



# Phosphorus Dynamics Associated With Organic Carbon Mineralization by Reduction of Sulfate and Iron in Sediment Exposed to Fish Farming

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The expansion of the aquaculture industry has resulted in accumulation of phosphorus (P)-rich organic matter via uneaten fish feed. To elucidate the impact of fish farming on P dynamics, P speciation, and benthic P release along with partitioning of organic carbon (C<sub>org</sub>) mineralization coupled to sulfate reduction (SR) and iron reduction (FeR) were investigated in the sediments from Jinju Bay, off the southern coast of South Korea, in July 2013. SR in the farm sediment was 6.9-fold higher than the control sediment, and depth-integrated (0–10 cm) concentrations of NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3-</sup>, and H<sub>2</sub>S in pore water of the farm sediment were 2.2-, 3.3-, and 7.4-fold higher than that in control sediment, respectively. High biogenic-P that comprised 28% of total P directly reflected the impact of P-rich fish feed, which ultimately enhanced the bioavailability (58% of total P) of P in the surface sediment of the farm site. In the farm sediment where SR dominated C<sub>org</sub> mineralization, H<sub>2</sub>S oxidation coupled to the reduction of FeOOH stimulated release of P bound to iron oxide, which resulted in high regeneration efficiency (85%) of P in farm sediments. Enhanced P desorption from FeOOH was responsible for the increase in authigenic-P and benthic P flux. Authigenic-P comprised 33% of total P, and benthic P flux to the overlying water column accounted for approximately 800% of the P required for primary production. Consequently, excessive benthic P release resulting directly from oversupply of P-rich fish feed was a significant internal source of P for the water column, and may induce undesirable eutrophication and harmful algal blooms in shallow coastal ecosystems.

**Keywords:** biogenic apatite P, benthic nutrient flux, P regeneration, aquaculture, phosphorus speciation, sulfate reduction, iron reduction

## INTRODUCTION

Phosphorus (P) is a key nutrient, not only regulating primary production as a limiting nutrient in aquatic ecosystems, but also inducing eutrophication that can stimulate undesirable algal blooms in coastal ecosystems (Tyrrell, 1999; Diaz and Rosenberg, 2008; Middelburg and Levin, 2009; Lomnitz et al., 2016). In shallow coastal ecosystems, sediment serves either as a source (i.e., regeneration

and release) or a sink (i.e., adsorption and precipitation) of P for the water column (Slomp, 2011; Kraal et al., 2015; An et al., 2019). P dynamics in coastal sediments are tightly coupled to the rate and partitioning of  $C_{org}$  mineralization and the resulting interaction between iron and sulfur (Rozan et al., 2002; Canfield et al., 2005; Kraal et al., 2013; Slomp et al., 2013; Andrieux-Loyer et al., 2014; An et al., 2019). P release from sediments into pore water first proceeds from  $C_{org}$  mineralization, relying on various terminal electron-accepting processes, such as aerobic respiration, denitrification, and reduction of Mn and Fe oxides and sulfate (Canfield et al., 2005; Holmer et al., 2005). In particular, under organic-rich and sulfidic conditions in which sulfate reduction dominates  $C_{org}$  mineralization, abiotic reduction (i.e., reductive dissolution) of Fe oxides coupled with  $H_2S$  oxidation to form Fe sulfide minerals can intensify P desorption from Fe oxides (**Supplementary Table 1**). Because the interaction of P with Fe and S has a profound impact on P dynamics in anoxic sediment (Rozan et al., 2002; An et al., 2019), quantification of the relative significance of SR and FeR, which regulate the availability of Fe to react with the P, is particularly important to better understand the P dynamics in coastal sediments.

World aquaculture production has grown rapidly since the late 1980s. It accounted for 47% of total fish production in 2016, while capture-fishery production has remained relatively stable (FAO, 2018). Despite its economic importance, irresponsible aquaculture activities have led to massive accumulations of organic matter induced by uneaten fish feed and fecal production in coastal sediments (Hall et al., 1990; Holby and Hall, 1991; Holmer et al., 2002, 2003; Husa et al., 2014). This has generated various environmental issues including accumulation of chemically reactive and toxic  $H_2S$  and regeneration of reduced inorganic nitrogen ( $NH_4^+$ ) and phosphate ( $PO_4^{3-}$ ) in the sediment (Holmer et al., 2005; Hyun et al., 2013; Choi et al., 2018, 2020). The release of inorganic nutrients into the water column may result in changes in composition and diversity of macroalgae, eutrophication, and (harmful) algal blooms in coastal ecosystems (Holmer et al., 2008; Price et al., 2015; Ferrera et al., 2016; Stigebrandt and Andersson, 2020).

Given the overall economic and environmental significance of the aquaculture industry, relevant proxies for assessing the condition of fish-farm sediment are required (Ministry of Oceans and Fisheries (MOF), 2016; Choi et al., 2020). P enrichment of sediment has been recognized as a useful indicator for assessing the environmental impact of fish farms that make use of large amounts of feed (Holmer and Frederiksen, 2007; David et al., 2009), as natural variation of P in sediment is small compared with that of C and N (Soto and Norambuena, 2004). In particular, biogenic apatite P (Bio-P) has been proposed to be an important representative indicator of the impact of fish farming (Matijević et al., 2008). On the other hand, it is also speculated that the precipitation of authigenic-P (Aut-P) could be stimulated during the enhanced deposition and mineralization of  $C_{org}$  and subsequent P release in farm sediment as it was reported in various anoxic sediments with high organic matter loading (Anschutz et al., 2007; Goldhammer et al., 2010; Tsandev et al., 2012; Joshi et al., 2015; Kraal et al., 2017). Most studies

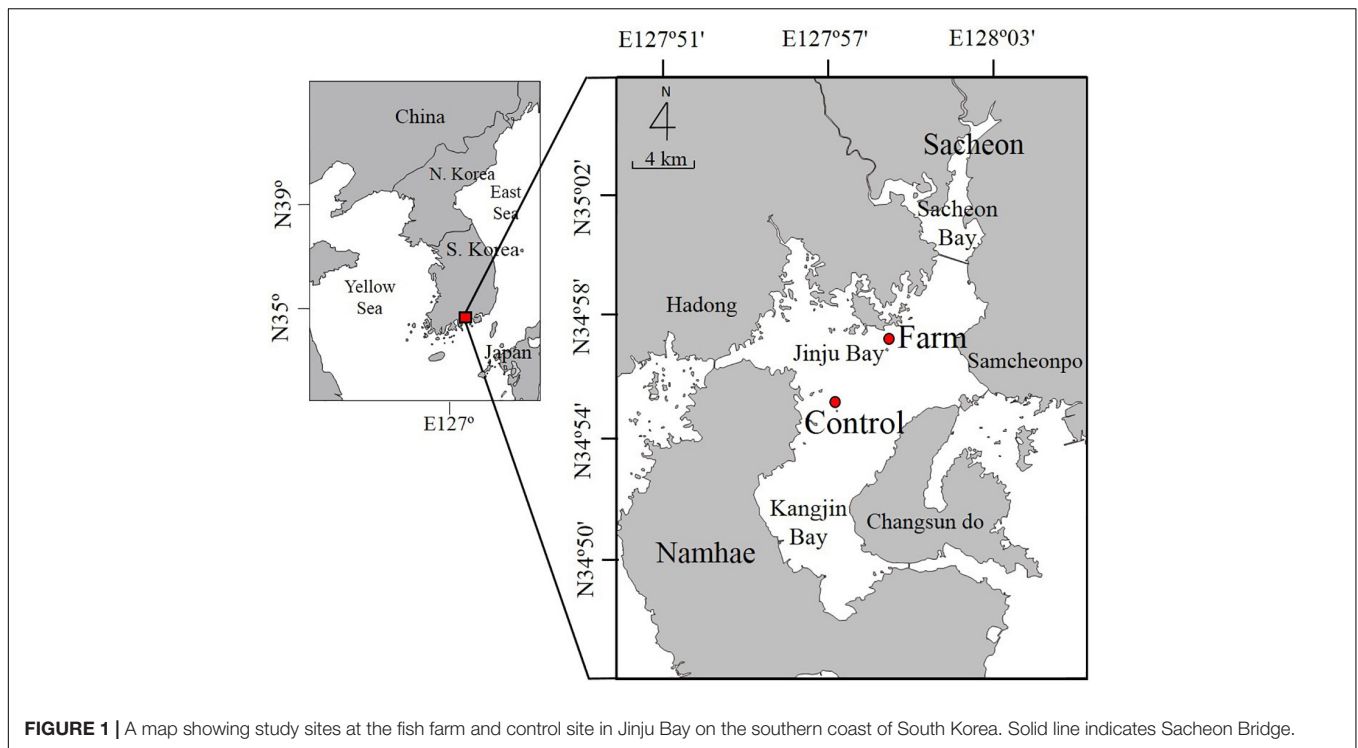
that assess aquaculture impact associated with P evaluated total P and major P forms (Kassila et al., 2001; Matijević et al., 2008; David et al., 2009; Jia et al., 2015; Morata et al., 2015). Although several papers have suggested that sulfide accumulation enhances benthic P release in sediment affected by aquaculture (Heijls et al., 2000; Holmer et al., 2002, 2003; Nielsen et al., 2003; Holmer and Frederiksen, 2007), any comparative biogeochemical process studies on P dynamics accompanied by direct estimation of the rates and partitioning of FeR and SR have not been conducted in coastal ecosystems (Valdemarsen et al., 2012; An et al., 2019), especially in fish farm sediments where high P-containing fish feed would have significant impacts on P dynamics in sediments. The objectives of this article are: (1) to present the impact of fish farming on P enrichment and speciation in sediment, with special emphasis on biogenic-P and authigenic-P, (2) to elucidate P dynamics (i.e., adsorption and desorption) related to variation in Fe and S cycles associated with FeR and SR, the two major  $C_{org}$  mineralization pathways in coastal sediments, and (3) to evaluate the potential significance of farming-induced benthic P release as an internal source of P that can stimulate (harmful) algal blooms in shallow coastal ecosystems via benthic-pelagic coupling.

