MINERALOGICAL AND GEOCHEMICAL CONTROLS ON POLYPHOSPHATE TRANSFORMATION AND MINERALIZATION IN MARINE SEDIMENTARY ENVIRONMENTS

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MINERALOGICAL AND GEOCHEMICAL CONTROLS ON POLYPHOSPHATE TRANSFORMATION AND MINERALIZATION IN MARINE SEDIMENTARY ENVIRONMENTS

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polyphosphate (2 mM as total P), γ-Al$_2$O$_3$ 0.4 g/L, pH 6.0 and 8.0, with/without 1 mM Ca^{2+}. Solid lines are the first-order kinetic model fitting results.

birnessite (g–i), hematite (j–l), and boehmite (m–o) at pH 6 and 8. Error bars indicate standard deviation (SD) of the replicate experiments.

LIST OF SYMBOLS AND ABBREVIATIONS

Al	aluminum
ACP	amorphous calcium phosphate
AMP	adenosine 5′-monophosphate
AOS	average oxidation state
ASW	artificial seawater
ATP	adenosine 5′-triphosphate
BET	Brunauer–Emmett–Teller gas adsorption analysis
C	carbon
CFA	calcium fluorapatite
DI	deionized water
DIP	dissolved inorganic phosphorus
DOP	dissolved organic phosphorus
ED	electron diffraction
EDX/EDS	energy dispersive X-ray spectroscopy
EELS	electron energy loss spectroscopy
EXAFS	extended X-ray absorption fine structure
Fe	iron
FTIR	Fourier transform infrared spectroscopy
GP	β-glycerophosphate
G6P	D-glucose 6-phosphate
HA	humic acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRTEM	high resolution transmission electron microscopy
ICP-MS	inductively coupled plasma-mass spectrometry
IHP	*myo*-inositol hexakisphosphate
IP	inorganic phosphorus
LCF	linear combination fitting
Mn	manganese
MOPS	3-(N-morpholino)propanesulfonic acid
N	nitrogen
NaN_3	sodium azide
NMR	nuclear magnetic resonance
NOM	natural organic matter

NP	β-nicotinamide adenine dinucleotide
O	oxygen
OP	organic phosphorus
P	phosphorus
pyroP/P_2	pyrophosphate
triP/P_3	tripolyphosphate
PIP	particulate inorganic phosphorus
POP	particulate organic phosphorus
PP	particulate phosphorus
p-NPP	*p*-nitrophenyl phosphate
polyP	polyphosphate
SEM	scanning electron microscopy
STEM	scanning transmission electron microscopy
TEM	transmission electron microscopy
UV/vis	UV/visible spectrophotometry
XANES	X-ray absorption near edge structure
XAS	X-ray absorption spectroscopy
XRD	X-ray diffraction

SUMMARY

Phosphorus (P) is an essential and limiting macronutrient that regulates marine primary productivity and affects the cycling of carbon (C) and other major elements such as nitrogen (N). Despite the critical roles of marine P cycle in global biogeochemical processes, the mechanisms leading to P removal from the ocean are not fully understood. The largest pathway for marine P removal is the *in situ* (or authigenic) formation of stable calcium (Ca) phosphate minerals (e.g., apatite). However, under marine conditions, apatite formation is often kinetically inhibited. Polyphosphate (polyP) has been proposed to potentially mediate authigenic apatite formation. PolyP is a group of polymeric molecules with at least three phosphate groups joined by phosphoanhydride (O–P–O) bonds. PolyP can be widely synthesized by most microorganisms in aquatic environments and has important physiological roles. PolyP release to the local marine environment can be a potential orthophosphate (orthoP) source to induce the nucleation of Ca-phosphate minerals, such as amorphous Ca-phosphate (ACP; a precursor of crystalline Ca-phosphate) and apatite. Ca-phosphate mineral precipitation from exogenous polyP intermediates was reported to significantly contribute to P burial in global marine sediments. However, despite the widespread presence of polyP and its relevance to authigenic apatite formation in marine environments, biotic and abiotic factors controlling polyP transformation and mineralization are not well understood, and the underlying mechanisms are poorly constrained.

This dissertation investigates the mechanisms of polyP-mediated Ca-phosphate precipitation under marine conditions approached by laboratory controlled experiments and mesocosm sediment incubations. The *first aim* is to investigate, at molecular level, mineral-catalyzed hydrolysis of linear polyP by representative environmental metal oxide minerals, such as iron (Fe), aluminum (Al), and manganese (Mn) oxides. Wet chemistry analyses were combined with advanced spectroscopic and microscopic techniques to reveal the reaction kinetics and

molecular level mechanisms. Linear polyP can be degraded to orthophosphate by all three metal oxides and this process was significantly faster in the presence of Ca^{2+}. Mineral type, structure, and particle size, as well as dissolved metal cations and solution pH strongly influence the hydrolysis rate and extent.

Four representative Mn oxides were studied, including α-MnO_2, δ-MnO_2, birnessite, and β-MnO_2 (Chapter 2). All four Mn oxides can rapidly hydrolyze polyP via one-by-one cleavage of terminal P$-$O$-$P bonds, and the hydrolysis rates are in the order of α-MnO_2 > δ-MnO_2 > birnessite > β-MnO_2. The hydrolysis rates for longer chained polyP are higher than those of shorter chained ones. The presence of dissolved metal cations (Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}) promotes polyP hydrolysis by δ-MnO_2. Formation of cation-polyP ternary surface complexes is likely the dominant mechanism for cation promotion of polyP hydrolysis on Mn oxides. Solid calcium polyP granules can also be hydrolyzed by Mn oxides and transform into ACP solids, and the content of ACP solids increases as pH increases.

In the suspensions of the Al oxide γ-Al_2O_3 (of three different sizes 5, 35, and 70 nm) with 1 mM Ca^{2+}, the rate of polyP hydrolysis decreases with increasing mineral particle size (Chapter 3). The main surface P species on γ-Al_2O_3 are explored using single pulse magic angle spinning (SP/MAS), $^{31}P\{^1H\}$ cross-polarization MAS (CP/MAS), 2-dimensional (2D) $^{31}P\{^1H\}$ heteronuclear correlation (HetCor), ^{27}Al SP/MAS NMR, and P K-edge X-ray absorption near edge structure (XANES) spectroscopy. The surface P species include: 1) amorphous calcium phosphate precipitates, 2) phosphate groups in polyphosphate that formed direct bonds with the mineral surface as inner-sphere complexes, and 3) phosphate groups in polyphosphate that were not directly bonded to the mineral surfaces.

Fe oxides can also strongly hydrolyze polyphosphate in the presence of Ca^{2+}, and the hydrolysis rates follow the order of lepidocrocite > hematite > ferrihydrite > goethite (Chapter 4).

A terminal-only pathway via one-by-one cleavage of terminal phosphate groups is the dominant hydrolysis mechanism. Under alkaline pH conditions, polyP hydrolysis leads to the precipitation of ACP in the presence of Ca^{2+}, and the fraction of ACP increases as the pH value increases. The newly formed ACP eventually transforms to crystalline hydroxyapatite upon long-term aging. However, the hydrolysis rate of polyphosphate and the percentage of ACP formed is relatively low in artificial seawater, possibly due to the strong aggregation of Fe oxides at high ionic strength and the resulting reduced reactive surface area.

In addition to polyP, organic and short chained condensed phosphates are also viewed as important P sources that can potentially mediate authigenic apatite formation. Chapter 5 systematically compares the hydrolysis rates of six organic and three condensed phosphates by two common phosphorus enzymes (acid and alkaline phosphatases) and three representative metal oxide minerals (hematite, birnessite, and boehmite). Phosphatases rapidly hydrolyze organic phosphate monoesters and condensed phosphatases, whereas hematite and birnessite quickly hydrolyze condensed phosphates. By normalizing the reaction rates and considering the abundance and reactivity of these enzymes and minerals in soils/sediments, the hydrolysis rates of condensed phosphates on hematite and birnessite are comparable to those by acid and alkaline phosphatases. Interestingly, phosphatases and minerals show different preferential hydrolysis for organic phosphate monoesters and condensed phosphates. The results provide the first direct quantitative comparison on the hydrolysis rates of different complex P molecules by enzymes and minerals.

