



Phosphorus Forms in Sediments of a River-Dominated Estuary

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Estuaries are biologically productive transition zones between land and sea that play a vital role in transforming, recycling, and sequestering nutrients and organic matter, thus influencing nutrient loading to coastal systems. Yet, the processes involved in phosphorus (P) transformation and cycling among inorganic and organic P forms are poorly known in estuaries. To better understand the potential for P transformation and sequestration, we identified P forms and estimated their contributions to total P in intertidal wetland sediments of a river-dominated estuary (Columbia River, Oregon, USA) using solution ³¹P nuclear magnetic resonance spectroscopy (P-NMR). Inorganic P forms dominated sediment P extracts throughout the estuary, with orthophosphate accounting for 71–84% of total extracted P. However, biologically-derived inorganic and organic P forms were also detected. Polyphosphates were found in sediment extracts throughout the estuary, contributing as much as 10% of extracted P. Similar to other wetlands, orthophosphate monoesters and diesters made approximately equal contributions (~ 20%) to total extracted P. However, monoesters (e.g., phytate) were more abundant in sedimentary environments characterized by low organic matter content, while diesters (e.g., DNA) were more abundant in sedimentary environments with high organic matter, regardless of salinity. Collectively, the data show strong evidence for P transformation in sediments of a large, river-dominated estuary, which influences its transport to the coastal Pacific Ocean via the expansive Columbia River plume.

Keywords: phosphorus, ³¹P-nuclear magnetic resonance, sediments, river, estuary

INTRODUCTION

Phosphorus (P) is an essential but often limiting nutrient in freshwater aquatic and terrestrial ecosystems (Daniel et al., 1998); however, downstream systems are increasingly under threat from nutrient-stimulated eutrophication (Conley et al., 2009; Tiessen et al., 2011). Watershed losses result from inefficient recycling, or poor sequestration, and lead to P transport from soils to surface waters; ultimately, excess P ends up in estuaries and coastal systems where it often results in nutrient-driven eutrophication. Eutrophication can lead to hypoxia, acidification, and harmful algal blooms (Paerl, 2006; Bricker et al., 2008) and represents a growing worldwide threat to human health and well-being (Millennium Ecosystem Assessment, 2006).

Estuaries are biologically productive transition zones comprising a variety of habitats including wetlands, mudflats, and seagrass beds between freshwater and marine systems. They play a vital role in transforming, recycling, and sequestering nutrients and organic matter (OM) prior to entering

the coastal ocean (Barbier et al., 2011). Tidal mixing of fresh and saltwater within estuaries leads to particle flocculation (Sholkovitz, 1976) and the localized resuspension of bottom material in turbidity maxima (van Beusekom and Brockmann, 1998; Small and Prahl, 2004). Combined with downstream transport in rivers, estuaries are vulnerable to nutrient pollution because dissolved inorganic P (orthophosphate) desorption from sediment surfaces occurs when anions in seawater compete with phosphate anions for binding sites, leading to P efflux (Fox et al., 1986; Froelich, 1988; House and Warwick, 1999; Monbet et al., 2010). Moreover, the reducing conditions associated with intense OM remineralization increase P desorption, setting up a positive feedback loop whereby eutrophic conditions contribute to P mobilization and limit nutrient sequestration (Howarth et al., 2011; Li et al., 2013). This impairs the critical function of estuaries and wetlands, which is to reduce nutrient loads before they reach coastal waters (Barbier et al., 2011).

Phosphorus retention, or sequestration, in wetlands can occur via chemical, physical, or biological means. For example, orthophosphate is retained in sediment pore waters through adsorption from the water column, particularly in sediments dominated by clays and minerals (Reddy and DeLaune, 2008). Phosphorus (P) buffering is defined as the equilibrium reached between adsorption and desorption of orthophosphate at the water/sediment interface, which reduces variability in orthophosphate concentrations irrespective of input or removal processes (Froelich, 1988). High concentrations of inorganic P and minerals (i.e., Fe, Al, Ca, and Mg) in sediments result in the formation of both soluble and insoluble P compounds, predominantly Fe and Al minerals in acidic soils and Ca compounds in near-neutral to alkaline soils (Stevenson, 1986; Reddy et al., 1999; Richardson and Reddy, 2013). However, seasonality in hydrology, including flooding and drying, produces changes in soil redox conditions, which play a governing role in transformation, storage, and transport of P in wetlands.

In comparison, organic P in wetlands is associated with living systems including plants, microbes, detritus and organic matter, and commonly accounts for large fractions of total P (Vaithiyathan and Richardson, 1997; Reddy et al., 1999). Although plant storage material is often the dominant source of organic P in wetland soils (Reddy and DeLaune, 2008), soil microbes play a key role in controlling degradation of organic matter and cycling of P in wetlands. For example, microbes secrete enzymes that readily mineralize organic P and can quickly assimilate orthophosphate released into pore water (Richardson, 1985; Kellogg and Bridgham, 2003; Noe et al., 2003). Microbes also regulate P in sediments through biomass, which can account for up to 25–50% of the total P in wetland mineral sediments (Wright et al., 2001; McDowell and Sharpley, 2003) and up to 70% of total P in hydrologically isolated sediments (Williams and Silcock, 2001).

Because the mobility and retention of P depends upon its chemical forms, the determination of chemical structures is critical for identifying possible sources and for estimating the potential for retention, sequestration, and transformation (Celi and Barberis, 2005; Condrón et al., 2005; Quiquampoix and