## MATERIALS AND METHODS

### Study Area

The study area was located in semi-enclosed Jinju Bay near Sacheon and Namhae Island on the southern coast of South Korea ( $34^{\circ}55'–34^{\circ}57'N$ ,  $127^{\circ}57'–127^{\circ}59'E$ ) (**Figure 1**). Fish farming activities have been ongoing for several years in which most fish were harvested in November (fall), and restocking of empty cages with fry took place in the following March to May (spring) (Choi et al., 2020). Sampling was conducted in July 2013 at a farm site ( $34^{\circ}57'08''N$ ,  $127^{\circ}59'07''E$ ) where sea bass (*Lateolabrax japonicus*), gray mullet (*Mugil cephalus*), and black porgy (*Acanthopagrus schlegelii*) were intensively cultivated. Annual fish production in the farming zone with an area of 1,323  $m^2$  was approximately 445 tons in 2013. Fish feed, consisting of a mix of trash fish and extruded pellets (EP), was continuously supplied in accordance with the growth stage of the fish (Choi et al., 2020). In July 2013, only EP was used, and the input of EP at the fish farm was approximately  $1.2 \text{ kg m}^{-2} \text{ d}^{-1}$ . The EP used in the farming zone contain protein, fat, calcium, phosphorus, etc. Among other ingredients, the P content in fish feed varies depending on the growth stage of fish and can be up to 1.5–2.7% (NIFS)<sup>1</sup>. In order to evaluate the impact of fish farming on P dynamics in farm sediment, a control site ( $34^{\circ}55'08''N$ ,  $127^{\circ}57'09''E$ ) was selected in the center of the bay, 4.5 km away from the farm site. Although current speed ranging from 28 to 33  $\text{cm s}^{-1}$  in the study area (Ro et al., 2007) was fairly high enough to reduce the accumulation of sinking organic particles onto the sediment, sedimentation flux of particulate material at the farm ( $126.2 \text{ g m}^{-2} \text{ d}^{-1}$ ) was almost two-fold higher than that of the control site ( $77.7 \text{ g m}^{-2} \text{ d}^{-1}$ ) (NIFS, 2013). Accordingly, the density of infaunal polychaetes at

<sup>1</sup><http://www.nifs.go.kr/fishfeed>



**FIGURE 1** | A map showing study sites at the fish farm and control site in Jinju Bay on the southern coast of South Korea. Solid line indicates Sacheon Bridge.

the farm site (about 2,500 inds.  $m^{-2}$ ) was 25-fold higher than at the control site (about 100 inds.  $m^{-2}$ ).

## Sampling and Handling

To minimize surface sediment disturbance, sediment samples were collected by SCUBA divers using acrylic cores (40 cm in diameter, 40 cm in length). At both farm and control sites, approximately 30 sediment cores were collected to analyze the geochemical properties of pore water and sediments and to measure microbial metabolic activities such as the rate of sulfate reduction, iron reduction and total anaerobic organic carbon mineralization. On the vessel, triplicate sub-samples for geochemical analysis were collected using acrylic cores (6.5 cm in diameter, 25 cm in length).

Sediment temperature was measured using an electronic thermometer (Multi thermometer, DT400, Summit) within a depth of 10 cm. The sediment cores for analysis of geochemical constituents in pore water were transferred to  $N_2$ -filled glove bags, in which the sediment was sectioned and loaded into polypropylene centrifuge tubes (BD, Franklin Lakes, NJ, United States). The tubes were tightly capped and centrifuged for 10 min at 3,500 rpm. After reintroduction to the  $N_2$ -filled glove bag, pore water was sampled to measure  $NH_4^+$ ,  $PO_4^{3-}$ ,  $Fe^{2+}$ ,  $SO_4^{2-}$ , and  $H_2S$  and filtered through cellulose acetate syringe filters (0.2- $\mu m$  pore size, ADVANTEC, Toyo Roshi Kaisha, Ltd., Japan). The samples for determination of  $NH_4^+$  were stored at 4°C after adding  $HgCl_2$  (125 mM). The samples for  $PO_4^{3-}$  were frozen at  $-25^\circ C$  until analysis. The samples for  $Fe^{2+}$  and  $SO_4^{2-}$  were frozen at  $-25^\circ C$  after adding HCl (0.1 M final concentration). The pore water samples for  $H_2S$  and sediment

samples for acid volatile sulfides ( $AVS = H_2S + FeS$ ), chromium reducible sulfur ( $CRS = FeS_2 + S^0$ ) and elemental sulfur ( $S^0$ ) were fixed in a zinc acetate dehydrate (20% w/v) solution and frozen at  $-25^\circ C$  until analysis. Sediment samples for analysis of solid-phase iron, chlorophyll-a (Chl *a*), total organic carbon (TOC), and total nitrogen (TN) were frozen at  $-25^\circ C$  until analysis.

*In situ* water temperature and salinity were measured using a CTD meter (19 plus; Sea-Bird Electronics, Bellevue, WA, United States). Approximately 20 water samples were collected at the surface and bottom layers using a Niskin water sampler to measure concentrations of Chl *a* and inorganic nutrients. Duplicate samples were filtered using GF/F filters (47 mm in diameter) and kept in the dark at  $-20^\circ C$  before analysis in the laboratory.

## Laboratory Analysis

Concentrations of  $NH_4^+$  and  $PO_4^{3-}$  in pore water were analyzed using the flow injection analysis (FIA) method (Hall and Aller, 1992) and a nutrient autoanalyzer (QUAATRO, Seal Analytical), respectively. Dissolved  $Fe^{2+}$  in pore water was determined by colorimetry with a ferrozine solution (Stookey, 1970).  $SO_4^{2-}$  concentration was measured using ion chromatography (819 IC detector using an A-Supp 5 column, Metrohm, Swiss; 1 mM  $NaHCO_3$  + 3.2 mM  $Na_2CO_3$  eluent). Dissolved sulfide was determined using the methylene blue method (Cline, 1969). Reproducibility of  $NH_4^+$  was better than 10%. The detection limit of  $H_2S$  and  $Fe^{2+}$  was 3 and 1  $\mu M$ , respectively.

Acid volatile sulfides and CRS in sediments were determined using a two-step distillation method with cold 12 M HCl and boiling 0.5 M  $Cr^{2+}$  solution (Fossing and Jørgensen,

1989), and reacted with the Cline solution for extracted S compound analysis (Cline, 1969). Elemental sulfur ( $S^0$ ) was defined as the sulfur extracted with methanol from sediment and measured as cyclo- $S_8$  by reversed-phase high-performance liquid chromatography (Zopfi et al., 2004). Oxalate-extractable Fe(II), hereafter Fe(II)<sub>(oxal)</sub>, was extracted in 0.2 M anoxic oxalate solution (pH 3) for 4 h (Phillips and Lovley, 1987), and total Fe<sub>(oxal)</sub> was extracted from an air-dried sediment in anoxic oxalate (Thamdrup and Canfield, 1996). Fe(II)<sub>(oxal)</sub> and total Fe<sub>(oxal)</sub> were determined with ferrozine as described above. Solid Fe(III)<sub>(oxal)</sub> was defined as the difference between total Fe<sub>(oxal)</sub> and Fe(II)<sub>(oxal)</sub>. Chl *a* in surface sediment (0–2 cm) was extracted using 90% acetone for 24 h, and determined using a spectrophotometer (Shimadzu, UV-2401PC) (Parsons et al., 1984). TOC and TN content in sediment was analyzed using an elemental analyzer (EURO EA 3000, Eurovector S.P.A., via Tortona 5, Milan, Italy) after removal of CaCO<sub>3</sub>.