The *second aim* of this dissertation is to explore the roles of enzyme *vs.* mineral catalyzed reactions in polyP mineralization and transformation into Ca-phosphate minerals under marine sedimentary conditions. Mesocosm sediment incubations are conducted to test polyP hydrolysis and transformation into Ca-phosphate minerals in enzyme or mineral amended sediments, and the incubation products are characterized by a variety of diffraction, spectroscopic, and microscopic

techniques. The extents and rates of polyphosphate hydrolysis in sediment incubations roughly follow the order of alkaline phosphatase > acid phosphatase $\geq$ birnessite > hematite > boehmite $\approx$ unamended sediments. Additionally, regardless of the sterilization state and methods for the sediments, the trends and extents of orthophosphate production are similar across five different sterilization treatments (no treatment, autoclaved, high temperature, UV-light, and NaN_3). This suggests that microbial activity has limited effect on polyphosphate hydrolysis within the experimental time frame under the incubation conditions. Upon polyP hydrolysis, ACP forms first but its transformation into crystalline Ca-phosphate minerals is not observed, likely due to the presence of highly concentrated Mg^{2+}. When Mg^{2+} is removed from the reaction solution, equilibrium P concentrations are significantly lower due to ACP formation and its potential transformation m into hydroxyapatite during the late stage of sediment incubation. For sediments obtained from different depth (surface vs deep), no obvious differences on solid P speciation is observed in the reaction products, both mainly contain adsorbed P species and ACP. The rates of mineral-catalyzed hydrolysis in deep sediments are higher than those in surface sediments, likely due to the mineralogical difference in these two sediments.

This dissertation provides new insights into the roles of enzymes and minerals in the transformation of polyP and the subsequent precipitation of Ca-phosphate minerals in marine sedimentary environments. Considering the ubiquity of metal oxide minerals in sediments, this research, for the first time, reveals the role of these oxide minerals in P mineralization and burial in oceanic environments. More detailed understanding of polyP marine cycling can provide important insights into the roles of P as a nutrient and an element that reacts extensively with other major elements. This study greatly advances our current understanding of global P cycle and marine P burial under the influences of mineralogical and biological controls and provides new insights for understanding the occurrence of crystalline Ca-phosphate minerals in marine environments.

CHAPTER 1. INTRODUCTION

Phosphorus (P) is an essential but often limiting macronutrient for terrestrial biological production and aquatic primary productivity (Paytan and McLaughlin, 2007; Ruttenberg, 2014). As one of the most abundant elements on Earth, P has an average crustal abundance of 0.1% by weight (Canfield et al., 2005). Total P contents are estimated to be 170–21,000 mg kg^{-1} in surface and subsurface environments (e.g., soils and sediments) (Klein et al., 2019). A variety of P-containing compounds are found in natural environments, including orthophosphate (orthoP), organic phosphate (OP) esters, condensed phosphates, phosphite, and phosphonates, etc. (Figueroa and Coates, 2017; Paytan and McLaughlin, 2007; Van Mooy et al., 2015). Orthophosphate is typically considered as the most common P species in nature and the only P species (in the forms of HPO_4^{2-} and $H_2PO_4^-$) that can be used directly and effectively by most living organisms, though recent studies showed that some microorganisms can take up low molecular weight dissolved organic P and reduced P species (Karl, 2014; Van Mooy et al., 2015). Orthophosphate forms a variety of P-bearing minerals in the environments, and the most common P minerals are apatite [$Ca_2Ca_3(PO_4)_3(OH, F, Cl)$], strengite ($FePO_4 \cdot 2H_2O$), vivianite [$Fe_3(PO_4)_2 \cdot 8H_2O$], and wavellite [$Al_3(PO_4)_2(OH)_3 \cdot 5H_2O$] (Arai and Sparks, 2007; Rothe et al., 2016). In soils and sediments, phosphate strongly adsorbs on natural oxide and clay minerals, which accounts for a significant fraction in solid P pool (Arai and Sparks, 2007; Gu et al., 2020; Ruttenberg, 2014). The cycling of these P compounds/minerals has significant impacts on P bioavailability, global biogeochemical processes, terrestrial/aquatic primary productivity, and agricultural production (Ruttenberg, 2014; Van Mooy et al., 2015). To predict P bioavailability and understand the global P cycle, the main biotic and abiotic processes controlling the transformation of different P-containing compounds/minerals in various environmental settings need to be systematically studied.

1.1 Marine phosphorus cycling

In the oceans, P limits primary productivity across vast geographical areas over both modern and geologic timescales, thus controlling the biogeochemical cycles of other major elements such as carbon (C), nitrogen (N), and oxygen (O) (Benitez-Nelson, 2000; Schulz and Schulz, 2005; Slomp and Van Cappellen, 2007). Marine photosynthetic (micro)organisms can take up dissolved inorganic phosphate (DIP), carbon dioxide (CO_2), nitrate (NO_3^-), and other essential nutrients (e.g., trace elements) to build biomass using energy from the sunlight, and produce O_2 and organic matters (Paytan and McLaughlin, 2007; Ruttenberg, 2014). Marine P cycle eventually links to the marine C and N cycles by the microscopic floating marine plants or phytoplankton that are the base of marine food web (Paytan and McLaughlin, 2007; Ruttenberg, 2014). P is typically considered to be a main limiting macronutrient for primary productivity of marine ecosystems (Krom et al., 1991; Tyrrell, 1999).

The source-sink balance of P in the ocean controls primary productivity on geological timescales (Benitez-Nelson, 2000; Goldhammer et al., 2010). Riverine influx (0.032–0.058×10^{12} moles P per year for dissolved P and 0.59–0.65×10^{12} moles P per year for particulate P) is thought to be the primary P input, whereas atmospheric P deposition (0.01–0.05×10^{12} moles P per year) has recently been considered as another important marine P source (Figure 1.1a) (Benitez-Nelson, 2015; Paytan and McLaughlin, 2007; Ruttenberg, 2014). The dominant sink for P is sedimentary deposition and burial (0.093–0.341×10^{12} moles P per year). After transformation from dissolved to particulate forms, P is lost to the sediment on continental shelves and a smaller portion to deep-sea sediments (Benitez-Nelson, 2000; Paytan and McLaughlin, 2007; Ruttenberg, 2014). The P reservoir in marine water columns is dominated by dissolved forms at about 3×10^{15} moles

$(2.9 \times 10^{15}$ moles in deep water and 0.1×10^{15} moles in surface water) (Paytan and McLaughlin, 2007), but the sedimentary P reservoir is large, at about 1.3×10^{20} moles P (Benitez-Nelson, 2000).

Figure 1.1 Illustration of the marine phosphorus cycle (a) and phosphorus transformations in water columns and marine sediments (b). Modified based on Ruttenberg (2014), Paytan and McLaughlin (2007), and Defforey and Paytan (2018). P: phosphorus; DIP: dissolved inorganic phosphorus; DOP: dissolved organic phosphorus; IP: inorganic phosphorus; OP: organic phosphorus; PIP: particulate inorganic phosphorus; POP: particulate organic phosphorus.

Several P-containing compounds are found in marine environments, including orthophosphate, pyrophosphate (pyroP), polyphosphate (polyP), and organic phosphates (e.g., OP monoesters, OP diesters, phosphonates, etc.), etc. (Pasek, 2008; Paytan and McLaughlin, 2007). In terrestrial environments, phytate is the most abundant OP and a main P storage form for organisms (Baldwin, 2013; Suzumura and Kamatani, 1995; Turner et al., 2005). PolyP is the main P reserve in marine microorganisms (Omelon and Grynpas, 2008; Rao et al., 2009). OPs are also very abundant in marine environments since P can provide the phosphate-ester backbone for DNA and RNA, is crucial in the transmission of chemical energy through ATP molecule, and is a

structural constituent in many cell components such as phosphoproteins and phospholipids in cell membranes (Paytan and McLaughlin, 2007). Recently, dissolved phosphonate (P in +3 oxidation state) was found to be ubiquitous in seawater and marine sediments (Defforey and Paytan, 2018; Sannigrahi and Ingall, 2005; Van Mooy et al., 2015) and a vast oceanic P redox cycle was dominated by colonial nitrogen-fixing cyanobacteria (Van Mooy et al., 2015).