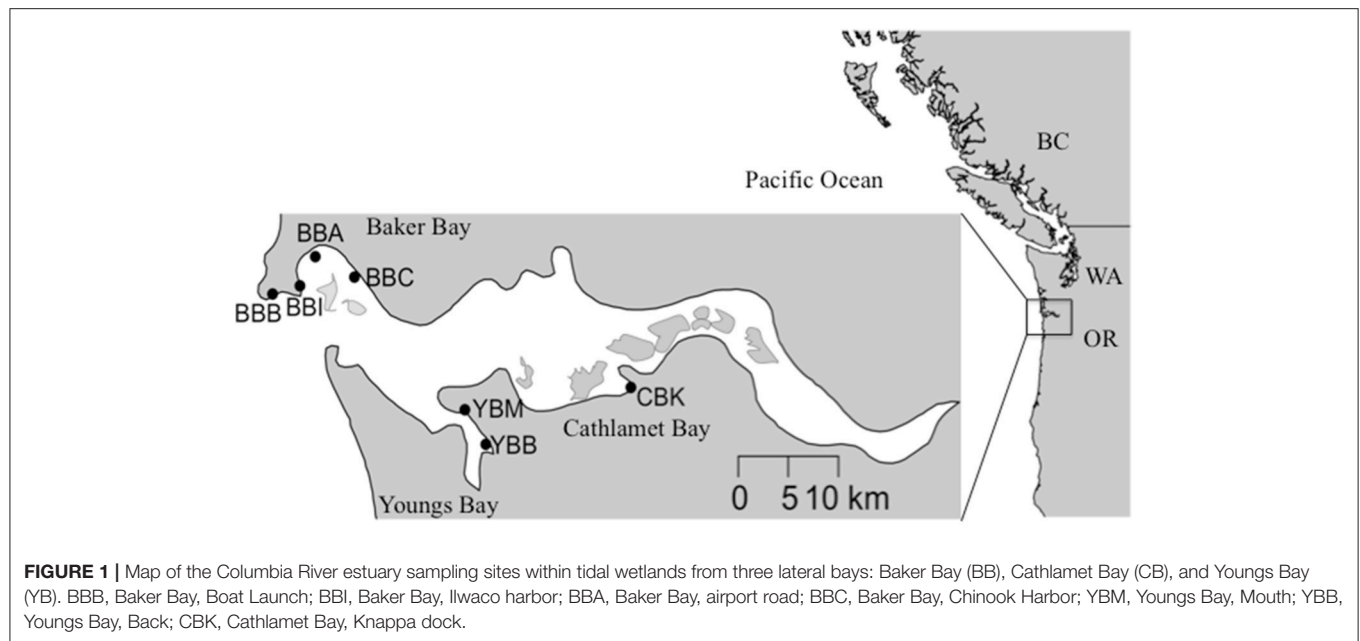
Mousain, 2005). The goal of this study was to investigate P forms and their contributions in a mesotidal, river-dominated system characterized by rapid flushing and short water residence times (1–5 d; Chawla et al., 2008; Columbia River estuary, Oregon, USA). Previous work in this estuary showed that water-column dissolved inorganic phosphate (DIP) exhibited seasonal non-conservative increases along a salinity gradient, suggesting the presence of P sources that were not accounted for when orthophosphate was measured in river and ocean end members (Gilbert et al., 2013). To address the knowledge gap, our goal was to determine what P forms are present in the peripheral bay sediments that might contribute to fluxes of DIP and to characterize variability in estuarine P forms as a function of sediment properties.

MATERIALS AND METHODS

Field Sites

The Columbia River is the second largest by discharge in the USA (Sherwood et al., 1990), culminating in a river-dominated, salt-wedge estuary with strong seasonality in salinity and biogeochemical properties (Roegner et al., 2011). The U.S. Pacific Northwest coast is strongly influenced by summer upwelling when northerly winds promote the offshore transport of surface waters, which are replaced by nutrient-rich subsurface oceanic water (Huyer, 1983; Grantham et al., 2004). The Columbia River discharge varies with season, peaking at $>10,000 \text{ m}^3 \text{ s}^{-1}$ during the snowmelt-driven spring freshet (April–June) and declining to $<2,000 \text{ m}^3 \text{ s}^{-1}$ in the dry season (July–October). Salt intrusion into the estuary is greatest during periods of low discharge, which typically occur coincidentally with upwelling-favorable conditions between June and September (Chawla et al., 2008). The estuary exchanges the majority of water in north and south paths that run along the peripheral bays, including Baker Bay (north) and Youngs and Cathlamet Bay (south; **Figure 1**).

Compared to the estuary at large, the three peripheral bays included in this study share some similarities in terms of sediment characteristics (Sherwood et al., 1984; Sherwood and Creager, 1990); however, there are differences among them in terms of the composition (i.e., percent silt and clay vs. sand) and grain size. Although Baker and Youngs Bays receive considerable deposits of fine sand, silts, and clays, the sources of sediment deposits differ (Sherwood and Creager, 1990; Simenstad et al., 1990). Baker Bay and Cathlamet Bay receive fine sediments from suspended material, while Youngs Bay received fortnightly inputs of fine sediment from ephemeral estuarine turbidity maximum events (Sherwood and Creager, 1990). In addition, there are seasonal differences in depositional patterns, with the western side of Baker Bay receiving coarser sediment deposits than the east side in February and June. In Youngs Bay, the upstream reaches tend to accumulate a combination of long-term deposits of coarse and fine sediments, with the latter dominating on the fringes. Closer to the estuary's south channel, sediment deposits are coarser than in the other peripheral bay areas. Salinity varies throughout the year in peripheral bays as a function of river discharge; Baker Bay on the northern side of the estuary is mesohaline (i.e., brackish), with a range of 5–20 practical salinity



units (PSU) during periods of relatively low flow (i.e., summer and autumn), while Youngs and Cathlamet Bays on the southern side of the estuary are oligohaline (0–5 PSU). The furthest upriver site, Cathlamet Bay, includes a diverse region of islands, tidal flats and marshes that experience seasonal exposure to salinity at depth.

Sample Collection and Chemical Analysis

Sediment texture (percent clay, silt) was determined using the hydrometer method (AgSource Laboratories, OR) on sediment samples from August 2013 prior to the collection of samples for the analysis of P forms. From September 2013–August 2014, sediment samples for the analysis of P forms were collected 1–2 h before low tide at sites within Baker Bay (four sites: BBB, BBI, BBA, BBC), Youngs Bay (two sites: YBB, YBM) and Cathlamet Bay (one site: CBK; **Figure 1**), all on exposed mudflats lacking any emergent vegetation at low tide. Cores were collected by pressing 50 mL Falcon conical centrifuge tubes with the bottom cut off into sediments. A full tube represented a sediment volume of $\sim 43 \text{ cm}^3$ (with a core diameter of 2.3 cm and a length of 10 cm). The tubes were transported back to the laboratory on ice (~ 2 –6 h) and kept at -20°C pending analysis. For this study, sediment cores from March–April and May–August were coarsely sieved to remove large debris, air-dried in a fume hood, ground and homogenized prior to P-NMR extraction (Cheesman et al., 2013). Total P concentrations in the unextracted sediments were determined by ignition followed by extraction with dilute H_2SO_4 (SmartChem 170, Unity Scientific, MA). Following combustion, dissolved P from the sediment extracts was determined colorimetrically (Murphy and Riley, 1962) and total OM content was estimated by loss on ignition (LOI) at 500°C (Nelson and Sommers, 1996).