## Metabolic Rate Measurement

To determine anaerobic C<sub>org</sub> mineralization rates, sediment cores (10 cm in diameter, 20 cm in length) were transferred to a glove bag filled with N<sub>2</sub> gas and sliced to a depth of 10 cm at 2-cm intervals. The sliced sediments were treated in the same way as the analysis of geochemical constituents. Ten 50-ml centrifuge tubes that were filled with sediment without headspace were incubated in N<sub>2</sub> filled gas-tight bags in the dark at *in situ* temperature. Two tubes were then taken at regular intervals (i.e., 0, 1, 2, 3, and 5 days), and pore water was extracted as described above. Anaerobic C<sub>org</sub> mineralization rates were determined by linear regression of the accumulation of total dissolved inorganic carbon (DIC) in the pore water with added HgCl<sub>2</sub> (125 mM) (Hyun et al., 2009) after correcting for CaCO<sub>3</sub> precipitation (Thamdrup et al., 2000; Hyun et al., 2017). CaCO<sub>3</sub> precipitation was calculated from decreasing dissolved Ca<sup>2+</sup> concentration during incubation:

$$\text{CaCO}_3 \text{ precipitation} = \Delta[\text{Ca}^{2+}]_{\text{sol}} \times (1 + K_{\text{Ca}}) \quad (1)$$

where  $K_{\text{Ca}}$  is the adsorption constant for Ca<sup>2+</sup> ( $K_{\text{Ca}} = 1.6$ ) (Li and Gregory, 1974). Then the anaerobic C<sub>org</sub> mineralization rate corrected for CaCO<sub>3</sub> precipitation was calculated as:

$$\begin{aligned} \text{anaerobic C}_{\text{org}} \text{ mineralization} &= \text{DIC accumulation} \\ &+ \text{CaCO}_3 \text{ precipitation} \quad (2) \end{aligned}$$

Sulfate reduction rates (SRRs) were determined with a radiotracer method (Jørgensen, 1978). Triplicate intact cores (35 cm long with a 3-cm internal diameter) were collected at each site. Two  $\mu\text{Ci}$  of  $^{35}\text{SO}_4^{2-}$  (Institute of Isotopes Co. Ltd.) were injected into the injection port at 1-cm intervals, and cores were incubated for 2 h at *in situ* temperatures. At the end of incubation, sediment was sliced into sections, fixed in Zn acetate (20%), and frozen until processing in the laboratory. Extraction of reduced  $^{35}\text{S}$  was performed by two-step distillation (Fossing and Jørgensen, 1989), and the radioactivity of the reduced

$^{35}\text{S}$  was quantified using a liquid scintillation counter (Tri-Carb 2910 TR; PerkinElmer Inc., Waltham, MA, United States). Background radioactivity of  $^{35}\text{S}$  at the farm and control sites was  $53.9 \pm 5.4 \text{ cpm cm}^{-3}$  ( $n = 9$ ) and  $53.9 \pm 5.4 \text{ cpm cm}^{-3}$  ( $n = 9$ ), respectively. Detection limits of the SRR estimated according to Fossing et al. (2000) ranged from 0.58 to 2.29 nmol cm<sup>-3</sup> d<sup>-1</sup>. The C<sub>org</sub> mineralization by SR was calculated from the stoichiometric equation (S:C = 1:2) presented in **Supplementary Table 2** (Hyun et al., 2017).

To determine iron reduction rates (FeRR), the sediment that was used for the incubation experiment of the anaerobic C<sub>org</sub> mineralization rate measurement was homogenized in an anaerobic chamber, and Fe(II)<sub>(oxal)</sub> was extracted in an anoxic oxalate solution as described above. FeRRs were determined by linear regression of the increase in solid Fe(II)<sub>(oxal)</sub> concentration with time (Hyun et al., 2009, 2017). The separation of microbial and abiotic processes in FeR and the contribution of FeR to anaerobic C<sub>org</sub> mineralization were calculated based on the stoichiometric equation (Fe:S = 2:3, Fe:C = 4:1) presented in **Supplementary Table 2** (Hyun et al., 2017).

## Sediment Oxygen Demand and Benthic Nutrient Flux

Sediment cores for measuring sediment oxygen demand (SOD) and benthic nutrient flux (BNF) were collected using an acrylic incubation chamber (10 cm diameter, 22 cm length). The benthic chambers were transported to the laboratory immediately after sampling. Incubation began within 6 h of sampling. The benthic chambers were incubated in the dark at *in situ* temperature, after careful sealing to keep out air bubbles. Overlying water in the benthic chamber was continuously mixed using a geared pump (PQ-12, Greylor Co., flow rate =  $0.14 \pm 0.02 \text{ l min}^{-1}$ ). Overlying water for measurement of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) flux was sampled using the attached syringe of circulation tubing every 1–2 h during incubation. Concentrations of NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup> were analyzed using the nutrient autoanalyzer (QUAATRO, Seal Analytical). SOD and BNF at the sediment-water interface were calculated as follows (Eq. 3):

$$F = \frac{dC}{dt} \times \frac{V}{A} \quad (3)$$

where  $F$  is the flux of oxygen and nutrients ( $\text{mmol m}^{-2} \text{ d}^{-1}$ ),  $(dC/dt)$  is the slope of the linear regression line derived from the concentration change with time ( $\text{mmol L}^{-1} \text{ d}^{-1}$ ),  $V$  is the volume of benthic chamber ( $\text{m}^3$ ), and  $A$  is the area of sediment-water interface ( $\text{m}^2$ ). The regression lines were derived from first 4 h of incubation before oxygen was depleted from the fish farm sediment sample.

## Phosphorus Speciation in the Sediment

Phosphorus speciation in the sediment was determined using the extraction scheme proposed by Anschutz and Deborde (2016) modified from Schenau and De Lange (2000) and Ruttenberg et al. (2009). The extraction procedure of each P fraction is summarized in **Table 1**. We separated six major P fractions:

**TABLE 1** | Sequential extraction scheme for P fractions in the sediments.

Extracted fraction	Extractant	Symbol
Loosely bound + Exchangeable P	NaHCO <sub>3</sub> + toluene (pH 8, 24 h, 3 times)	Lsor-P
Iron bound P	0.3 M Na-citrate + 1 M NaHCO <sub>3</sub> /~1 g Na-dithionite (8 h) 1 M MgCl <sub>2</sub> wash	Fe <sub>(CDB)</sub> -P
Biogenic apatite + Carbonate-associated P	2 M NH <sub>4</sub> Cl [pH 7, 4 h, 13 (or 10) times]	Bio-P
Authigenic apatite	1 M Na-acetate (pH 4-acetic acid buffered, 16 h) 1 M MgCl <sub>2</sub> wash	Aut-P
Detrital apatite + Other inorg. P	1 M HCl (16 h) MQ water wash	Detr-P
Organic P	18 M H <sub>2</sub> SO <sub>4</sub> (16 h)	Ref-OP

**TABLE 2** | Environmental parameters in the water column of the fish farm and control sites.

Site	Water depth (m)	Sampling layer	Temperature (°C)	Salinity	DO (μM)	DO saturated (%)	DIN (μM)	DIP (μM)	Chl <i>a</i> (μg L <sup>-1</sup> )
Farm	12.5	Surface	25.5	31.5	180	83.5	4.45	0.33	8.11 (±0.23)
		Bottom	22.4	32.3	114	51.5	4.13	0.24	3.83 (±0.46)
Control	5.5	Surface	26.6	31.0	260	122.4	4.07	0.27	1.90 (±0.37)
		Bottom	22.8	32.1	227	100.4	4.73	0.29	2.38 (±0.45)

(1) loosely sorbed and exchangeable P (Lsor-P); (2) easily reducible/reactive Fe-bound P [Fe<sub>(CDB)</sub>-P], (3) biogenic apatite and carbonate-associated P (Bio-P), (4) authigenic apatite (Aut-P), (5) detrital apatite and other inorganic P (Detr-P), and (6) organic P (Ref-OP). Approximately 0.1 g of wet sediment was subsampled and placed into a 15-mL polypropylene conical tube. Sediment handling and extraction of Lsor-P and Fe<sub>(CDB)</sub>-P phases were conducted under N<sub>2</sub>-atmosphere to avoid the exposure to air during drying and to protect resorption of released P in the leaching solution (Kraal and Slomp, 2014; Anschutz and Deborde, 2016). Total P was calculated from the sum of the six different P fractions. Concentrations of phosphorus were measured with an ultraviolet-visible light spectrophotometer at 885 nm as the molybdenum-blue complex (Anschutz and Deborde, 2016), except in the case of Fe<sub>(CDB)</sub>-P. Because the dithionite and citrate in the Fe<sub>(CDB)</sub>-P extraction step interfered with the molybdate reagent, concentrations of Fe<sub>(CDB)</sub>-P were measured by inductively coupled atomic emission spectroscopy (ICP-AES, JY 138, Ultrace, Jobin Yvon, France) (Kraal and Slomp, 2014).

