthonous (at stations 41, 51 and 56) sources. In addition, the higher the Chl-*a* concentration the higher the DOP and COP concentrations in the water column, as supported by the correlation between the COP and Chl-*a* concentrations (Supplementary Fig. S2b).

4.3. The importance of colloidal phosphorus

High abundances of colloidal P and its role in the biogeochemical cycling of P have been reported in soil solution and river/estuarine waters (Hens and Merckx, 2002; Shand et al., 2000; Turner et al., 2004; Cai and Guo, 2009; Lin et al., 2012). Colloidal P, especially the higher molecular weight COP, have different environmental fate and play more active roles than other forms of P (especially dissolved P in which it is typically considered) in the biogeochemical cycling of P in aquatic environments (Fig. 3; Haygarth et al., 1997; Lin et al., 2012). In addition to the abundances of bulk colloids, knowledge about detailed size distributions of colloidal P and their changes from river to estuarine/coastal waters is needed.

The percent of CIP in the DIP pool ranged from < 10% in the river/inner bay waters to undetectable in open bay waters (Table 4), indicating that DIP occurs mainly in the <1 kDa LWM phase even in Fox River waters. Low CIP abundance in the Fox River is consistent with those observed in the Mississippi River (Cai and Guo, 2009) and the Milwaukee River (Lin and Guo, 2016a), but lower than those reported for small black water rivers in the Gulf coast (Cai and Guo, 2009; Lin et al., 2012) or soil solutions (Hens and Merckx, 2002; Turner et al., 2004). The difference in CIP abundance among rivers is probably due to differences in water chemistry, soil type, and the abundance of DOM, iron-containing and other inorganic colloids (Stolpe et al., 2010; Baken et al., 2016; Gottselig et al., 2017). In contrast to low or undetectable CIP abundance, a major fraction of DOP was measured in the HMW-DOP or COP forms in the study area (Table 4; Fig. 6). On average, COP comprised from $49 \pm 4\%$ of the bulk DOP pool in river waters to as high as $59 \pm 5\%$ in open bay waters, showing an initial decrease from 52% in river water to 33% in the mixing zone, and then an increase from river plume to open Green Bay (Fig. 6). These results suggest two different COP sources with terrestrial COP from river water and autochthonous COP (algal exudates) from open bay water. In addition, the terrestrially derived COP appeared to be rapidly removed at the river-lake interface and/or an active exchange between colloidal and dissolved phases during estuarine mixing, similar to that observed in a marine estuarine environment (Lin et al., 2012).

In addition to bulk colloids, previous studies also reported the molecular size-dependent abundance and composition of colloidal

organic matter in surface waters (Xu and Guo, 2017), as also evidenced by the DOC/DOP ratio in the Fox River plume (Table 2). Within the bulk COP pool, the large sized colloidal fractions (both the 3–10 kDa and 10 kDa–0.7 μm) increased gradually from the Fox River to open Green Bay, while the 1–3 kDa small COP decreased along the same transect (Fig. 7). These changes in COP abundance seemed to support a dynamic coagulation and peptization of colloids regulated by DOM and ionic strength (Mosley et al., 2003; Xu et al., 2018b). On the other hand, changes in the colloidal size distribution of DOP along the river-bay transect could be mostly the result of shifting in colloidal sources and freshness of colloids across the hydrologic/trophic interface along the river-bay transect. For example, freshly photosynthesized and/or liberated DOP or DOM would be mainly HMW materials (Qu et al., 2012; Benner and Amon, 2015). Therefore, the assimilation and utilization of DIP by phytoplankton in open water could generate fresh DOP, increasing the COP abundance and the larger sized colloids (Fig. 7). These results indicated that the colloidal size distribution of DOP is source-specific and regulated by environmental settings (e.g., pH and ionic strength) as well as biological and physicochemical processes. Overall, COP in the Fox River is mainly allochthonous in nature and composed of mostly smaller sized colloids (from highly degraded soil DOP), while COP in open bay waters is predominantly autochthonous and contains mostly larger sized colloids (from algae and their exudates) (Fig. 7).