Samples collected from waters overlying sediments were filtered in the field by syringe (60 mL) through combusted (4 h

at 450°C) $0.7 \mu\text{m}$, 25 mm GF/F filters (Whatman, NY) into 60 mL acid-cleaned HDPE bottles (Nalgene) and transported to the lab on dry ice in a cooler, where they were stored at -20°C pending processing. Total nitrogen (N) and total P concentrations were determined on unfiltered water samples. A rapid flow analyzer (Astoria Analyzer, Astoria Pacific, Clackamas, OR) was used for colorimetric determination of dissolved nitrate+nitrite (NO_x), ammonium, nitrite, and DIP (here measured as molybdate-reactive orthophosphate) concentrations and for measurement of total N and P after alkaline persulfate digestion (US EPA, 1983a,b; Antweiler et al., 1996; Zimmerman and Keefe, 1997; Patton and Kryskalla, 2003). Further, salinity and temperature measurements were checked during time of sampling by *in situ* sensor platforms (SATURN04, Cathlamet Bay; SATURN07, Baker Bay; SATURN09, Youngs Bay) in the Columbia River from a publicly available online database (www.stccmop.org) maintained by the Center for Coastal Margin Observation and Prediction (Baptista et al., 2015).

Extraction of Phosphorus

Using a modification of the Cade-Menun and Preston (1996) procedure, approximately 3.0 g of air-dried sediment was suspended in 30 mL of NaOH-EDTA (0.25 M NaOH, 50 mM Na_2EDTA) and sonicated (Branson Digital, Cleveland, OH) for 1 min at 20 kHz ($\sim 55 \text{ W}$) in an ice bath followed by shaking at 150 rpm for 4 h. Samples were centrifuged for 10 min at $1,200 \times g$ and the supernatant was removed, neutralized with 10% HCl and frozen at -80°C prior to lyophilization. An aliquot of each NaOH-EDTA extract was analyzed for Al, Ca, Fe, Mg, manganese (Mn), and P (mg L^{-1}) by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using a Plasma 400 emission spectrophotometer (PerkinElmer, Waltham, MA).

Solution P-NMR

Lyophilized extracts were redissolved in a solution combining 0.6–0.65 mL each of D₂O, H₂O, NaOH-EDTA solution, and 10 M NaOH, vortexed, centrifuged at 1,500 × g for 20 min, and stored at 4°C within 12 h of analysis. If precipitate formed (observed in two samples, from BBA and BBC), it was filtered out using 0.2-μm polycarbonate syringe filters prior to analysis. Spectra were collected on a Bruker Avance 500 MHz NMR spectrometer with a 10 mm broadband probe. The NMR delay times were determined based on the ratio of concentrations of P/(Fe+Mn) within the extracts (McDowell et al., 2006). A temperature of 21°C and a 45° pulse were used, with a pulse delay of 4.5 s. Acquisition time was 0.5 s and 2,800–5,830 scans (~4–8 h) were collected. Samples were analyzed with no spinning and no proton decoupling.

Data Analysis and Statistics

The NMR spectra were processed using NMR Utility Transform Software (NUTS; Acorn NMR, Livermore, CA, 2000 edition). Processing included standardizing the orthophosphate peak for each sample at 6,000 ppm. Phosphorus forms (P forms) were identified by their chemical shifts using published libraries for known peaks, with spiking for confirmation (Cade-Menun, 2015). Spectra were processed with 7–10 Hz line broadening for full spectra and 3 Hz line broadening to identify peaks in the orthophosphate monoester and diester regions (Cade-Menun and Liu, 2014). Corrections were made to account for orthophosphate diester degradation by summing the peak areas associated with degradation products (α- and β-glycerophosphate and mononucleotides, degraded from phospholipids and RNA respectively), subtracting this sum from the total monoester area, and adding it to the total diester area (Young et al., 2013; Schneider et al., 2016).

A constrained ordination was performed on the transformed P forms (abundance) and sediment environmental data (TotalP, LOI, Fe, Mn, Ca, and Mg). A redundancy analysis (RDA) is an extension of principal components analysis (PCA) designed to identify or reduce complexity of environmental data. The principal components were constrained to be linear combinations of the environmental variables, with the goal being to explain variation of the dependent variables (P form abundance) as much as can be explained by independent variables (sediment properties; Legendre and Legendre, 1998; Ramette, 2007). Phosphorus forms and sediment data were normalized with centered log ratio (clr) transformations (Abdi et al., 2015). Statistical analyses were performed with R using the vegan package (R Core Team, 2015).

RESULTS

Chemical Analysis

Physicochemical characteristics associated with each sample are shown in **Table 1**. It should be noted that the small number of samples analyzed in this study limits spatial and temporal comparisons made across sites and environmental characteristics. Total sediment P concentrations varied by more than two-fold, with the highest value observed at CBK (1,366 mg kg⁻¹) and the lowest at BBC (601 mg kg⁻¹). The OM (LOI%) content mirrored total sediment P (11.3% at CBK, 2.2% at BBC). Sediment texture analysis demonstrated the largest percentage of clay and silt at the western side of Baker Bay (42.4%, BBB) and at the mouth of Youngs Bay (35%, YBM), which come from different sources in the estuary including fine suspended material and estuarine turbidity maxima events, respectively (Sherwood and Creager, 1990).

TABLE 1 | Physicochemical characteristics of water and sediments collected from the estuary.