## Statistical Analysis

Statistical analysis was conducted in SPSS Statistics 26. To compare differences in geochemical properties, metabolic rates, and P distributions in the sediment of farm and control sites, Student's (independent-samples) *t*-test was performed. Before analysis, to assess the homogeneity of variance of samples, Levene's test was used. The level of significance was taken at 5% ( $P < 0.05$ ).

## RESULTS

### Environmental Parameters

Water temperature and salinity ranged from 22.4 to 26.6°C, and from 31.0 to 32.3, respectively (Table 2). Dissolved oxygen (DO) concentrations in seawater were lower at the farm site (114–180 μM) than at the control site (227–260 μM). Low DO

saturation (51.5%) was observed in the bottom water of the farm site. DIN and DIP concentrations in seawater ranged from 4.07 to 4.73 μM and from 0.24 to 0.33 μM, respectively. Chl *a* concentrations in seawater were higher at the farm (3.83–8.11 μg L<sup>-1</sup>) than at the control site (1.90–2.38 μg L<sup>-1</sup>) (Table 2). Temperatures in the surface sediment of farm and control sites were 22.8 and 22.3°C, respectively. TOC, TN, and Chl *a* concentrations in the surface sediment were greater in the farm sediment than in the control sediment (Table 3).

### Pore-Water and Solid-Phase Constituents

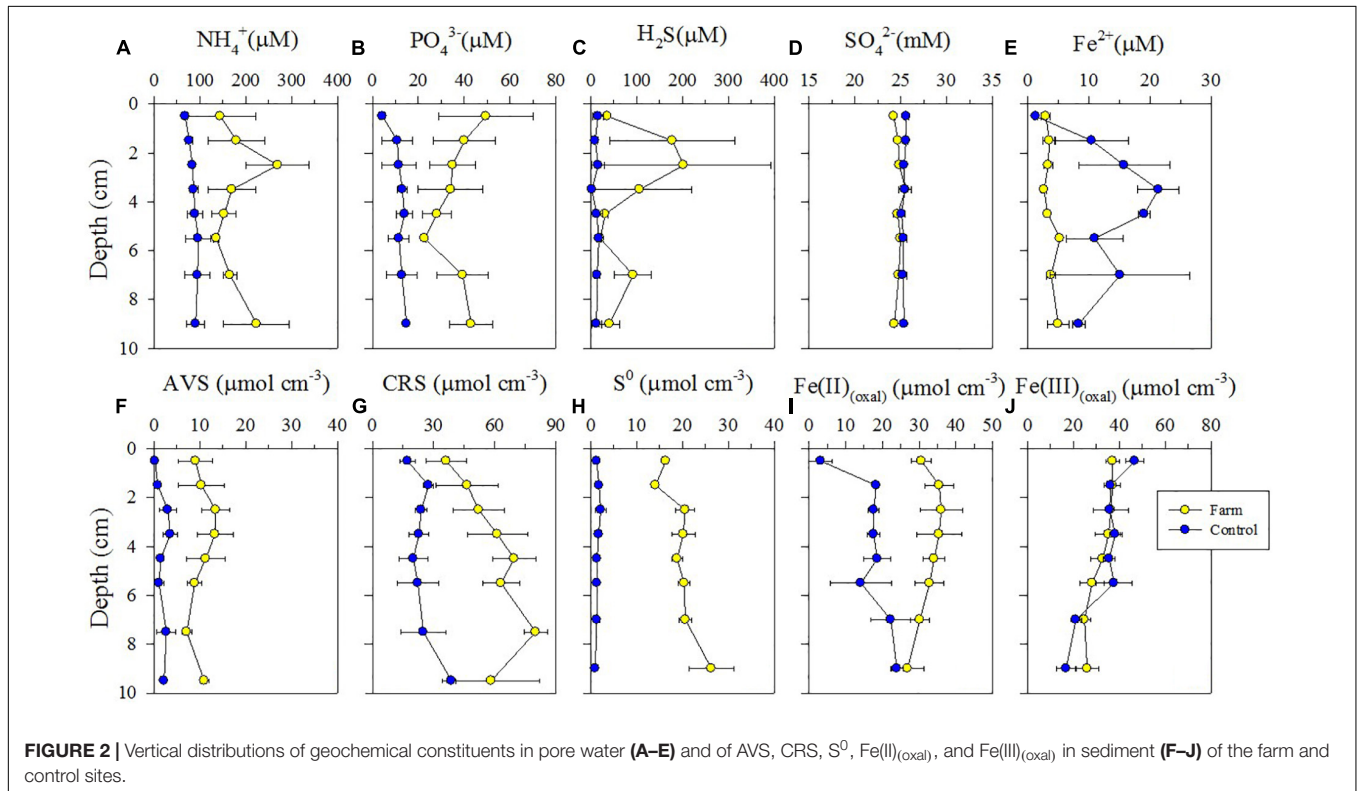
Concentrations of NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3-</sup>, and H<sub>2</sub>S in pore water were significantly higher at the farm than at the control site (Figures 2A–C,  $P < 0.05$ ). Depth-integrated (0–10 cm) concentrations of NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3-</sup>, and H<sub>2</sub>S in pore water at the farm were 2-, 3-, and 7.4-fold higher than at the control site, respectively (Table 4). SO<sub>4</sub><sup>2-</sup> concentrations, which ranged from 24.2 to 25.6 mM in pore water from both sites, showed a vertically homogenous distribution patterns (Figure 2D,  $P = 0.414$ ). In contrast, Fe<sup>2+</sup> concentrations in pore water from the farm sediment were 3.0-fold lower than at the control site (Figure 2E and Table 4,  $P < 0.05$ ).

Reduced sulfur compound content (i.e., AVS, CRS, and S<sup>0</sup>) was significantly higher at all depths of farm sediment compared with control sediment (Figures 2F–H,  $P < 0.001$ ). Depth-integrated (0–10 cm) content of AVS and CRS in the sediments were 5.2- and 2.3-fold higher at the farm than at the control site, respectively (Table 4). Depth-integrated S<sup>0</sup> content in the farm sediment was 13.6-fold greater than in the control sediment (Table 4). Solid Fe(II)<sub>(oxal)</sub> content was significantly higher within 6 cm depths of the farm sediment compared with the control sediment (Figure 2I,  $P < 0.05$ ), whereas solid Fe(III)<sub>(oxal)</sub> content in sediment was similar at both sites (Figure 2J,  $P = 0.737$ ). Depth-integrated (0–10 cm) Fe(II)<sub>(oxal)</sub> content in sediment was 1.8-fold greater at the farm compared with the control site, whereas Fe(III)<sub>(oxal)</sub> content in sediment was similar at the two sites (Table 4). Overall, dissolved and solid-phase constituent analysis

**TABLE 3** | Environmental parameters in the surface sediment (0–2 cm) of the fish farm and control sites.

Site	Temperature (°C)	Chl <i>a</i> (mg m <sup>-2</sup> )	TOC (% dry wt.)	TN (% dry wt.)
Farm	22.8	257 (±16.8)	2.16	0.32
Control	22.3	205 (±15.3)	1.47	0.19

Values represent average ± 1 SD on the duplicate or triplicate samples.

**FIGURE 2** | Vertical distributions of geochemical constituents in pore water (A–E) and of AVS, CRS, S<sup>0</sup>, Fe(II)<sub>(oxal)</sub>, and Fe(III)<sub>(oxal)</sub> in sediment (F–J) of the farm and control sites.**TABLE 4** | Depth integrated (0–10 cm) concentrations of the pore water and solid phase constituents in sediments (mmol m<sup>-2</sup>).

Site	Pore water					Solid phase					
	NH <sub>4</sub> <sup>+</sup>	PO <sub>4</sub> <sup>3-</sup>	Fe <sup>2+</sup>	SO <sub>4</sub> <sup>2-</sup>	H <sub>2</sub> S	AVS	CRS	S <sup>0</sup>	Fe(II) <sub>(oxal)</sub>	Fe(III) <sub>(oxal)</sub>	Total Fe
Farm	14.4 (±1.76)	2.95 (±0.24)	0.30 (±0.02)	1947 (±7.96)	6.68 (±3.85)	1023 (±58)	6054 (±896)	1982 (±118)	3187 (±298)	3086 (±171)	6273 (±171)
Control	6.57 (±0.97)	0.91 (±0.20)	0.95 (±0.17)	1940 (±9.41)	0.94 (±0.05)	197 (±88)	2614 (±268)	146 (±39)	1725 (160)	3064 (±152)	4789 (±152)

revealed that fish farm sediment exhibited highly reduced conditions, with higher concentrations of NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3-</sup>, and H<sub>2</sub>S in pore water and greater AVS, CRS, S<sup>0</sup>, and Fe(II) content in sediments compared with control site sediment (Figure 2).