Biological cycling and remineralization are the primary mechanisms for the transformation of dissolved phosphorus (DP) and are dominant in surface seawater (Figure 1.1a). Most of the P in the open ocean is associated with biogenic particles as particulate phosphorus (PP) (Ruttenberg, 2014). In upper seawater column, DP is lost from surface waters via downwelling and biological uptake as particulate organic phosphorus (POP) and returns to surface waters via upwelling (Figure 1.1b) (Ruttenberg, 2014). Reactive PP and DP undergo biotic and abiotic transformations throughout the water columns and in the sediments, while detrital P are exported to the seafloor without such processes and passively buried (Figure 1.1b) (Defforey and Paytan, 2018). At the sediment-water interface, a series of diagenetic processes act to enrich pore water P concentration above the seafloor and result in an appreciable benthic flux of P from the seabed to overlying bottom waters (Figures 1.1 and 1.2a). The main processes include i) microbial respiration of organic matter in marine sediments, ii) microbial reduction and dissolution of iron (Fe) and manganese (Mn) (oxyhydr)oxides with subsequent release of associated P (mainly as Fe/Mn-bound P), and iii) sulfidization of P-bearing Fe minerals by H_2S originated from microbial sulfate reduction and resultant liberation of Fe-P (Krom and Berner, 1981; Ruttenberg, 2014).

Dissolved inorganic phosphorus (DIP), mainly as orthoP, is usually assimilated by phytoplankton in surface seawater and transformed into OP compounds (Cotner and Wetzel, 1992; Ruttenberg, 2014). Phytoplankton cell lysis releases cellular dissolved IP and OP to surrounding

environments and some of the organic P compounds can be hydrolyzed by enzymes synthesized by bacteria and phytoplankton, and subsequently assimilated (Figure 1.1a) (Ghyoot et al., 2015; Paytan and McLaughlin, 2007; Ye et al., 2010). Much of DIP uptake takes place in photic zone during the photosynthesis and OP hydrolysis (in both particulate and dissolved forms) occurs through the water column, eventually resulting in the classic depth profile of oceanic DP (Faul et al., 2005; Paytan and McLaughlin, 2007; Ruttenberg, 2014). DOP and DIP can also adsorb onto particulate matter such as natural organic matter (NOM) and natural oxide and clay minerals, then sink in the water column. The sinking particulate P pool consists of POP (~40%), authigenic particulate inorganic phosphorus (PIP) such as calcium fluorapatite (CFA) (~25%), and labile PIP (21%), with less amounts of nonreactive detrital P (~13%) (Faul et al., 2005). P associated with NOM, P adsorbed on mineral particles, and P in authigenic apatite are often considered as the three primary sedimentary sinks for P sequestration from oceans. However, the vast majority of P is remineralized within the water column, and approximately 1% of P is carried to the deep sea by these falling particles and removed from the ocean reservoir into the deep ocean sediments (Paytan and McLaughlin, 2007).

After deposition into marine sediments, the exchange of P between reactive particulate phases occurs because of biological and diagenetic alterations, leading to the release of P or conversion of P into less labile forms (Defforey and Paytan, 2018; Ruttenberg, 2014). The transformation processes are usually affected by respiration of organic matter and changing redox conditions, including microbial breakdown of OP compounds and the production of DIP and DOP, uptake and release of pore water P via sorption onto mineral phases [e.g., Fe and Mn oxides, calcium carbonate ($CaCO_3$)] and the redox dissolution of Fe and Mn minerals, and the precipitation of P into authigenic minerals (e.g., vivianite and CFA) (Defforey and Paytan, 2018; Egger et al.,

2015; Kraal et al., 2017; Ruttenberg, 2014). Under anoxic bottom-water conditions, bacterial apatite formation contributes to significant P sequestration in organic-rich sediments with low reactive Fe content, indicating that microorganisms are important in mineral precipitation in the sediments of highly productive upwelling regions and possibly sediments in systems with expanding water-column anoxia (Goldhammer et al., 2010). Extracellular phosphatases, especially alkaline phosphatase (APs), produced by a majority of marine microorganisms, have the potential to hydrolyze dissolved OP monoesters. The activity of extracellular phosphatases tends to decrease with sediment depth as organic matter content decreases and determines the P dynamics in marine sediments (Defforey and Paytan, 2018; Hans-Georg, 2003; Taylor et al., 2009). P association with $CaCO_3$ and transformation of Fe oxide-bound P to vivianite are recently proposal to play critical roles in P burial in oceanic sediments (Dijkstra et al., 2018; Egger et al., 2015; Kraal et al., 2017). Thus, microbial activity, organic matter mineralization, and dissolution of minerals (such as Fe/Mn oxides and $CaCO_3$) are generally considered to be important factors in controlling P dynamics in marine sediments (Paytan and McLaughlin, 2007; Ruttenberg, 2014).

1.2 Polyphosphate in marine environments

Polyphosphate (PolyP) is a group of P polymers with at least three phosphate groups joined by phosphoanhydride (O–P–O) bonds and can exist in ring, branched, or linear structures (meta- and ultra-phosphates, respectively), though linear structure is the most common form in natural environments (Azevedo and Saiardi, 2014; Kornberg et al., 1999). PolyP is ubiquitous in marine environments, representing 1–13% of total P not only in planktonic organisms (Diaz and Ingall, 2010; Martin and Van Mooy, 2013; Rao et al., 2009), but also in the sediments (Sannigrahi and Ingall, 2005) and the dissolved and particulate pools of seawater (Martin et al., 2014; Paytan et al., 2003b; Young and Ingall, 2010). In wastewater treatment plants, polyP can account for 15–75%

of total P in raw sewage due to microbial polyP accumulation during enhanced biological phosphorus removal (EBPR) process and 5–40% in secondary effluents (Guan et al., 2007; Mullan et al., 2006). PolyP is widely found in archaea, bacteria, algae, fungi, protists, plants, insects, and mammals, and typically represents 10–20% dry weight of some organisms (e.g., yeast) (Albi and Serrano, 2016; Docampo et al., 2005; Kornberg et al., 1999; Rao et al., 2009). PolyP has many important functions for microorganisms, such as serving as an ATP substitute, energy source, P reservoir, chelator of metal ions (e.g., Mn^{2+}, Ca^{2+} and Mg^{2+}), pH buffer, channel for DNA entry, and cell capsule, as well as a regulator for P stress, survival, and biological evolution (Kornberg et al., 1999; Kulaev et al., 2005; Rao et al., 2009).

PolyP can be synthesized via orthoP dehydration at high temperatures in industry application or via orthoP condensation by some kinase enzymes (Kulaev et al., 2005; Omelon and Grynpas, 2008). PolyP formed by high temperature condensation divides into linear condensed phosphate, cyclic condensed phosphate, and cross-linked condensed phosphate (Albi and Serrano, 2016; Cini and Ball, 2014). Some specific enzyme proteins are involved in polyP biosynthesis by bacteria, yeasts, and parasitic protists, and are confirmed to widely exist in marine sediments (Jones et al., 2016). For example, inorganic polyP is mainly synthesized by polyphosphate kinase 1 (PPK1) which catalyzes the reversible transfer of the energy-rich γ-phosphate from ATP to elongate the polyP chain ($polyP_n + ATP \leftrightarrow polyP_{n+1} + ADP$) (Albi and Serrano, 2016; He et al., 2007b). An alternative enzyme (PPK2) efficiently uses either GTP or ATP to synthesize polyP and is also suggested to be a polyP-degrading enzyme, serving as a polyP:AMP phosphotransferase and polyP:ADP phosphotransferase ($polyP_n + AMP \leftrightarrow polyP_{n-1} + ADP$) (Ishige and Noguchi, 2000; Ishige et al., 2002; Rao et al., 2009). In the slime mold *Dictyostellium discoideum*, a new type of PPK, named *Dd*PPK2, was identified and is a complex of three actin-related proteins (Arp),

which polymerizes into an actin-like filament concurrently with the reversible synthesis of polyP chain from ATP (Albi and Serrano, 2016; Gomez-Garcia and Kornberg, 2004). In oceanic sediments, exopolyphosphatase (PPX) was found, which catalyzes the sequential hydrolysis of the terminal phosphate residue ($polyP_n + H_2O \leftrightarrow polyP_{n-1} + P_i$) (Achbergerová and Nahálka, 2011; Jones et al., 2016). Additionally, APs display strong PPX activity and have the ability to recycle polyP in marine environments (Lorenz and Schröder, 2001; Martin et al., 2018a).