Site	Date (Time)	Water [†]							Sediment [‡]		
		DIP μM	TotP μM	NO _x μM	TotN μM	NH ₄	Salinity PSU§	Temp °C§	Total P mg kg ⁻¹	LOI %	Clay + Silt %
CBK	4/29/14 (1:10)	0.59	1.5	27.0	39.3	3.14	0	11.7	1,366	11.3	13.4
YBM	3/28/14 (18:55)	0.30	1.2	1.64	21.7	1.68	3	10.8	712	3.8	35.0
YBB	3/28/14 (19:19)	0.94	5.1	36.4	80.7	4.10	1	10.8	1,221	9.6	–
BBB	3/28/14 (7:04)	0.54	13.7	24.7	114.7	2.61	4	9.4	853	6.5	42.4
BBI	4/29/14 (7:30)	0.85	1.9	5.64	30.2	4.82	4	13.8	630	2.5	15.8
BBC	5/29/14 (10:15)	0.98	2.9	1.20	29.8	3.25	6	15.1	601	2.2	11.8
BBA	8/25/14 (7:50)	4.79	10.5	0.10	56.9	16.0	18	17.9	752	1.2	13.4

[†]Water collected above the sediments; DIP, dissolved inorganic phosphorus (P); TotP, total P; NO_x, NO₃⁻+NO₂⁻; TotN, total dissolved nitrate/nitrite; NH₄⁺, ammonium; PSU, practical salinity units; and Temp, temperature.

[‡]Total P (sediment) mg kg⁻¹ dry sediment weight, and organic matter was calculated as loss-on-ignition (LOI); Clay and Silt were analyzed for sediments collected in August 2013.

§Salinity and temperature collected from local autonomous sensors (SATURN04, Cathlamet Bay; SATURN07, Baker Bay; SATURN09, Youngs Bay).

Monthly measurements of DIP, NO_x, and ammonium (NH₄) at the seven sites over a nine-month period (Jan–Sep 2014) are provided in Supplemental Materials (Figure S2). Only the measurements that coincide with the analysis of sediment P forms are indicated in Table 1. Water column DIP concentrations were low from March–May; in contrast, the sample taken in August at site BBA was one of the highest observed (4.79 μM; Table 1). The 9-month time series confirmed that DIP levels were low in the winter and spring but increased in the late summer (Figure S2). Higher salinity (6–18 PSU vs. 0–4 PSU) and higher DIP concentrations were observed in the August water sample compared to the others. High concentrations of NO_x in overlying waters were observed in samples collected from March–May, with the exception of the BBC site; NO_x concentrations were lowest in late summer (Table 1, Figure S2). Ammonium concentrations mirrored DIP throughout the year (Figure S2), with the lowest concentrations observed in spring and the highest value in the late summer (Table 1).

The P recovery following sediment NaOH-EDTA extraction varied from a low of 1.5 at site BBA to a high of 24.5% at YBB (Table 2). The samples with the lowest OM tended to have the lowest Fe and Mn concentrations in the extracts (e.g., 0.45 and 0.34 mg kg⁻¹, respectively at BBC; Table 2), and therefore may have required longer delay times to be fully quantitative for extracted P. Higher concentrations of paramagnetic ions in samples (e.g., Fe, Mn) shorten ³¹P nuclei relaxation times, reducing the delay time required between pulses (McDowell et al., 2006), but potentially increasing line-broadening of peaks. Since it is unknown whether variation in the efficiency of P extraction among the samples led to any biases due to preferential extraction of certain P forms, quantifications should be interpreted with caution. Of the cations extracted with P, Ca was most abundant, ranging from 110 to 2077 mg kg⁻¹ across samples (Table 2), while Fe concentrations ranged from 1.5 mg kg⁻¹ (BBA) to 73.7 mg kg⁻¹ (YBB), however caution should be taken in

interpretation as these cation concentrations do not necessarily represent their actual concentrations in sediments.

Phosphorus Forms and Their Contributions to P in Sediments

Figure 2 shows example P-NMR spectra, which demonstrate peaks associated with P forms detected in estuarine sediments of the Columbia River; the remaining spectra are included as Supplemental Data (Figure S1). Chemical shifts associated with the peaks are summarized in Table S1 and the percentages of total extracted P accounted for by identified P forms are in Table S2.