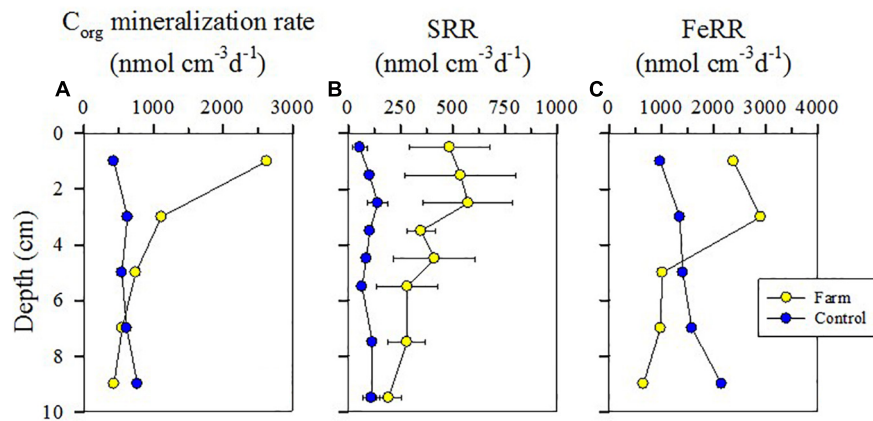
### Rates of Anaerobic C<sub>org</sub> Mineralization, Sulfate Reduction, and Iron Reduction

Both SR and FeR in the farm sediment were significantly greater than in the control sediment ( $P < 0.05$ ), especially in the surface layer (Figure 3). SRR (11.0 mmol S m<sup>-2</sup> d<sup>-1</sup>) and FeRR (47.8 mmol Fe m<sup>-2</sup> d<sup>-1</sup>) within a depth of 2 cm of the farm sediment were 6.9-fold and 2.4-fold higher than those measured at the control site (1.58 mmol S m<sup>-2</sup> d<sup>-1</sup> and 19.6 mmol Fe m<sup>-2</sup> d<sup>-1</sup>), respectively (Supplementary Table 2).

Total anaerobic C<sub>org</sub> mineralization rates (1872 ± 1071 nmol cm<sup>-3</sup> d<sup>-1</sup>) within a depth of 4 cm of the farm sediment was 3.6-fold greater than that (527 ± 139 nmol cm<sup>-3</sup> d<sup>-1</sup>) of the control sediment, although this difference was not significant ( $P = 0.22$ ) (Figure 3 and Supplementary Figure 1). In particular, the anaerobic C<sub>org</sub> mineralization rate within 2 cm of the farm sediment (52.6 mmol C m<sup>-2</sup> d<sup>-1</sup>) was up to 6.1-fold higher than that measured in the control sediment (8.56 mmol C m<sup>-2</sup> d<sup>-1</sup>) (Supplementary Table 2).

### Sediment Oxygen Demand and Benthic Nutrient Release

Sediment oxygen demand, as determined by linear regression of the decrease in DO concentration with time (Figure 4),



**FIGURE 3** | Vertical profiles of anaerobic  $C_{org}$  mineralization rates (A), sulfate reduction rates (SRRs) (B), and iron reduction rates (FeRRs) (C) in the fish farm and control sediment.

was 2.5-fold higher in the farm sediment ( $107 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) compared with the control sediment ( $43.1 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) (Supplementary Table 2). The flux of DIN and DIP was estimated from concentration variation in the overlying water of the chamber during incubation (Figure 4). Benthic  $\text{NH}_4^+$  fluxes in the farm and control sediments were  $11.4$  and  $4.61 \text{ mmol N m}^{-2} \text{ d}^{-1}$ , respectively, and  $\text{NO}_x$  fluxes were  $-0.25$  and  $0.11 \text{ mmol N m}^{-2} \text{ d}^{-1}$ , respectively. Consequently, the DIN fluxes calculated from the sum of  $\text{NH}_4^+$  and  $\text{NO}_x$  fluxes were 2.4-fold higher in the farm sediment ( $11.1 \text{ mmol N m}^{-2} \text{ d}^{-1}$ ) compared with the control sediment ( $4.72 \text{ mmol N m}^{-2} \text{ d}^{-1}$ ) (Table 5). The DIP flux in the farm sediment ( $4.13 \text{ mmol P m}^{-2} \text{ d}^{-1}$ ) was 24.3-fold greater than that measured in the control sediment ( $0.17 \text{ mmol P m}^{-2} \text{ d}^{-1}$ ) (Table 5).

## Speciation of Phosphorus in the Sediments

The content and distribution of P forms in sediment varied significantly between the farm and control site (Figures 5, 6,  $P < 0.05$ ) except for Lsor-P ( $P = 0.532$ ). Within a depth of 10 cm, total P content in the farm sediment ( $39\text{--}256 \mu\text{mol g}^{-1}$ ) was 4.5-fold greater than measured in the control sediment ( $14\text{--}40 \mu\text{mol g}^{-1}$ ) (Supplementary Table 3). The total P profile in the farm sediment was largely determined by the distribution of  $\text{Fe}_{(CDB)}\text{-P}$ , Bio-P, and Aut-P, whereas the distribution of Lsor-P and Detr-P was mostly responsible for the shape of total P in the control sediment (Figure 6). Vertically, the three major P fractions, i.e.,  $\text{Fe}_{(CDB)}\text{-P}$ , Bio-P, and Aut-P, were highest in the surface layer (0–1 cm) of the farm sediment and decreased with depth (Figure 5).

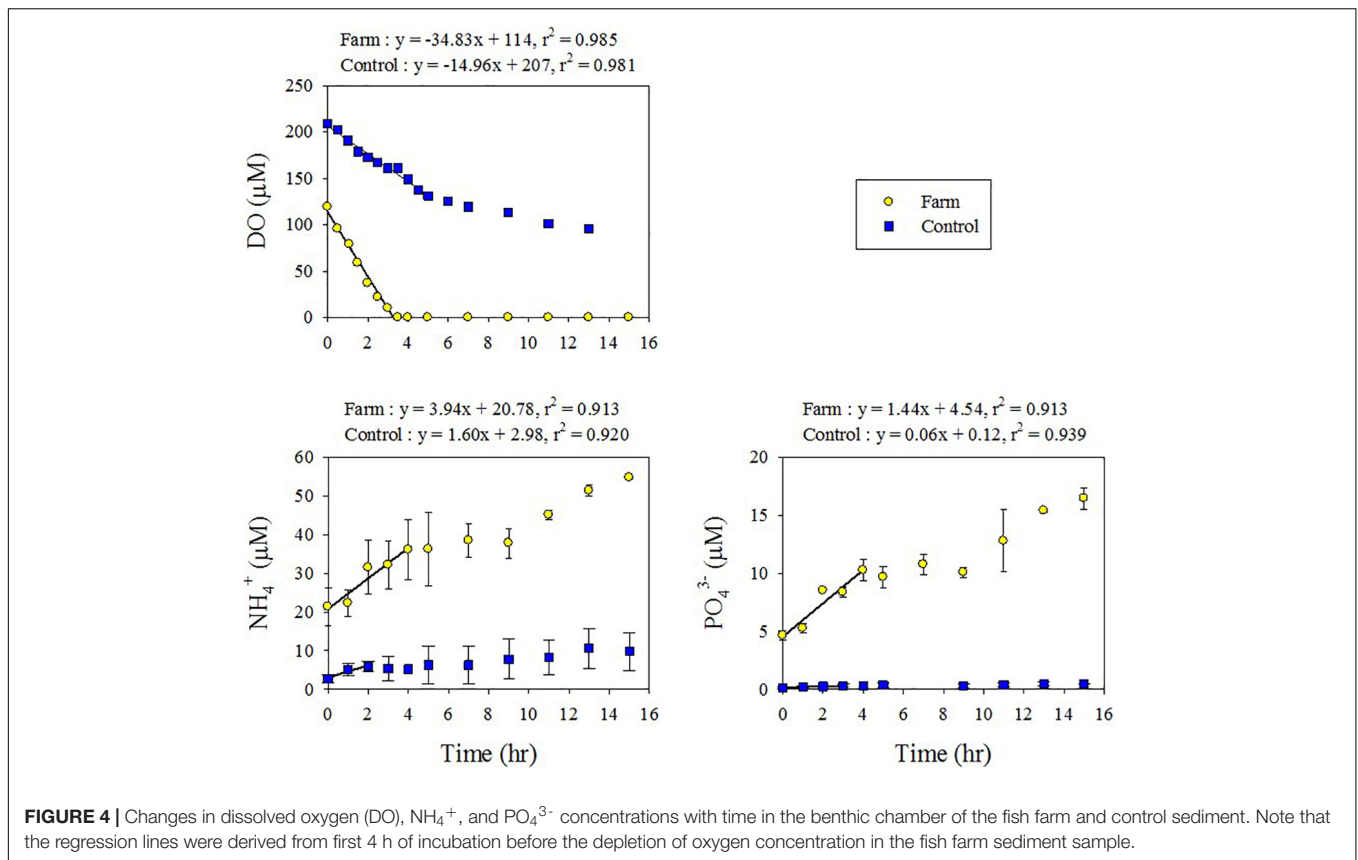
Lsor-P content was similar in both sites, ranging from 4 to  $10 \mu\text{mol g}^{-1}$  and from 3 to  $11 \mu\text{mol g}^{-1}$  in the sediments of the farm and control site, respectively, and the concentration decreased with depth (Figure 5). Lsor-P constituted a minor P form in the farm sediment, accounting for 4–15% of total P within 10 cm of depth, whereas it comprised 16–28% of total P in the control sediment (Figure 6 and Supplementary Table 3).