In aquatic environments, magnetotactic bacteria of the *Magnetococcaceae* family can accumulate a large amount of polyP in the cell, appearing as P hotspots in the particulate fraction at the oxic-anoxic interface of water columns (Rivas-Lamelo et al., 2017). Marine bacteria and phytoplankton can also store and concentrate orthoP as amorphous polyP granules (Diaz et al., 2008; Hupfer et al., 2007; Omelon et al., 2013). A portion of the biologically internal (i.e., endogenous) polyP is eventually released to the surrounding environments, thereby becoming biologically external (i.e., exogenous) P (Diaz et al., 2008; Hupfer et al., 2004). As a result, substantial levels of polyP are routinely detected in both dissolved and particulate fractions of seawater and marine sediments (Diaz et al., 2008; Sannigrahi and Ingall, 2005; Young and Ingall, 2010). In "biological pump", polyP accounts for 7% of total P in bulk plankton community (surface water biomass), 7% of total P in sinking particles, 11% of total P in dissolved P phase in euphotic zone, and 8% of total P in surface sediments (Diaz et al., 2008). However, the spatial distribution, vertical profile in water columns and sediments, and the specific roles of polyP dynamics in marine P cycling remain poorly constrained (Martin et al., 2014; Martin et al., 2018a; Omelon and Grynpas, 2008).

Figure 1.2 (a) Summary of the main P transformation processes in natural systems, modified from Defforey et al., (2018) and Kruse et al., (2015). Arrows indicate the transfer of P between different reservoirs via biotic (e.g., mineralization, uptake, release, and assimilation) and abiotic processes (e.g., adsorption, desorption, dissolution, and precipitation). (b) Illustration of marine polyphosphate transformation processes in water columns and sediments (summarized from literatures, detailed in *Section 1.3*). PolyP: polyphosphate; NOM: natural organic matter.

Recent studies showed that the oceanographic distribution of polyP varied remarkably by latitude and longitude (Martin et al., 2014; Martin et al., 2018a). An identical latitudinal trend with fivefold increase in the ratio of polyP:total particulate phosphate (TTP) from the offshore temperate western North Atlantic (TWNA) to the Sargasso Sea was observed, though with a low concentration of absolute polyP (Martin et al., 2014). Martin et al. (2018) found that, in the Indian Ocean, particulate polyP concentrations around the equator were relatively higher than those in the north and south. Vertical profiles of polyP concentrations showed similar trends at the North Pacific Subtropical Gyre at station ALOHA (a P-limited system) and the Indian Ocean (a Fe- or N-limited system) (Diaz et al., 2016; Martin et al., 2018a). Diaz et al. (2016) found that the concentrations of particulate polyP and total particulate P synchronously decreased with increasing water depth in the upper 150 m of the North Pacific Subtropical Gyre, and were similar in both

spring and fall, although a steady ratio of polyP to total particulate phosphate (TPP) was maintained. However, both polyP concentrations and polyP:TPP ratios sharply decreased with depths and sinking particles at 150 m depth contained little polyP in the Sargasso Sea and Indian Ocean (Martin et al., 2014; Martin et al., 2018a). The difference between these two studies might relate to biological response to environmental P stress and AP activity in seawater columns (Martin et al., 2018a; Saad et al., 2016). Cultivation experiments of *Thalassiosira pseudonana* in P replete and limited conditions showed that P esters (> 90% of DOP) are the dominant P species in dissolved organic matter (DOM) produced under P replete conditions with small or negligible contributions from phosphonates or glycerol P and polyP, but the relative abundance of polyP was measured in low P cultures (Saad et al., 2016). Only limited studies reported high polyP concentration in marine sediments, whereas abundant polyP was found in water columns, possibly due to the low stability of polyP and the difficulties in accurate determination of polyP concentration in natural samples (Defforey and Paytan, 2018; Kraal et al., 2015; Miller and Arai, 2017; Sannigrahi and Ingall, 2005). For example, abundant polyP was detected in the surface sediment (1–2 cm) at Effingham Inlet overlain by oxic waters (Sannigrahi and Ingall, 2005). P K-edge X-ray absorption near edge structure (XANES) indicated that most P (including polyP) was located in 0.6–8 μm sized P-rich particles and consisted of calcium phosphate and polyP granules, confirming the important roles of polyP in the P cycle and P sequestration in marine sediments (Brandes et al., 2007; Diaz et al., 2008). Yet, polyP cycling and its roles in the recycling of total P in water columns and sediments still remain poorly understood; more research is needed to determine the transformation of this important P species at the sediment-water interface and its roles in global P cycling (Figure 1.2b).

1.3 Polyphosphate transformation and precipitation into calcium phosphate minerals

Polyphosphate palys an important role in marine P sequestration and the nucleation of marine calcium phosphate minerals, both controlled by a range of biotic and abiotic processes (Figure 1.2b) (Diaz et al., 2008; Jones et al., 2016). Marine bacteria and diatoms store and concentrate P as polyP complexes/granules (Diaz et al., 2008; Omelon et al., 2013). Subsequent polyP release from these microoganisms into local environments leads to biologically induced calcium phosphate nucleation (Omelon and Grynpas, 2008). Intracellular and extracellular amorphous granules, rich in Ca^{2+} and polyP, have been observed in apatite-biomineralizing vertebrates, protists, and atremate brachiopods, and the polyP transformations can directly dicated apitate formation in organisms as polyP can be cleaved by APs to release orthoP, increasing the local concentration of reactive orthoP available for apatite bioformation (Omelon et al., 2013; Omelon et al., 2014; Omelon and Grynpas, 2011). This tranformation pathway is also considered to be important for P burial in marine sediments (Diaz et al., 2008; Goldhammer et al., 2010), but direct envidence for this pathway is still lacking (Figure 1.2b).

Similar to other polyoxyanions, polyP can strongly complex with multivalent cations such as Zn^{2+}, Ca^{2+}, and Fe^{3+} (Figure 1.2b) (Irani and Callis, 1960; Irani and Morgenthaler, 1963; Omelon and Grynpas, 2008). The complexation of polyP with metal cations not only allows the formation of new solid phases, but also has significant impacts on the stability and degradation pathway of polyP (Irani and Callis, 1960; Kura, 1987b; Rulliere et al., 2012). Binding with multivalent cations affects the hydrolysis of polyP chains and this effect is more pronounced for cations with higher charge and smaller radius (Kura, 1987b; Rashchi and Finch, 2000). For example, Ca^{2+} allowed a faster thermal degradation (at 120 °C) of long chained polyP with a higher yield of cyclic trimetaphosphate ($P_3O_9^{3-}$) and pyrophosphate ($P_2O_7^{4-}$) than in the absence of Ca^{2+}

(Rulliere et al., 2012), though the mechanism was not fully understood. The interaction between Ca^{2+} and polyP starts with Ca^{2+} coordination to the oxygen atoms of polyP molecules, which can lead to the formation of insoluble, neutrally charged, and nano-sized calcium phosphate sol particles, which then undergo condensation into a gel, and these precipitates are all X-ray amorphous (Nilles and Plank, 2012).

The interaction between humic acid (HA) and two model polyP compounds ($Na_5P_3O_{10}$ and $Na_{58}P_{56}O_{169}$) was investigated (Figure 1.2b) and this reaction is an exothermic processes (Fang et al., 2015). A stable polyP-HA complex was formed through the non-covalent interaction and hydrogen bond was another main driving force for the binding process via the complexation of the proton-accepting groups of polyP (e.g., P=O and P–O$^-$) with the oxygen containing functional groups in HA (Fang et al., 2015). Interaction of polyP with HA (or other NOM) in the presence of Ca^{2+} may be widely present in marine sediments and can affect polyP transforamtion, which is not well understood.