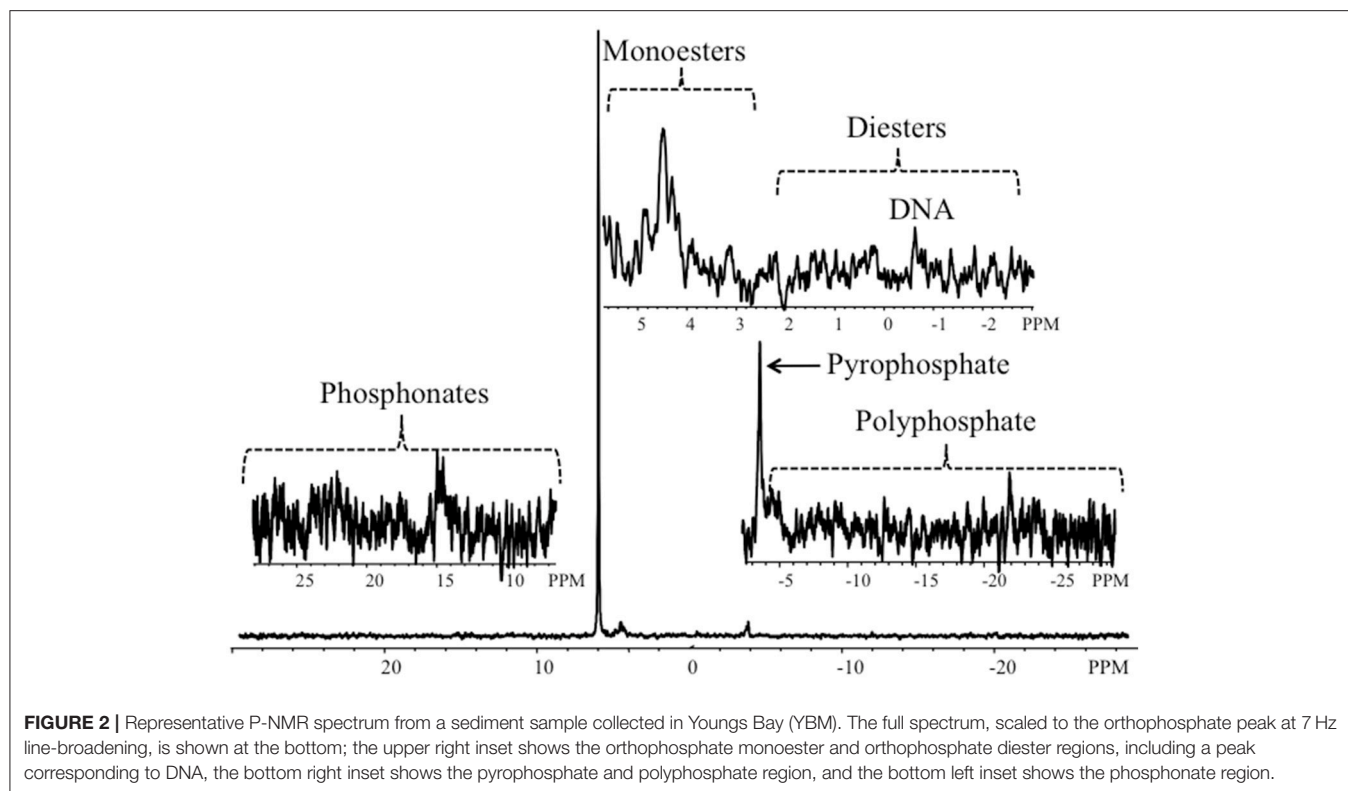
Total extracted P was dominated by inorganic forms (P_i; Figure 3A), which exceeded contributions from organic P (P_o) by 2–6 times. Orthophosphate, which varied twenty-fold across sites (8.1–231.3 mg kg⁻¹ at BBA and YBB, respectively), accounted for 70.7–84.4% of total P [Σ (P_i + P_o)] and 87–99% of P_i (Table S2). Both pyrophosphate and polyphosphate were detected at all sites except BBA in the summer (where only polyphosphate was detected). There was little variation in concentration among most sites (Figure 3B), with pyrophosphate and polyphosphate accounting for 1–13% of P_i and 0.8–10.6% of total P, respectively (Table S2). The largest total polyphosphate (pyrophosphate + polyphosphate) concentrations were observed at the west end of Baker Bay (6.2 mg kg⁻¹, BBI), while the lowest were observed in Cathlamet Bay, located in a southern bay of the estuary (CBK, 0.8 mg kg⁻¹; Table 3).

Concentrations of P_o ranged from 2.9 to 59.5 mg kg⁻¹, accounting for 13.5–26.6% of total extracted P in sediments (Figure 3C). Orthophosphate monoesters made the largest contribution to P_o, where they accounted for 66–94% of P_o and 9.4–25% of total extracted P. Identified monoesters comprised stereoisomers of inositol hexakisphosphate (IHP), including *chiro*, *myo*, *neo*, *scyllo*-IHP, as well as α - and β -glycerophosphate and mononucleotides. The IHP stereoisomers were broadly

TABLE 2 | Concentrations of P and selected cations in alkaline extracts from estuary lateral bay sediments.

Location	Sample	Date (Time)	NaOH-EDTA extracts						P Recovery [†] %
			P	Fe	Mn	Al	Mg	Ca	
			mg kg ⁻¹						
Cathlamet Bay	CBK	4/29/14 (1:10)	111.0	34.1	60.3	77.0	24.5	1553.5	8.1
Youngs Bay	YBM	3/28/14 (18:55)	47.7	1.69	0.56	13.4	36.3	193.6	6.7
	YBB	3/28/14 (19:19)	298.8	73.7	34.0	205.1	16.1	1077.7	24.5
Baker Bay	BBB	3/29/14 (7:04)	204.3	12.0	6.01	58.5	73.3	1044.1	23.9
	BBI	4/29/14 (7:30)	100.1	3.29	1.39	49.6	87.6	575.5	15.9
	BBC	5/29/14 (10:15)	33.9	0.45	0.34	16.7	10.6	109.7	5.7
	BBA	8/25/14 (7:50)	11.1	1.50	0.19	16.5	12.9	79.4	1.5

[†]P Recovery, Percentage of total sediment P extracted by NaOH-EDTA.



detected in estuarine sediments at concentrations that varied considerably ($1.4\text{--}54.1\text{ mg kg}^{-1}$) over short distances, indicating high local heterogeneity (Table 3). For example, the lowest and highest IHP concentrations were observed within Baker Bay (BBB and BBI, respectively while BBA had no detectable IHP). Stereoisomers of IHP (*chiro*, *myo*, *neo*, *scyllo*) contributed approximately equally to IHP with the exception of BBB, where contributions from *myo*-IHP were small (Table 3).

Orthophosphate diesters made up 0.8–7.4% of extracted P, contributing $0.4\text{--}28.7\text{ mg kg}^{-1}$ of P (Table S2; Figure 3C). The highest concentration of total orthophosphate diesters was observed at YBB (28.7 mg kg^{-1}). DNA was detected throughout the estuary, contributing as little as 0.2 mg kg^{-1} of P (BBC) and as much as 4.5 mg kg^{-1} of P (YBB), which also had the highest concentration of diester degradation products (α - and β -glycerophosphate and mononucleotides; Table 3). Though present at low concentrations, phosphonates were detected throughout estuarine sediments ($0.7\text{--}1.4\%$ P), ranging from 0.33 to 2.1 mg kg^{-1} of total extracted sediment P (Figure 3C).