$\text{Fe}_{(CDB)}\text{-P}$  content ranged from 6 to  $49 \mu\text{mol g}^{-1}$ , and from 1 to  $7 \mu\text{mol g}^{-1}$  in the farm and control sediment, respectively, and the concentration of  $\text{Fe}_{(CDB)}\text{-P}$  decreased with depth (Figure 5). The  $\text{Fe}_{(CDB)}\text{-P}$  form accounted for 12–24% and 7–18% of total P within a depth of 10 cm in the farm and control sediment, respectively (Figure 6 and Supplementary Table 3). Bio-P content ranged from 12 to  $78 \mu\text{mol g}^{-1}$  and from 1 to  $3 \mu\text{mol g}^{-1}$  in the farm and control sediment, respectively, and the concentration of Bio-P decreased with depth (Figure 5). Bio-P constituted the major P form in the farm sediment, accounting for 20–39% of total P, but only 6–8% of total P in the control sediment (Figure 6 and Supplementary Table 3). Aut-P content ranged from 14 to  $102 \mu\text{mol g}^{-1}$  and from 5 to  $102 \mu\text{mol g}^{-1}$  in the farm and control sediment, respectively. Overall, Aut-P content decreased with depth (Figure 6). This form, together with Bio-P, constituted the major P form to a depth of 10 cm in the farm sediment, representing 12–44% of total P (Figure 6 and Supplementary Table 3). Detr-P contents ranged from 8 to  $21 \mu\text{mol g}^{-1}$  and from 5 to  $11 \mu\text{mol g}^{-1}$  in the farm and control sediment, respectively (Figure 5). Detr-P constituted the major P form in the control sediment, accounting for 28–37% of total P to a depth of 10 cm (Figure 6 and Supplementary Table 3). Ref-OP content, which ranged from 2 to  $5 \mu\text{mol g}^{-1}$  and from 2 to  $3 \mu\text{mol g}^{-1}$  in the farm and control sediment, respectively, was similar at both sites (Figure 5). This form accounted for 2–5% and 8–12% of total P within 10 cm of depth in the farm and control sediment, respectively, and thus constituted a minor P form together with Lsor-P in the farm sediment (Figure 6 and Supplementary Table 3).

## DISCUSSION

### Impact of Farming on Phosphorus Enrichment and Speciation

The most prominent features revealed by P speciation analysis were that: (1) high total P, more than four-fold greater than the control site, accumulated in fish farm sediment



**TABLE 5 |** Nutrient demand for primary production (PP), benthic nutrient flux (BNF), and contribution of BNF to PP.

Site	PP <sup>a</sup> (mmol m <sup>-2</sup> d <sup>-1</sup> )	Nutrient demand for PP <sup>b</sup> (mmol m <sup>-2</sup> d <sup>-1</sup> )		Benthic nutrient flux (mmol m <sup>-2</sup> d <sup>-1</sup> )		Contribution of BNF to PP (%)	
		N	P	N <sup>c</sup>	P <sup>c</sup>	N	P
Farm	54.3	8.2	0.5	11.1	4.13	135	826
Control	86.1	13.0	0.8	4.72	0.17	36.3	21.3

<sup>a</sup>PP data were taken from NIFS (2013).

<sup>b</sup>N and P demands for PP were calculated using Redfield's ratio of C:N:P = 106:16:1.

<sup>c</sup>DIN was calculated from the sum of  $\text{NH}_4^+$  and  $\text{NO}_x$  release rate, and DIP was calculated from  $\text{PO}_4^{3-}$  release rates (Figure 4).

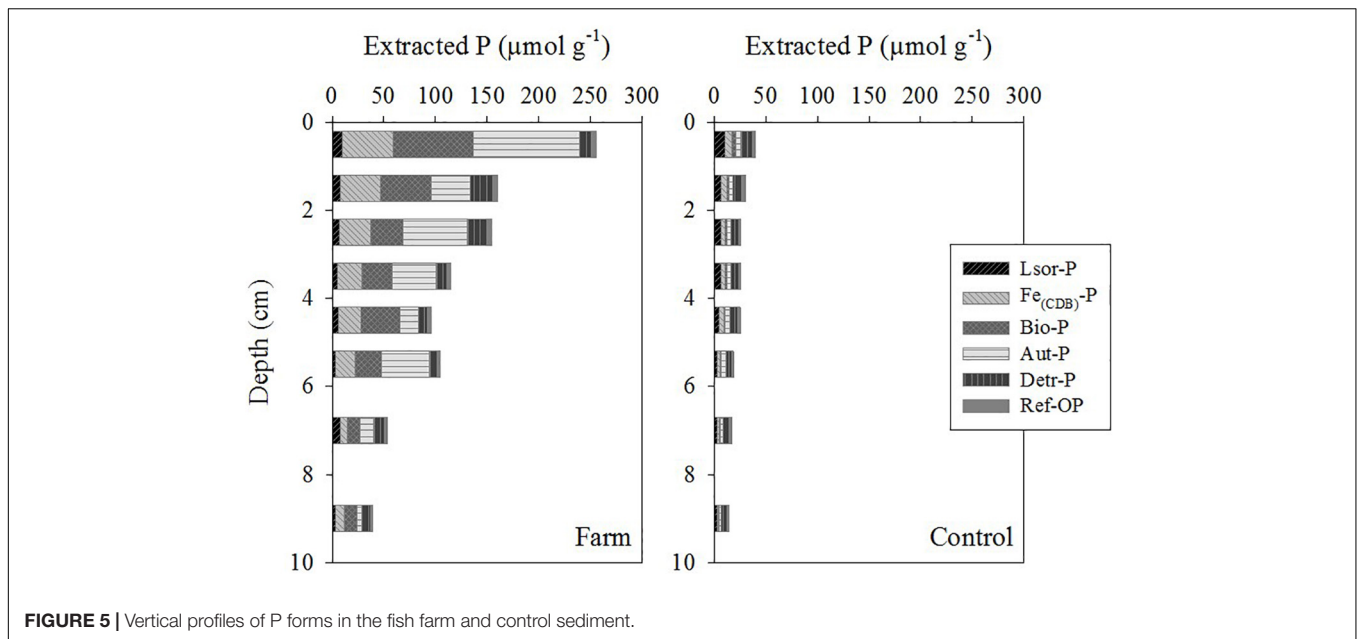
(Figure 5 and Supplementary Table 3), and (2) Aut-P and Bio-P were the dominant fractions of P forms (Figure 6). The higher accumulation of total P in farm sediment (Figure 5 and Supplementary Table 3) directly reflected the impact of P-rich fish feed in fish farm regions (Karakassis et al., 1998; Holmer et al., 2002; Soto and Norambuena, 2004; Porrello et al., 2005; Matijević et al., 2008). Several studies have estimated that 60–70% of the P in feed is discharged as waste into the marine environment (Olsen et al., 2008; Wang et al., 2012; Bouwman et al., 2013), which is equivalent to the discharge of 9.4–25 kg of P waste per ton of fish production (Holby and Hall, 1991; Islam, 2005; Wang et al., 2012). Based on the P content in fish feed (0.8%, Christensen et al., 2000) and the feed input from the present study area (Status of Fish Culture 2017)<sup>2</sup>, 8–16 kg of P waste per ton of fish production would be generated in the study area. If 53% of

the P in fish feed is accumulated in coastal sediment (Wu, 2001), the sinking of uneaten fish feed and fecal production from fish farms is directly responsible for P enrichment in sediment.