The main interfacial behaviors of polyP in geological environments include adsorption, complexation, precipitation, and hydrolysis (Figure 1.2b). These reactions can control polyP transformation and P dynamics in subsurface environments. The mechanism of polyP adsorption on natural oxide minerals such as Fe and aluminum (Al) oxides is likely via the exchange of phosphate groups with the surface hydroxyl groups to form surface inner-sphere complexes (Ogata et al., 2014; Rajan, 1976). However, the interfacial reactions of polyP with Mn oxides are likely to be different. First, Mn dioxides exhibit very low polyP sorption capacity because of the highly negative surface charge over a wide range of environmental pH conditions. Secondly, Mn dioxides can facilitate the hydrolysis of tripolyphosphate (tripolyP) to orthoP (with pyroP as a reaction intermediate) with the polarization of the adsorbed species by the surface or participation of surface

hydroxyl groups (Inman et al., 2001). A variety of Fe, Al, and Mn oxide minerals (e.g., goethite, hematite, ferrihydrite, α-Al oxide, TiO_2, and birnessite) were found to enhance the surface hydrolysis of *p*-Nitrophenyl phosphate, a model phosphate ester (Baldwin et al., 1995). Thus mineral-catalyzed hydrolysis of polyP by natural oxides may be widely present and play important roles in polyP transformation at the sediment/soil-water interface (Figure 1.2b).). In addition to mineral catalyzed hydrolysis of polyP, Ppx and APs are both found to be capable of hydrolyzing polyP in marine environments, thus enzyme-mediated polyP hydrolysis should also be considered as another important factor influencing marine polyP transformation (Figure 1.2b) (Jones et al., 2016; Martin et al., 2018a).

Homogenous polyP hydrolysis by proton (acid-catalyzed hydrolysis) or hydroxyl (alkali-catalyzed hydrolysis) was previously observed in highly acid and alkaline solution, both leading to the production of orthoP (de Jager and Heyns, 1998; Kura, 1987a; Rashchi and Finch, 2000). The mechanism of acid-catalyzed polyP hydrolysis includes the formation of a pentacovalent terminal P intermediate such that a terminal unit can be activated by protonation of the double-bond oxygen on that unit, followed by a nucleophilic attack of water. The transfer of proton from a P–OH group to a neighbor O–P–O bond leads to the breakage of terminal O–P–O bond, gradually shortening the phosphate polymer of polyP molecules (de Jager and Heyns, 1998). Alkali-catalyzed polyP hydrolysis involves ion-pair formation between PO^- and cations (e.g., Na^+, K^+, Li^+ and Cu^{2+}), which renders the P atom more positive by reducing the electron density around the P atom, making it more susceptible to nucleophilic attack by OH^- (Kura, 1987a, b). Due to the ready acid/alkali hydrolysis of polyP, polyP concentrations are likely underestimated in environmental samples using common P classification methods such as sequential sediment extraction (SEDEX) and solution ^{31}P NMR (both methods typically involve the utilization of

highly concentrated acid or alkali) (Miller and Arai, 2017). Because of the low stability and possible underestimation of polyP, polyP interfacial behaviors in marine environments and its role in marine P cycling is needs to be further constrained.

In nature, a variety of calcium phosphate minerals are produced by geological (igneous apatite), geochemical/geomicrobiological (phosphorite), and biological (biological apatite) processes (Omelon et al., 2013; Wang and Nancollas, 2008). Marine P burial via authigenic formation of stable calcium phosphate minerals (e.g., apatite) in sediments is a major pathway for the oceanic sinking of reactive P (Ingall, 2010). PolyP derived from diatoms or sulfur bacteria play a critical role in the formation of calcium phosphate minerals in marine sediments (Figure 1.2b) (Brandes et al., 2007; Diaz et al., 2008; Schulz and Schulz, 2005), but the direct link of the apatite formation with polyphosphate transformation is still lacking. The family of apatite minerals, a main calcium phosphate phase in natural environments, has a generalized formula of $A_5(X)_3Z$, where A is a divalent cation (e.g., Ca^{2+}), X is PO_4^{3-}, and Z is an anion (e.g., F^-, Cl^-, OH^-) (Omelon et al., 2013; Wang and Nancollas, 2008). The saturation of apatite is defined by the reactivities of Ca^{2+}, PO_4^{3-}, and other anions such as F^-, Cl^-, OH^- at a given temperature (Omelon and Grynpas, 2008). Marine bacteria and phytoplankton can concentrate orthoP as intracellular polyP under oxic conditions. When the surrounding environments become anoxic, polyP is depolymerized as the source of energy and alternative P, which can be utilized directly or released into the environments (Nathan et al., 1993; Omelon et al., 2013). The formed polyP complexes/granules are often enriched in Ca^{2+} (Brown and Kornberg, 2004) and the association of Ca^{2+} with polyP may consequently reduce the concentration of reactive Ca^{2+}. However, the hydrolytic degradation of polyP within a closed system changes both Ca^{2+} and P speciation and therefore increases the activity of both orthoP and Ca^{2+} (Omelon and Grynpas, 2011; Omelon and Grynpas, 2008). The

total concentrations of dissolved Ca^{2+} and P from calcium polyphosphate glass were reported to be over 3 orders of magnitude greater than those required to saturate the solution with respect to hydroxyapatite precipitation (Bunker et al., 1984). A previous study showed that, in marine sediments, 3 to 14 months of constant phosphate release (6–20 nmol liter^{-1} s^{-1}) from polyP hydrolysis by sulfur-oxidizing bacteria led to the formation of hydroxyapatite (Schulz and Schulz, 2005). The combined properties of Ca^{2+} sequestration, susceptibility to hydrolytic degradation, and the reluctance to form Ca-polyP precipitates make polyP an interesting potential precursor for sedimentary apatite formation. In regions with high concentrations of polyP associated with Ca^{2+}, the degradation of these polyP complexes/granules led to eventual apatite formation without extensive interaction with the free sedimentary P pool (Diaz et al., 2008; Omelon and Grynpas, 2008).

The surface sediments (1–2 cm) at the oxic site of Effingham Inlet contained polyP species at ~8%, but abundant polyP was not observed in other sediments at anoxic site and the deeper sediments at the same oxic site (Sannigrahi and Ingall, 2005). Similar trends have been reported in lake sediments, where polyP accounted for up to 11% of the total P in the top 0.5 cm of sediments, but steeply disappeared in deep sediments (0.5–2 cm) (Hupfer et al., 2004). Schulz and Schulz (2005) reported large benthic P fluxes in shelf sediments resulted from polyP utilization by the sulfide-oxidizing bacterium *Thiomargarita namibiensis*. The absence of polyP in deep or anoxic sediments is possibly due to their low production, high utilization by microorganisms, or fast degradation in the surface sediments (Diaz et al., 2008; Hupfer et al., 2004). The sharp peaks of P concentration (in the pore water) and total P content (dried samples) observed in the sediment profiles of Namibian shelf were restricted to a narrow sediment horizon (about 3 cm thick), corresponding to the sediment depths where *Thiomargarita namibiensis* is most abundant (Schulz

and Schulz, 2005). Microbial breakdown of polyP by *Thiomargarita namibiensis* was an auxiliary metabolism, which can explain why it occurred only episodically and why P did not continuously accumulate in the deeper sediment (Schulz and Schulz, 2005). An incubation study on the diversity and activity of polyP-utilizing microorganisms in marine sediments indicated that orthoP release to solution increased in anoxic sulfidic incubations, decreased in oxic incubations, and did not change in sediment-free controls (Jones et al., 2016). Transcripts encoding PPK2 were 6–22 times more abundant in metatranscriptomes from the anoxic incubations, suggesting that reversible polyP degradation by PPK2 was an important metabolic response to anoxia for marine microorganisms and sulfur-oxidizing microorganisms (Jones et al., 2016). Laboratory ^{33}P-radiotracer incubations of Namibia marine sediment demonstrated that under both anoxic and oxic conditions, large sulfur-oxidizing bacteria accumulated ^{33}P in the cells and catalyzed the nearly instantaneous conversion of orthoP to apatite (Goldhammer et al., 2010). The enhanced recovery of ^{33}P from the organic and cell-internal fraction under oxic conditions supported a model of P uptake under oxic conditions by *Beggiatoa* and *Thiomargarita* and orthoP polymerization to polyP. Under anoxic conditions, polyP was hydrolyzed to orthoP, which increased orthoP concentration and led to the precipitation of ^{33}P labeled phosphate into apatite (Goldhammer et al., 2010). A direct evidence linking the formation of apatite to polyP transformation was provided by Diaz et al. (2008). Among the hundreds of P-enriched particles identified in Effingham Inlet sediments, ~50% P was polyP with the remaining fraction composed of apatite, thus dispersed grains of sedimentary polyP might promote apatite nucleation and growth directly and non-episodically (Diaz et al., 2008). These studies all suggested that marine P burial/sequestration as apatite is highly related to polyP transformation and more detailed research is needed to fill the current knowledge gaps on polyP cycling at the sediment-water interface.