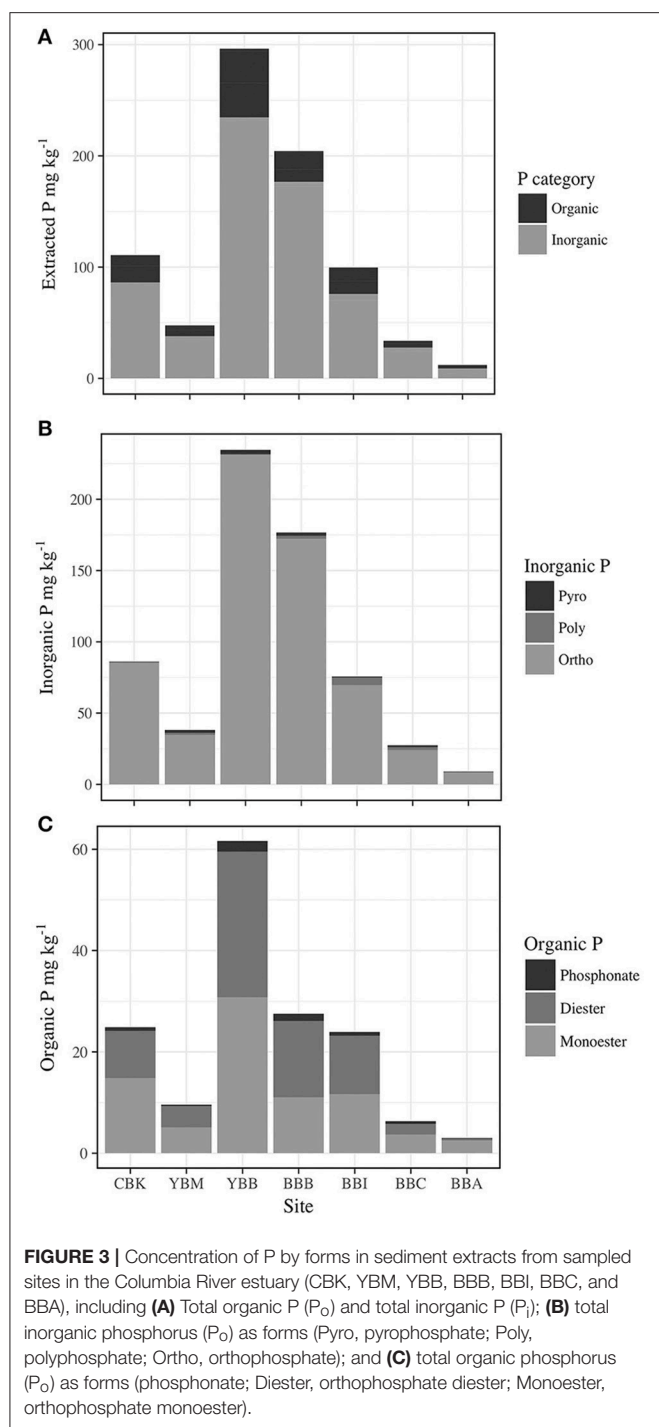
Redundancy Analysis

An RDA was performed to explore possible relationships among P forms and sediment characteristics within lateral bay sediments (Figure 4). The biplots of the RDA model display ordination between response variables (P form abundance) and explanatory variables (TotalP, LOI, Fe, Mn, Ca, and Mg; Figure 4A), sites (non-quantitative centroids; CBK, YBM, YBB, BBB, BBI, BBC, and BBA) and explanatory variables (Figure 4B). The dominant constraining variable (RDA1) was explained by variation in

organic matter (LOI; 90.6%) and secondarily (RDA2) by Mg concentrations (Mg; 6.7%), which together explained 97.3% of the variation observed in P forms.

An ordination biplot was generated to look for correlative relationships among sediment characteristics and P form abundances (Figure 4A). For example, among the response variables (i.e., P form abundances), phytate (IHP)—and to a lesser degree, total monoesters (Mono)—were associated with low organic matter (LOI) found at BBA, BBC, BBI, and CBK (Figures 4A,B). In contrast, diesters (Diester), and to a weaker extent DNA were associated with high concentrations of organic matter, Ca, and Mg (LOI, Ca, Mg; Figure 4A), observed at YBM, YBB and BBB. Orthophosphate and phosphonates were weakly associated with high OM (LOI), and total polyphosphates (TotPoly; pyrophosphate and polyphosphates) were not associated with any explanatory variables. Differences in overlying water properties (total P, NO_x , DIP, temperature, salinity) did not explain patterns in P form abundance (data not shown).

An ordination plot of sample sites and sediment characteristics split up sites into two groups according to their associative sediment characteristics (Figure 4B). For example, sites BBA, BBC, CBK and to a lesser extent BBI all had low organic matter, total sediment P and concentrations of cations (Ca, Mg, Fe, and Mn; Figure 4B), compared to sites YBB, YBM and BBB. The BBA site separated from the other sites because of its very low organic matter (LOI) and cation concentrations (Figure 4B). Correlation of explanatory variables can be obtained by taking the cosine of angles between vectors;



this analysis revealed that sediment Ca and organic matter (LOI) were highly correlated, with angles $\leq 15^\circ$ between most vectors ($R = 0.97$; **Figures 4A,B**).

DISCUSSION

There are few studies reporting P forms in estuarine environments, and therefore the small data set presented here provides useful information about the potential for P

cycling and retention in a river-dominated estuary. The time and costs associated with P-NMR often limit the number of samples that can be analyzed; in addition, samples are often homogenized and pooled, limiting potential replication. In addition, standardization of methods is being stressed in the literature so that P-NMR results can be compared across environments (Cade-Menun and Liu, 2014). For this reason, extracts were prepared from 10 cm diameter cores, which represents the homogenization of at least 1 year's worth of sediment deposition (based on rates of $\sim 6 \text{ cm year}^{-1}$; Sherwood and Creager, 1990). In addition, the extraction of organic P forms is notoriously difficult (Cade-Menun et al., 2006), therefore we used an alkaline extraction that optimizes the extraction of organic P forms; however, this tends to produce low extraction efficiencies, and this must be kept in mind when interpreting the data. The recovery rates reported here for NaOH-EDTA extracted P (6–24%) are comparable to other P-NMR studies in wetlands with similar sediment characteristics. For example, P recovery was 6–16% in calcareous soils and 16–30% in sediments with low OM and neutral pH (Cheesman et al., 2014). In contrast, higher recoveries (25–84%) have been reported in wetland sediments that are acidic (pH 3.6–4.8) or that have high OM (56–94%; Turner and Newman, 2005; Cheesman et al., 2014). It is likely that the residual P that was not extracted in our protocol was inorganic, alkali-stable, and associated with Ca or Mg minerals, since these forms are not readily extracted using NaOH-EDTA (Turner and Newman, 2005; Defforey et al., 2017). Nevertheless, despite low recovery of P in NaOH-EDTA extracts, we detected a range of P forms in estuarine sediments.