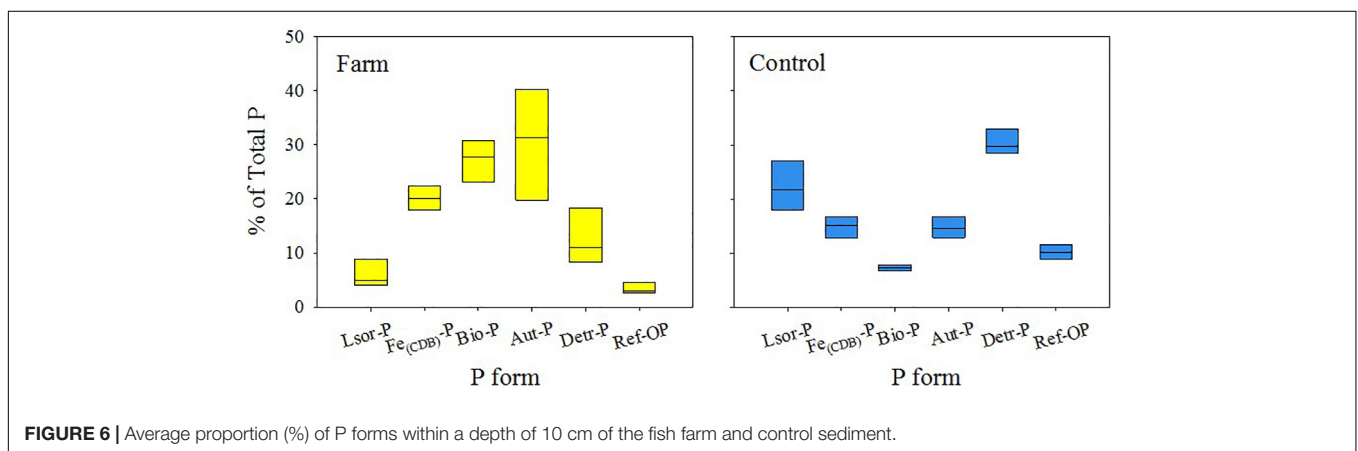
In addition to the insight it provided into P enrichment, P-speciation analysis clearly demonstrated the proportional order of each form of P in farm sediment [Aut-P > Bio-P > Fe<sub>(CDB)</sub>-P > Detr-P > Lsor-P > Ref-OP] and control sediment [Detr-P > Lsor-P > Fe<sub>(CDB)</sub>-P > Aut-P > Ref-OP > Bio-P] (Figure 6). The down-core increase in Aut-P in control sediment is resulted from the precipitation of Ca<sup>2+</sup> with P that is released by C<sub>org</sub> mineralization, which is responsible for the mirror image observed in the relative abundance of Fe<sub>(CDB)</sub>-P and Aut-P with depth (i.e., sink-switching between Fe-P and Aut-P) (Supplementary Figure 2; Ruttenberg, 2003). However, this sink-switching model did not appear in the farm sediment where the relative abundance of Aut-P was highest in surface layer (Supplementary Figure 2).

<sup>2</sup><http://kosis.kr>





**FIGURE 5** | Vertical profiles of P forms in the fish farm and control sediment.

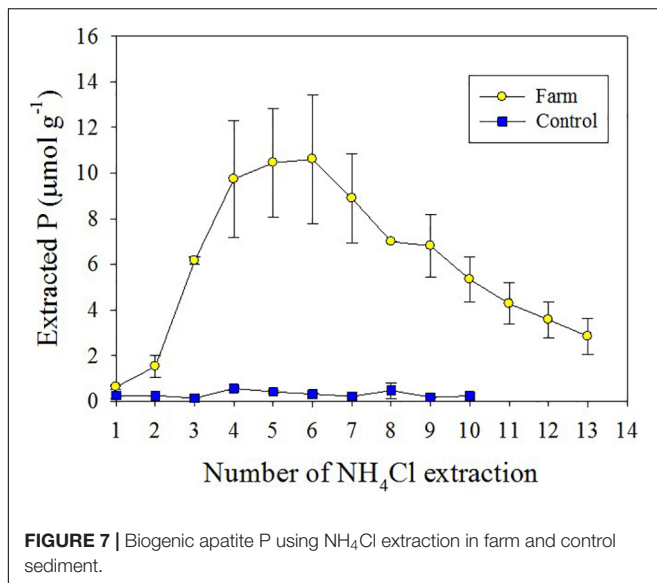


**FIGURE 6** | Average proportion (%) of P forms within a depth of 10 cm of the fish farm and control sediment.

The high proportion of Aut-P together with the high  $\text{PO}_4^{3-}$  concentration in surface sediment at the farm site is primarily related to mineralization of organic matter (i.e., P-rich fish feed). The P released during  $\text{C}_{\text{org}}$  remineralization resulted in the P supersaturation in pore water with respect to apatite, which ultimately forms Aut-P (Slomp et al., 1996; Schenau et al., 2000; van der Zee et al., 2002; Andrieux-Loyer et al., 2014). In organic-rich sediment in particular, the regeneration of inorganic P from  $\text{C}_{\text{org}}$  remineralization was the predominant process for Aut-P precipitation (Anschutz et al., 2007; Tsandev et al., 2012; Joshi et al., 2015; Kraal et al., 2017). The enhancement of  $\text{C}_{\text{org}}$  mineralization rate and reductive dissolution of Fe oxides resulting from the reaction of FeOOH with sulfide in the organic-rich farm sediment were directly responsible for the strong surface enrichment of Aut-P ( $102 \mu\text{mol g}^{-1}$ , 40% of total P) in the farm sediment (Figure 5; van der Zee et al., 2002; Brock and Schulz-Vogt, 2011; Slomp, 2011; Andrieux-Loyer et al., 2014; Supplementary Table 1). In particular, the accumulation of  $\text{S}^0$  in

the sediments may result from the reaction between FeOOH and  $\text{H}_2\text{S}$  (Figure 2H and Supplementary Table 1). Furthermore, as biogenic hydroxyapatite (Bio-P) is more soluble than fluorapatite, additional phosphate is released into pore water during the Bio-P dissolution process after deposition, which results in a subsequent precipitation of P into the CFA (Froelich et al., 1988; Kassila et al., 2001; Schenau and De Lange, 2001).

The abundance of Bio-P in farm sediment is associated with P-rich fish feed. In sequential extraction methods, authigenic apatite generally includes authigenic CFA, biogenic apatite, and  $\text{CaCO}_3$ -associated P (Jensen et al., 1998; Ruttenberg et al., 2009). However, Schenau and De Lange (2000) recognized that a significant amount of reactive P was removed through high preservation of fish debris consisting of hydroxyapatite in surface deposits from the Arabian Sea, which suggested the need for identification of P associated with biogenic apatite (Bio-P) from authigenic apatite. In this regard, an additional attempt was made to separate biogenic P (Bio-P) associated with fish debris



from Aut-P to assess the impact of fish farming on coastal environments through repeated NH<sub>4</sub>Cl extraction (Matijević et al., 2008). Indeed, repeated NH<sub>4</sub>Cl extraction revealed that the concentrations of extracted P in farm sediment increased sharply from the third extraction step, and then remained high until the seventh extraction, whereas NH<sub>4</sub>Cl-extracted P in the control sediment remained low throughout the extraction procedure (Figure 7). As a result, Bio-P in the farm sediment (78 µmol g<sup>-1</sup>, 31% of total P) was up to 25 times higher in surface layers compared with that in the control sediment (3 µmol g<sup>-1</sup>, 8% of total P). These results clearly demonstrate that the deposition of feed was directly responsible for the high surface accumulation of Bio-P in the fish farm sediments, and thus directly affected P speciation in sediment (Figures 5, 6).

### Impact of Fish Farming on P Dynamics Associated With Iron- and Sulfate Reduction

In the present study, despite the high sinking particle flux (126.2 g m<sup>-2</sup> d<sup>-1</sup>, NIFS, 2013) at the farm site, the organic content in the fish farm sediments (2.16% TOC) was lower than the values reported in other fish farm sediments (Holmer and Kristensen, 1992; Holmer et al., 2002; á Norði et al., 2011; Bannister et al., 2014). The high current speed (average 28–33 cm s<sup>-1</sup>, Ro et al., 2007) and high polychaete density (approximately 2,500 inds. m<sup>-2</sup>, NIFS, 2013) likely explain this low organic matter accumulation. For example, Holmer and Frederiksen (2007) reported low organic content (average 0.51–1.60% POC) despite high sinking particle fluxes (32.3–63.8 g m<sup>-2</sup> d<sup>-1</sup>) in farm sediments from the Mediterranean Sea and attributed it to dispersion of waste products under rapid water exchange (>5.5 cm s<sup>-1</sup>) and/or the consumption of waste products by benthic fauna, i.e., up to 400 inds. m<sup>-2</sup> polychaetes (*Hermodice carunculata*) (Heilskov et al., 2006) or up to 60 inds. m<sup>-2</sup> sea urchins (Ruiz et al., 2001).