1.4 Research scope and objectives

Many questions remain on the transformation of polyP into apatite. For example, why does polyP disappear so rapidly in surficial marine/lake sediments? How do microbial, chemical, and mineralogical factors affect polyP transformation? Does polyP transformation lead to the direct formation of apatite or via the transformation of ACP precursors? This dissertation focuses on filling these knowledge gaps on sedimentary polyP cycling and revealing the roles of polyP in marine P burial/sequestration. Specifically, this dissertation is guided by the four overarching questions:

(1) What biogeochemical conditions are conductive to exogenous polyP-mediated precipitation of calcium phosphate minerals in marine environments?

(2) What is the relative potential of calcium phosphate nucleation by various polyP phases (dissolved *vs.* particulate) and compositions (e.g., chain lengths and cation type/content)?

(3) Does exogenous polyP-mediated precipitation of marine calcium phosphate occur via heterogeneous or homogeneous nucleation?

(4) How can we extend the results from controlled laboratory settings to marine sedimentary conditions?

This dissertation seeks to lay the foundation for a better understanding of the chemical and biological processes governing polyP transformation and mineralization in various environmental settings. These processes include the hydrolysis of polyP by several representative phosphatase enzymes and the interfacial behaviors of polyP on common natural oxide minerals (e.g., Fe, Al, and Mn oxides). Additionally, sediment mesocosm incubation experiments were conducted to explore the roles of enzyme- *vs.* mineral-catalyzed processes in the transformation of polyP and

precipitation of calcium phosphate minerals under marine sedimentary conditions. Chapters 2–4 detail the kinetics and mechanisms on the transformation of polyP with varied chain length in the presence of various Fe, Al, and Mn oxides, as well as the subsequent precipitation of calcium phosphate minerals (including ACP and hydroxyapatite). Chapter 5 compares the mineralization of several P-containing molecules (e.g., OP monester, OP diester, pyroP, and polyP) by common phosphatases and oxide minerals. The results underline the important roles of natural minerals not only on absorbing phosphates on mineral surfaces (as a typical P sink) but also on catalyzing the degradation of complex P molecules and production of orthoP (as a potential P source). Chapter 6 reports mesocosm sediment incubation experiments using polyP, and the goal is to isolate and constrain the relative contributions of enzyme *vs*. mineral-catalyzed processes on the transformation of polyP and the precipitation of calcium phosphate minerals in sediment settings. By answering the questions on polyphosphate mineralization by biotic/abiotic factors and subsequent formation of calcium phosphate minerals, results from this dissertation will greatly advance our current understanding of P cycling at the sediment-water interface. Given the importance of P as an essential macronutrient and as an important element that can strongly react with other major elements, a more detailed understanding of marine P cycle can be applied across disciplines of ocean science and to the study of the biogeochemical cycles of other elements.

CHAPTER 2. MANGANESE OXIDE CATALYZED HYDROLYSIS OF POLYPHOSPHATE

2.1 Abstract

Polyphosphate (polyP) is a group of important phosphorus (P) species that plays critical roles in marine P cycle and potentially mediates phosphorus (P) burial in sediments. Revealing the transformation of polyP is important for understanding the P cycle in aquatic environments. This study systematically investigated the hydrolysis of polyP with different chain length by four representative types of manganese (Mn) oxides (α-MnO$_2$, β-MnO$_2$, δ-MnO$_2$, and birnessite) under varied solution conditions. All four Mn oxides can rapidly hydrolyze polyP, with the hydrolysis rate in the order of α-MnO$_2$ > δ-MnO$_2$ > birnessite > β-MnO$_2$. The hydrolysis rates for longer chained polyP were relatively higher than those of shorter chained ones. Results from kinetic studies and time-resolved ^{31}P solution nuclear magnetic resonance (NMR) spectroscopy indicated that the reaction mechanism was through a terminal-only hydrolysis pathway via one-by-one cleavage of terminal P$-$O$-$P bonds. The presence of Ca^{2+} obviously enhanced both the hydrolysis rate and extent. The presence of other common metal cations (Mg^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}) also showed promotion on polyP hydrolysis by δ-MnO$_2$. Formation of cation-polyP ternary surface complexes is likely the dominated mechanism of cation promotion on polyP hydrolysis on Mn oxides. P K-edge X-ray absorption near edge structure (XANES) analysis indicated that solid calcium polyphosphate (Ca-polyP) granules can be hydrolyzed by α-MnO$_2$ and transformed into amorphous calcium phosphate (ACP) phase, with increasing ACP content as pH increased. This

study highlights the rapid transformation of polyP at the mineral-water interface, and has significant implications for the alteration of P bioavailability in aquatic environments and for P burial in marine sediments.

2.2 Introduction

Phosphorus (P) is an essential nutrient for all life. Numerous research efforts have been devoted to explore P biogeochemical behaviors at mineral-water interfaces in both terrestrial and marine systems, which control the mobility, transformation, and bioavailability of P (Arai and Sparks, 2007; Ruttenberg, 2014). Polyphosphate (polyP) is an important group of phosphate-containing species, containing more than two phosphate (PO_4) groups joint by phosphoanhydride (P–O–P) bonds. PolyP can occur in ring and branched structures, although the linear structure is the most common form in nature (Azevedo and Saiardi, 2014; Kornberg et al., 1999). PolyP is found in a wide range of environments and represents 1–13% of total P in planktonic organisms (Diaz and Ingall, 2010; Rao et al., 2009), the dissolved and particulate pools of seawater (Martin et al., 2014; Paytan et al., 2003b), marine sediments (Sannigrahi and Ingall, 2005), 1.5–11.4 % of total P in lake sediments (Hupfer et al., 2004) and 0.4–7% of total P in soils (Ebuele et al., 2016). A portion of the biologically internal polyP is released to aquatic environments during common cell events such as extracellular release, lysis, and death, thereby becoming biologically external (Diaz et al., 2008). As a result, substantial levels of polyP are routinely detected in both dissolved and particulate fractions of lakes, seawater, and the corresponding sediments (Brandes et al., 2007; Diaz et al., 2008; Hupfer et al., 2004; Sannigrahi and Ingall, 2005). Additionally, polyP compounds are important industrial chemicals, frequently used as reagents for water treatment, medicine, fertilizers, flame retardants, and food additives (Kulakovskaya et al., 2012). The widespread industrial applications of polyP can ultimately lead to their release into soils, water bodies, and

sediments. The cycling of polyP in aquatic environments, which is strongly controlled by its interfacial biogeochemical behaviors (such as those involving mineral-water interfaces), is an important component of the global P cycle (Martin et al., 2018b).

Despite its wide occurrence in aquatic environments, polyP was mainly detected at top depth in lake and marine sediments, such as the top 0.5–2 cm of Effingham Inlet overlain by oxic waters and 22 European lakes based on solid-state ^{31}P nuclear magnetic resonance (NMR) spectroscopy analyses (Hupfer et al., 2004; Sannigrahi and Ingall, 2005). The lack of direct evidence for polyP presence in sediments at greater top depth was possibly due to its rapid transformation after entering sediment environments. Indeed, polyP have been reported to readily undergo acid or base hydrolysis (de Jager and Heyns, 1998; Kura, 1987a; Miller and Arai, 2017), as well as rapid transformation into orthophosphate (orthoP) during diagenesis in sediments and in water columns (Hupfer et al., 2004; Martin et al., 2018b). The preferential recycling of polyP relative to total particulate phosphate (TPP) in seawater columns was likely linked to polyP hydrolysis catalyzed by alkaline phosphatase (Huang et al., 2018b; Martin et al., 2018b). Yet, the roles of soil and sediment mineral components on the uptake and/or transformation of polyP still remain poorly understood.