Polyphosphates and pyrophosphates were detected in all estuarine sediment samples, where they contributed as much as 13% of inorganic P (10% of total extracted P). In almost half the samples, polyphosphate concentrations exceeded pyrophosphate, in contrast to other studies (Turner and Newman, 2005; Zhang et al., 2009; Cheesman et al., 2010). This might be explained by the fact that, unlike the previous work cited, we included a neutralization step prior to lyophilization, which increases the stability of polyphosphate (Cade-Menun et al., 2006). Degradation of polyphosphate to pyrophosphate (either *in situ* or during sediment extraction) may also account for the fact that only a handful of studies report detection of polyphosphate in wetlands (Zhang et al., 2009; Cheesman et al., 2014).

Polyphosphate is biologically produced across the domains of life, including by bacteria, fungi, protozoa, plants, and mammals (Kornberg, 1995; Brown and Kornberg, 2004; Kulaev et al., 2004) and is thought to be indicative of microbial activity rather than linked to particular soil or sediment types (Cheesman et al., 2014). Interestingly, we detected polyphosphate in sediments with different characteristics, from brackish sediments in Baker Bay where concentrations were highest BBI (low OM, low Fe, and Mn) and BBB (high OM, high Fe, and Mn), and further supported by our RDA which found total polyphosphates not affiliated with measured sediment properties. Further, the presence of diesters (e.g., DNA) as an indicator of microbial activity was associated with high OM, Fe, and Mn concentrations and total P in sediments, suggesting that microbial biomass may be a major contributor to organic matter in river-dominated

TABLE 3 | Phosphorus form contributions at estuary lateral bay sample sites.

Location	Samples	Inorg:Org [†]	Mono:Di	mg kg ⁻¹			cM:D	Myo:other IHP	Deg	DNA	mg kg ⁻¹	
				cMono	cDi	Total IHP					Total Poly	Pyro:Poly
Cathlamet Bay	CBK	3.5	5.2	14.8	9.3	7.8	1.6	0.7	5.4	1.7	0.8	1.3
Youngs Bay	YBM	4.0	3.5	5.1	4.2	2.5	1.2	0.6	2.2	0.5	3.4	1.5
	YBB	3.8	3.2	30.8	28.7	16.4	1.0	1.0	14.3	4.5	3.3	10.0
Baker Bay	BBB	6.4	2.8	11.0	15.1	1.4	0.7	0	8.2	2.3	4.3	1.1
	BBI	3.2	2.1	11.6	11.6	54.1	1.0	0.5	4.2	4.0	6.2	0.15
	BBC	2.1	9.7	3.7	2.1	2.3	1.8	0.4	0.9	0.2	3.6	0.7
	BBA	3.0	31.3	2.5	0.4	0	7.1	0	0.3	0.02	0.9	0

[†]Inorg:Org, ratio of inorganic to organic P; Mono:Di, ratio of Orthophosphate monoesters to diesters without correction for degradation; cMono, cDi, total orthophosphate monoester and diesters corrected for degradation; Total IHP, sum of myo-, scyllo-, neo- and D-chiro-inositol hexakisphosphate; Myo:other IHP, ratio of sum of myo-inositol hexakisphosphate to all stereoisomers (scyllo-, neo-, D-chiro-inositol hexakisphosphate); Deg, degradation compounds include α -glycerophosphate, β -glycerophosphate, and mononucleotides; DNA, sum of identified DNA peaks; Total Poly, sum of pyrophosphate and polyphosphate; Pyro:Poly, ratio of pyrophosphate to polyphosphate. Standard error (\pm) in P-NMR analysis for samples from native and tame grasslands and croplands (four replicates across land types) was 8.7% for extracted P at $\geq 10\%$, compared to 20.1% for extracted P at $< 10\%$ (personal communication B. Cade-Menun, Liu et al., 2018).

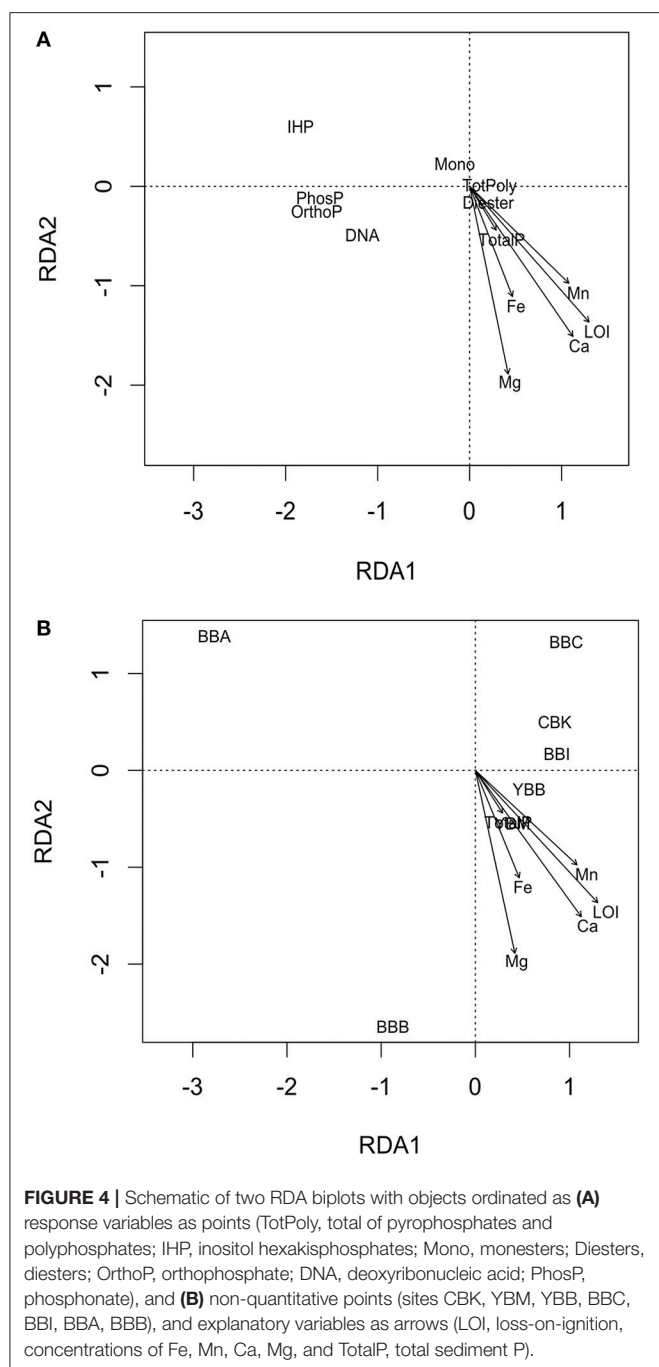
estuary sediments. Polyphosphates found throughout sediments produced by microbial activity may be a source of cycled P in dynamic, diel cycled environments such as estuaries, perhaps much more than has generally been assumed and further investigation is certainly warranted.