Analysis of geochemical constituents and metabolic rate measurement clearly revealed that high organic loading (126.2 g m<sup>-2</sup> d<sup>-1</sup>) in the fish farm greatly stimulated benthic metabolism (Figure 3 and Supplementary Table 2), which resulted in increased accumulation of mineralization products (i.e., NH<sub>4</sub><sup>+</sup>, H<sub>2</sub>S, and PO<sub>4</sub><sup>3-</sup>) in pore water from farm sediment (Figures 2A–C and Table 4). SR was a dominant C<sub>org</sub> mineralization pathway in the farm sediment, accounting for 70% of anaerobic C<sub>org</sub> mineralization (Supplementary Table 2). Stimulation of SR by aquaculture has been continuously demonstrated in several fish farms where feed or feces are deposited directly onto the sediment (Holmer et al., 2002, 2003; Holmer and Frederiksen, 2007; Holmer and Heilskov, 2008; Valdemarsen et al., 2009; á Norði et al., 2011; Hyun et al., 2013; Bannister et al., 2014; Choi et al., 2018, 2020). Along with enhanced SR, abundant Fe oxides (~40 µmol cm<sup>-3</sup>; Figure 2J) would ultimately stimulate FeR in surface sediments of the farm (Figure 3C; Thamdrup, 2000).

The enrichment of dissolved Fe<sup>2+</sup> in pore water can be ascribed to the stimulation of dissimilatory FeR or chemical reduction of Fe oxides coupled with H<sub>2</sub>S oxidation (Canfield, 1989; Hyun et al., 2013; An et al., 2019). However, despite the high FeRR in farm surface sediments, the Fe<sup>2+</sup> concentration in pore water of farm sediment was strikingly lower than that of the control sediment (Figure 2E). This decoupling of FeR and Fe<sup>2+</sup> was due to the precipitation of dissolved Fe<sup>2+</sup> with surplus sulfides derived from the high SR (Canfield et al., 2005; Firer et al., 2008), which subsequently stimulated reactions between Fe and S to form high FeS (AVS) and FeS<sub>2</sub> (CRS) content in the sediments (Figures 2E,G and Supplementary Table 1; Thamdrup et al., 1994; Jørgensen et al., 2019). In addition, under high C<sub>org</sub> loading conditions, SR dominates C<sub>org</sub> mineralization, which ultimately results in high P release by lowering the binding capacity of Fe to P due to the formation of FeS minerals (i.e., uncoupled cycling of Fe and P) (Lehtoranta et al., 2008, 2009; An et al., 2019).

### Enhanced Phosphorus Availability and Benthic Phosphorus Flux in Fish Farm Sediment

Higher SOD and benthic nutrient release (Figure 4) in fish farm sediment compared with control sediment indicate that the enhanced C<sub>org</sub> mineralization resulting from excess input of organic wastes was directly responsible for the stimulation of benthic nutrient release into the water column. Interestingly, the ratio of benthic release rate between ammonium (3.94 µmol N L<sup>-1</sup> h<sup>-1</sup>) and phosphate (1.44 µmol P L<sup>-1</sup> h<sup>-1</sup>) in the farm sediment (i.e., N/P = 2.74) was 9.7 times lower than that measured in the control sediment (i.e., N/P = 26.7) (Figure 4), which further suggests that benthic P release was greatly stimulated during the mineralization process of fish feed and subsequent P dynamics (Jia et al., 2015; Ferrera et al., 2016). Under SR-dominant conditions, the desorption of P via reduction of FeOOH coupled with H<sub>2</sub>S oxidation would be directly responsible for highly enhanced release of P from the sediment of the farm (Lehtoranta et al., 2008, 2009). The low P binding capacity (i.e., high P availability in pore water) in the farm sediment was further demonstrated through P regeneration efficiency (% P<sub>recyc</sub>), which

can be estimated from the percentage of benthic P flux ( $F_p$ ) directly measured from the benthic chamber incubation (**Table 5**) relative to the theoretical benthic P flux ( $F_{cp} = C_{org}$  oxidation rate  $\times 1/C:P$ ) based on Redfield stoichiometry ( $C:P = 106:1$ ) (Eq. 4, Ferrón et al., 2009a).

$$\% P_{recyc} = 100 \times (F_p/F_{cp}) \quad (4)$$

The measured benthic P flux ( $F_p$ ) in the farm sediment ( $4.13 \text{ mmol m}^{-2} \text{ d}^{-1}$ ; **Table 5**) was similar to the expected benthic P flux ( $4.86 \text{ mmol m}^{-2} \text{ d}^{-1}$ ). By contrast,  $F_p$  in the control sediment ( $0.17 \text{ mmol m}^{-2} \text{ d}^{-1}$ ; **Table 5**) was 3.3 times lower than  $F_{cp}$  ( $0.56 \text{ mmol m}^{-2} \text{ d}^{-1}$ ). Finally, the  $\% P_{recyc}$  calculated according to the Eq. 4 in the farm sediment (85%) was approximately three-fold greater than that estimated in the control sediment (30%). Here, the C:P ratio in the farm sediment was assumed to be lower than the Redfield ratio used in the control sediment (C:P = 106:1) due to the high P content in fish feed (Ferrón et al., 2009b), and thus we adopted the C:P ratio for organic matter (average 22.5) in the surface sediment measured by Holmer et al. (2007) in the case of the farm sediment. These results indicated that P could be quickly released to the water column through mineralization of organic matter from the farm sediment, whereas most P in the control sediment would be retained in the sediment. In addition, in the farm sediment, high  $P_{recyc}$  may be partly associated with P release through the chemical dissolution of Fe oxides and microbial metabolism (i.e., decomposition of polyphosphate) (Ferrón et al., 2009b; Brock and Schulz-Vogt, 2011).

The results also strongly suggest that, along with analysis of the P pool size, a separation of P forms in sediment is required to determine the upper limit of P availability associated with aquaculture in an aquatic ecosystem (Andrieux and Aminot, 1997; Hou et al., 2009). Hou et al. (2009) described bioavailable P as an integrated concentration of  $L_{sor}\text{-P}$ ,  $Fe_{(CDB)}\text{-P}$ , and Ref-OP. In the present study, Bio-P was additionally included in bioavailable P, due to the chemical dissolution properties of Bio-P (Schenau and De Lange, 2001). In the present study, the bioavailable P content within surface sediment to a depth of 2 cm (that is directly influenced by organic loading from the water column) was  $2,902 \text{ mmol m}^{-2}$  (58% of total P) in the farm sediment (**Supplementary Table 3**).

This high level of bioavailable P indicates that sediment below fish farms plays an important role as an internal source of P for coastal ecosystems (Ferrón et al., 2009b; Hou et al., 2009; Viktorsson et al., 2013; An et al., 2019). For example, Christensen et al. (2000) pointed out that aquaculture could have an impact on the entire coastal environment by supplying 42% of total P input in summer, even though fish farms occupy a relatively small area (0.02% of the total coastal area). In the present study, benthic release of N and P accounted for 135 and 826%, respectively, of the N and P required for primary production (**Table 5**). Because the occurrence of certain dinoflagellates (e.g., *Cochlodinium polykrikoides*) in coastal waters, including the study area, is often associated with excess P in the water column (Thomas and Smayda, 2008; Cho, 2010; Lee et al., 2015), the results suggest that the

excessive benthic P flux (or P availability) from aquaculture sediment could be a significant factor triggering harmful algal blooms (Rozañ et al., 2002; Stal et al., 2003; Joshi et al., 2015; Schoffelen et al., 2018).

## CONCLUSION

Input of fish feed associated with fish farming activity significantly enhanced the accumulation of biogenic apatite P in sediment, suggesting that it could be used as a sensitive indicator for the assessment of environmental conditions in fish farm sediments, especially those related to fish feed. High benthic metabolism by SR in farm sediment greatly stimulated P release into pore water, which ultimately altered P speciation by increasing the Aut-P fraction in total P. Enhanced P regeneration efficiency and high P bioavailability in the farm sediment suggests that the sediment below the fish farm acts as an internal source of P to support primary production in the coastal ecosystem. Overall results strongly suggest that variations in P forms and benthic P flux coupled with  $C_{org}$  mineralization and resulting C-Fe-S cycles in the sediment could provide important information for quantitative and qualitative assessments of the impact of aquaculture in coastal ecosystems.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

## AUTHOR CONTRIBUTIONS

J-SM and J-HH designed the study and conducted most writing of the manuscript. J-SM, AC, BK, and S-UA collected the samples and performed most laboratory analysis. All authors contributed to the discussion of the results and approved the submitted version.

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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.2021.645449/full#supplementary-material>

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