A recent study showed that triphosphate (P_3) adsorbed on goethite can be fully hydrolyzed at pH 4.5 and slightly hydrolyzed at pH 6.5 and 8.5 within 3 months (Hamilton et al., 2017). Our recent study showed that polyP can be hydrolyzed at the surface of γ-Al_2O_3 (an analog of natural Al oxides), which was affected by mineral particle size and solution chemistry such as pH and the presence of metal cations (Wan et al., 2019b). Amorphous MnO_2 showed high reactivity toward P_3 hydrolysis (i.e., complete degradation into three orthoP molecules within 2 hours at pH 4.0), and the hydrolysis rate in natural lake water was further promoted due to the presence of Ca^{2+} and

Mg^{2+} (Inman et al., 2001). Thus, mineral catalyzed hydrolysis is expected to play an important role in polyP transformation at environmental interfaces, yet related investigations are few.

Additionally, degradation of polyP can lead to local high concentrations of orthoP that can act as a potential source for the precipitation of Ca-phosphate minerals in the presence of Ca^{2+}. Our recent research on phosphatase-mediated hydrolysis of linear polyP revealed the fast release of orthoP into solution and the precipitation of amorphous Ca-phosphate solid(s) in the presence of Ca^{2+} (Huang et al., 2018b). Metal oxides (e.g., Mn, Fe, Al oxides) typically have high surface areas and large amounts of reactive surface sites that can facilitate processes such as adsorption or co-adsorption of cations and anions, thereby creating micro-environments with concentrated ions that can potentially induce or facilitate surface precipitation (Li et al., 2012b). For instance, co-adsorption and complexation of Ca^{2+} and phosphate on boehmite (γ-AlOOH) surface facilitated the surface precipitation of hydroxylapatite at pH 7–9 (Li et al., 2012b).

In this study, we systematically investigated the rapid hydrolysis of polyP by Mn oxides with varied structures (α-MnO_2, β-MnO_2, δ-MnO_2, and birnessite), as well as the effects of polyP phase (dissolved *vs.* solid) and structure (e.g., chain length), and type and concentration of common metal cations (Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}). The four MnO_2 are structurally distinct, and represent natural Mn oxide phases with dominating layered and tunneled structures (Huang et al., 2018a; Zhao et al., 2018). δ-MnO_2 and birnessite are two layered phyllomanganate phases, both with hexagonal symmetry but with different structural order. Birnessite has better crystallinity, larger particle size, more layer stacking, and lower surface area as compared to δ-MnO_2 (Zhao et al., 2018). α-MnO_2 (2×2 tunnels) and β-MnO_2 (pyrolusite, 1×1 tunnels) represent tunnel structured Mn oxides with varied tunnel size (Huang et al., 2018a; Meng et al., 2014). In this study, complementary batch experiments, solution ^{31}P NMR and X-ray absorption spectroscopy (XAS)

analyses were conducted to reveal the evolution of solution and solid phases during polyP interaction with Mn oxides. Results from this study can provide new insights for the abiotic transformation of polyP at environmental interfaces, which will aid the advancement of our understanding on P cycling in aquatic environments.

2.3 Experimental Section

2.3.1 Materials and characterization

Pyrophosphate and polyphosphates (with average chain length of 3–45) were used for the hydrolysis experiments. Sodium pyrophosphate tetrabasic decahydrate ($Na_4P_2O_7 \cdot 10H_2O$, hereafter P_2), sodium triphosphate pentabasic ($Na_5P_3O_{10}$, hereafter P_3), polyphosphate sodium salt (Na-polyP, hereafter P_{10}) and sodium phosphate glass (type 45, hereafter P_{45}) were purchased from Sigma-Aldrich. Sodium hexametaphosphate [$Na_6(PO_3)_6$, ring structure, hereafter P_6] was purchased from Alfa Aesar. All reagents were used as is. Information on the syntheses and characterization of calcium polyphosphate (Ca-polyP) granules, α-MnO_2, β-MnO_2, δ-MnO_2, birnessite and varied polyP are provided in Appendix A. *Text S1*.

2.3.2 Mn oxide catalyzed hydrolysis of polyphosphates

Prior to the hydrolysis experiments, 0.04 g Mn oxides (α-MnO_2, β-MnO_2, δ-MnO_2, or birnessite) and 0.58 g NaCl (as background electrolyte, equivalent to 0.1 mM) were added to 98.75 mL DI water in a 125 mL wide-mouth glass bottle and stirred for 18 h. After overnight dispersion, pH values of the suspension were manually adjusted to 6.0 ± 0.05 using 0.05 M HCl or NaOH. Buffers were not used to avoid potential interaction with polyP and/or mineral surfaces. To initiate polyP hydrolysis reaction, 1.25 mL of polyP (P_{10}) stock solution (containing 40 mM total phosphate) was added into the suspension, and the suspension pH was immediately adjusted to 6.0

using 0.05 M HCl or NaOH. During the reaction, pH of the suspensions was manually adjusted within the first 2 h, then at 3, 5, 7, 10, 24, 48, 72, and 96 h. At specific time points, 2 mL aliquots of the suspension were taken and immediately filtered through 0.22 μm Millipore membranes. The filtrate was analyzed for orthoP production and total P concentrations. OrthoP concentration was determined using the phosphomolybdate colorimetric assay (Murphy and Riley, 1962) on an UV–vis spectrometer (Carey 60, Agilent). For total P measurement, all P in the supernatant was first hydrolyzed to orthoP using the potassium persulfate autoclave digestion method (Das et al., 2014), then analyzed using the phosphomolybdate colorimetric assay. All experiments were performed in duplicate.

Several parallel experiments were conducted to explore the effects of metal cation type and concentration, polyP chain length, and polyP phase (dissolved *vs.* solid granules). (1) For experiments exploring Ca^{2+} effects, 1 mL stock solution of $CaCl_2$ (50 mM) was added into 97.75 mL DI water before the addition of varied Mn oxides and NaCl. The final Ca^{2+} concentration in the reaction suspension was 500 μM; (2) For hydrolysis experiments of polyP with varied chain length and α-MnO_2 in the presence or absence of Ca^{2+}, 1.25 mL of P_2, P_3, P_6, or P_{45} stock solution (each containing total P concentration of 40 mM) was added into 98.75 (no Ca^{2+}) or 97.75 mL (with 500 μM Ca^{2+}) prepared α-MnO_2 suspension; (3) For hydrolysis experiments of Ca-polyP solid granules by α-MnO_2, 0.02 g of the synthesized Ca-polyP granules was added into 100 mL of 0.4 g L^{-1} α-MnO_2 suspension with 0.1 M NaCl electrolyte at pH 6.0, 7.5, or 9.0; (4) For hydrolysis of P_{10} by δ-MnO_2 with different Ca^{2+} concentrations or different metal cations (Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}), calculated amount of the stock solution of the metal cations (chloride salts) was added into 97.75 mL deionized water to reach the desired final concentration (5, 10, 20, 50, 150, 500 μM for Ca^{2+}; 500 μM for Mg^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}), then 0.01 g δ-MnO_2 was added to prepare the

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dispersed suspension. During all experiments, concentrations of metal cations in the filtered supernatant were measured by inductively coupled plasma-mass spectroscopy (ICP-MS). In order to better isolate reaction processes such as surface adsorption and hydrolysis, our kinetic studies mainly focused on pH 6.0 (representative of freshwater environments), due to potential surface or solution precipitation of Ca^{2+} with polyP or orthoP (hydrolysis product) at high pH values. The relative high concentration metal cations (Cu^{2+}, Zn^{2+}, and Mn^{2+}) were used in order to clearly demonstrate their promotion effects on polyphosphate hydrolysis on the surface of Mn oxides and for consistency with Ca^{2+} and Mg^{2+} concentrations used. The effect of polyP phase (solid *vs.* dissolved) on its hydrolysis was investigated at pH 6–9 (representing fresh water to seawater conditions).