There was a significant negative correlation ($p < 0.01$) between COP and POP in the Fox River plume (Supplementary Fig. S3), indicating that surficial processes and colloid-colloid or particle-particle interactions, such as, flocculation/coagulation of colloids and disaggregation of particles, may play a significant role in controlling the partitioning and thus the biogeochemical cycling of P species in the water column. Indeed, the HMW-DOM or colloidal organic matter has been shown to have higher biological reactivity or degradation rates relative to its LMW counterparts (Benner and Amon, 2015) and higher chemical reactivity such as short residence time and young ^{14}C ages (Guo and Santschi, 1997; Guo et al., 2009) in aquatic environments. If fractions of the bulk colloidal organic P are biologically and chemically more reactive (Qin et al., 2015), the strong presence of COP observed here has implications for understanding P cycling and its relation to the development of eutrophication and seasonal hypoxia in coastal environments.

As shown in Fig. 5, values of $\log K_d$ for all three P phases decreased with increasing SPM concentrations, with a significant inverse correlation with $\log \text{SPM}$, consistently showing a “particle concentration effect” and similar to those observed for particle-reactive elements in different aquatic environments (Honeyman and Santschi, 1988; Santschi et al., 1995; Guo et al., 1997). Overall, consistently high $\log K_d$ values observed for different P species here again attest to its particle-reactive nature and the important role of colloidal phases and colloid related physicochemical processes, such as adsorption/desorption and coagulation/disaggregation in controlling the distribution, transport, and cycling pathways of P in aquatic environments during summer although biological processes are important in the production and consumption of colloids (Wang and Guo, 2001). Future studies could focus on elucidating the composition and reactivity of colloidal P and its role in the cycling of P, especially during other seasons and flow regimes that would enable the summer period that was described in detail here to be placed in an annual context, and focus on the relative importance of different biological and physicochemical processes.

5. Conclusion

Chemical speciation, phase partitioning, and transformation of P between dissolved, colloidal, and particulate phases were exam-

ined in the lower Fox River-Green Bay ecosystem, in northwest Lake Michigan, during midsummer. In general, concentrations of DIP, DOP, PIP, CIP and COP decreased but POP increased along the Fox River-Green Bay transect, with dynamic variations in the chemical speciation and partitioning of P. Within the total dissolved and particulate P pools, DIP and PIP were the dominant P species in river waters, while DOP and POP became predominant in the open Green Bay, showing highly variable P speciation and intensive transformation between P species through biological and physicochemical processes across the river-bay interface. Together with dynamic changes in $\delta^{13}\text{C}$ -POC signatures, the observed distinct particulate properties indicate a significant change in POM sources from largely terrigenous with low P contents (in mg-P/g) and higher C/N ratios and $\delta^{13}\text{C}$ -POC values in the Fox River to more autochthonous particles or algae with higher P contents (in mg-P/g) and lower C/N ratio and $\delta^{13}\text{C}$ -POC values in the open Green Bay.

The abundance of CIP was in general low and became undetectable in open bay water, showing DIP (or SRP) is exclusively present in the < 1 kDa LMW-phase. In contrast, COP abundances were high in both river water and open bay water, indicating source of terrigenous COP from the Fox River and autochthonous COP from open bay water. Within the bulk COP pool, highly degraded smaller sized-colloids (1–10 kDa) dominated in river water, while larger sized colloids (10 kDa–0.7 μm) dominated in open Green Bay and were mostly derived from algal exudates, showing a variable and highly heterogeneous DOP pool across the river-bay interface. High $\log K_d$ values were observed for both inorganic and organic P in the study area, demonstrating an evident “particle concentration effect” on the partitioning of P between dissolved and particulate phases and suggesting colloids/particles and thus surficial processes may play an important role in governing the abundance, speciation, and bioavailability of P in aquatic environments.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

We are grateful to colleagues from the Green Bay Metropolitan Sewerage District for their help in field sampling and data collections. We thank Patrick Anderson for his technical assistance in the measurements of stable isotopic composition and Jessica Garrett and two anonymous reviewers for their constructive comments/suggestions which greatly improved the manuscript. This work was supported in part by grants from University of Wisconsin-Milwaukee (DIG-101X405), the National Natural Science Foundation of China (No. 41706083), the Guangxi Natural Science Foundation (2018GXNSFDA281025), Guangxi “Marine Ecological Environment” Academician Workstation Capacity Building (No. Gui Science AD17129046), and the Distinguished Experts Program of Guangxi Province. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2021.117025](https://doi.org/10.1016/j.watres.2021.117025).

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