Although extracted P was dominated by inorganic forms, organic P accounted for $\sim 30\%$ of total P and was comprised of P forms that are considered both labile (e.g., RNA, phospholipids) and stable (e.g., IHP), despite rapid flushing and a low contribution of organic P compared to other freshwater wetlands (Turner and Newman, 2005; Turner et al., 2006; Cheesman et al., 2010, 2014) or lake sediments (Carman et al., 2000). The dominant organic P form in these estuarine sediments was the orthophosphate monoester myo-inositol hexakisphosphate (myo-IHP, also called phytate), which is derived from plants, and was detected in all but one sample. Inositol phosphates (myo-IHP, and stereoisomers scyllo-, neo-, and D-chiro-IHP), accrue in terrestrial soils and in aquatic sediments, particularly when clay content is high and pH is low; under these conditions, phytate tends to be the dominant form (Turner et al., 2002). In the river-dominated estuary, phytate was associated with low OM in both fresh and brackish sediments, with the highest concentrations occurring in samples from Baker Bay with relatively high salinity (BBA; > 15 PSU). Usually, sedimentary phytate concentrations tend to be lower in the presence of salt and under hypoxic conditions, (Suzumura and Kamatani, 1995a,b) due to the destabilization of phytate complexes (Gardolinski et al., 2004) formed through complexation with iron oxides in acidic soils or by minerals in sediments with high mineral content (Turner et al., 2002; Turner and Newman, 2005; Turner and Weckström, 2009; Cheesman et al., 2014). High variability in phytate concentrations in the higher-salinity samples may indicate a varying effect of salinity and/or sediment complexing agents as Baker Bay has the greatest variation in biogeochemical conditions.

Other Influences on P Forms and P Retention

Peripheral bay environments in this study are considered depositional environments, where fine material (mainly consisting of clays and silts) is deposited from suspended fluvial material (Sherwood and Creager, 1990). The composition of sediments and grain sizes varies seasonally in the estuary; however, the peripheral bays are consistently sites of long-term deposition of fine sediments. Differences in P forms found at the different sites within these bays could reflect differences in sediment sorting, and/or diagenetic processes. For example, sites differ in terms of the origin of deposited sediments, with Baker and Cathlamet Bay sediment originating from fluvial washload and Youngs Bay sediment originating from a combination of washload and deposits from ephemeral estuarine turbidity maxima.

In addition to sequestration of organic forms, P retention is influenced by abiotic buffering processes, which limit fluctuations in DIP through mineral precipitation with Fe, Mg, Ca, and Mn (e.g., Fe oxyhydroxides, Ca mineral precipitates; Richardson and Marshall, 1986; Froelich, 1988; Reddy et al., 1999; Bridgham et al., 2001). Retention also occurs through abiotic adsorption to sediment surfaces, particularly on fine particles (Li et al., 2013), which dominate the depositional environment of peripheral bays in the estuary. The relatively low and similar DIP concentrations in waters overlying Columbia River estuary sediments (Gilbert et al., 2013; **Figure S2**)—despite variations in total P within the sediments—may be explained by buffering of P (Froelich, 1988), which warrants further exploration as this would affect P retention in these depositional environments. In addition, the limited sample set suggests that YBB and BBB sites had high concentrations of extractable Ca²⁺, which readily forms mineral precipitates with P if in high concentrations in sediment environments, (e.g., CaHPO₄ from Ca²⁺ and HPO₄⁻²) at comparable rates with sediment P



sorption (Reddy et al., 1999; Li et al., 2013). Further studies should investigate relationships between P forms and total cation concentrations in different sedimentary environments within the estuary to determine how they might affect P mineralization and sequestration.

CONCLUSIONS

A variety of P forms were identified in river-dominated estuary sediment extracts, which were dominated by inorganic P.

Although orthophosphate made the largest contribution to inorganic P, polyphosphate and pyrophosphate were found throughout the lateral bays, constituting up to 13% of extracted inorganic P. Organic forms accounted for ~30% of total extracted P, and included both labile P forms (e.g., phospholipids, RNA) and resistant forms (e.g., IHP), which were detected at approximately equal concentrations throughout the estuary. Sample-to-sample variability was greatest among organic P forms, with orthophosphate monoesters such as phytate (*myo*-IHP) being most abundant when OM content was low. In contrast, orthophosphate diesters were more abundant in sediments with high OM, suggesting that microbial activity might play a larger role in P cycling in sediments with high OM. Low orthophosphate concentrations in overlying waters along with variable sediment P concentrations suggest P buffering may occur within peripheral bays. These data demonstrate that despite short water residence times and rapid flushing, sediments of a river-dominated estuary have the potential to sequester P in inorganic and inorganic forms, thus influencing P loading to the coastal ocean via the large Columbia River plume.

AUTHOR CONTRIBUTIONS

SW collected and analyzed all samples, wrote the manuscript, and managed communication among all the authors. BC-M ran the NMR experiments and analyzed spectra with SW. JN and SW analyzed water samples and JN contributed to sediment P analysis as well as design of the work. TP had substantial contributions to the conception and design of the work and along with BC-M critically revised the work for intellectual content and contributions.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2018.00302/full#supplementary-material>

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Conflict of Interest Statement: 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.

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