2.3.3 NMR analysis

In order to characterize the transformation of dissolved polyphosphates during mineral-catalyzed hydrolysis, parallel experiments were conducted for ^{31}P solution NMR characterization. Because total P concentration in the supernatant prepared in *Section 2.3.2* was not high enough, solution ^{31}P NMR spectra of the supernatant did not yield good signal-to-noise ratio. In order to obtain decent spectra within a reasonable time frame, the supernatant for NMR analysis was prepared at the same pH condition (6.0 ± 0.05) but by increasing the concentrations of polyP, Ca^{2+}, and Mn oxides by a factor of 6. At specific time points, 5 mL suspension was taken and immediately centrifuged and filtered through a 0.22-μm Millipore membrane, and the supernatant was used for solution ^{31}P NMR measurement. Solution ^{31}P NMR spectra were collected on a Bruker AMX 400 MHz spectrometer operated at 162 MHz and 297 K. A 90° pulse width, 6.5k data points (TD) over an acquisition time of 0.51 s, and relaxation delay of 15 s were applied. Chemical shift was calibrated using 85% H_3PO_4 as the external standard. At least 512 scans were

collected for each spectrum (equivalent to ~1 h).

2.3.4 P and Mn XAS analysis

For hydrolysis experiments using the synthesized Ca-polyP solid granules (details in Appendix A. *Text S1*), freeze-dried samples before and after reaction were analyzed by P K-edge X-ray absorption near edge structure (XANES) spectroscopy at Beamline 14-3 at the Stanford Synchrotron Radiation Lightsource (SSRL), Menlo Park, CA. Finely ground powders were brushed evenly onto P-free Kapton tape and mounted to a sample holder maintained under helium atmosphere. XANES data were collected in fluorescence mode using a PIPS detector. Energy calibration used $AlPO_4$ (edge position at 2152.8 eV). XANES spectra were collected at 2100–2485 eV. At least 2 scans were collected for each sample. Since Ca-polyP granules were used for studying the hydrolysis and precipitation, orthophosphate produced from the hydrolysis reaction can either adsorb onto α-MnO_2 or form Ca phosphate precipitates. Therefore, a suite of P reference compounds were prepared for XANES analysis: (1) polyphosphate sodium salt (Na-polyP) (Sigma Aldrich) and synthesized Ca-polyP granules, representing solid polyphosphates; (2) orthophosphate sorbed on α-MnO_2 (details in Appendix A. *Text S2*), representing Mn oxide associated P; (3) amorphous calcium phosphate (ACP), octacalcium phosphate (octaCa), and hydroxyapatite(Huang and Tang, 2016), representing calcium phosphate precipitates. XANES spectra of all reference compounds were collected in the same manner as for unknown samples. Data analysis used the software Ifeffit (Ravel and Newville, 2005). All spectra were carefully examined for energy calibration, merged, and normalized. Linear combination fitting (LCF) was conducted on the XANES spectra at energy range of 15–50 eV relative to the edge energy. The goodness of fit was evaluated using the residual factor (R-factor), and the fit with smallest R-factor was deemed the best fit. Mn K-edge XAS analysis was also conducted for the Mn oxides, with

experimental and data analysis details in Appendix A. *Text S3*.

2.4 Results and Discussion

2.4.1 Hydrolysis of polyphosphate (P_{10}) by Mn oxides and effect of mineral structure

Mn oxides with varied structures showed different reactivity toward polyP hydrolysis. The effect of four Mn oxides (α-MnO$_2$, β-MnO$_2$, δ-MnO$_2$, and birnessite) toward P_{10} hydrolysis is shown in Figure 2.1. Orthophosphate was produced and released to the solution during hydrolysis, and its production kinetics can be fitted by first-order kinetic model (Figures 2.2a-b and Table A.1). The degradation of polyP is a very complicated kinetic process involving the one-by-one hydrolysis of the terminal phosphate groups (the mechanism explained below) and the gradual shortening of the polyP chain length. First-order kinetic model showed the best fitting results when compared with zero-order and second-order fitting results. α-MnO$_2$ showed highest rate and extent toward polyP hydrolysis and was able to hydrolyze all P_{10} within 10 h (Figure 2.1a). This is consistent with the results from a previous study that α-MnO$_2$ showed the higher rate of hydrolysis for *p*-nitrophenyl phosphate than other Mn oxides (Baldwin et al., 1995). The hydrolysis rate roughly followed the order of α-MnO$_2$ > δ-MnO$_2$ > birnessite > β-MnO$_2$, regardless of Ca^{2+} presence (Figures 2.2a-b and Table A.1). The higher hydrolysis rates of α-MnO$_2$ and δ-MnO$_2$ can be related to their higher surface areas, both are ~70 times of β-MnO$_2$ and ~5 times of birnessite. In the control experiment, only 2.8% polyP was degraded after 5 d experiment in 500 µM Ca^{2+} solution without the existence of MnO$_2$. The presence of 500 µM Ca^{2+} enhanced the hydrolysis rates for all four Mn oxides, especially for δ-MnO$_2$ and birnessite (Figures 2.2a-b and Table A.1). For example, in the presence of 500 µM Ca^{2+}, α- and δ-MnO$_2$ were able to hydrolyze all P_{10} within 5 and 24 h, respectively.

Figure 2.1 Phosphate release as a function of time during polyphosphate P₁₀ reaction with α-MnO₂ (a), β-MnO₂ (b), δ-MnO₂ (c), and birnessite (d) at pH 6.0 with or without 500 µM Ca²⁺.

The change of total P concentration in solution was used to evaluate the sorption of both polyP and orthoP (released from hydrolysis) onto mineral surfaces (Figure A.4). The evolution of total P concentration in solution during mineral-catalyzed hydrolysis differed from the adsorption of orthoP or organic phosphate on metal oxides, where dissolved P concentration typically continuously decreases with the increase of reaction time (Yan et al., 2014b). For our polyP-

mineral hydrolysis system, in general, solution total P increased drastically during the first few hours of reaction, followed by steady state, suggesting polyP interface behavior to be an adsorption-hydrolysis processes. At the initial stage, polyP rapidly adsorbed onto Mn oxides. As the reaction proceeded, the hydrolysis of polyP and the release of orthoP led to the increase of total P concentration in the supernatant. From the change of total P concentration in solution, δ-MnO$_2$ showed a higher affinity for both polyP and orthoP species and adsorbed a relatively larger amount of polyP and orthoP at the surface than other Mn oxides (Figure A.4). Figure A.5a showed that δ-MnO$_2$ has the highest content of surface adsorbed Ca^{2+}. Adsorbed Ca^{2+} can likely promote polyP and orthoP adsorption by compensating negative mineral surface charge, increasing the electrostatic attraction of polyP and orthoP toward the mineral surface, and potentially promoting the formation of ternary surface complexes (Inman et al., 2001; Li et al., 2012b; Wan et al., 2017b). Such higher coverage of polyP due to Ca^{2+} presence likely resulted in the enhanced hydrolysis of polyP by Mn oxides, as discussed later.

Solution ^{31}P NMR spectroscopy revealed the alteration of P speciation in the filtrate after 5-day hydrolysis of polyP by Mn oxides (Figure 2.3). For δ-MnO$_2$ and birnessite systems, the presence of 500 µM Ca^{2+} remarkably increased the intensity of NMR signal for orthoP at 0.43 ppm, and decreased the NMR signal for polyP at −8 and −22 ppm (Figures 2.3c-d) (for polyP terminal and middle phosphate groups, respectively) (Huang et al., 2018b). Such enhancement is consistent with batch experiment results that the presence of Ca^{2+} significantly enhanced polyP hydrolysis by δ-MnO$_2$ and birnessite, but had minimal effects on α-MnO$_2$ system (which already had the highest hydrolysis rate). For example, δ-MnO$_2$ hydrolyzed most of polyP over 5 d; when Ca^{2+} added, no residual polyP was detected in the filtrate by NMR. In the α-MnO$_2$ system, only a single NMR peak at 0.43 ppm was observed, suggesting that no polyP remained after 5-d reaction

even without Ca^{2+} addition, consistent with the result of batch experiments (Figures 2.1a,3a). The difference of intensity for the orthoP peak in Figure 2.3a is due to the difference in numbers of scans (2048 scans for experiments without Ca^{2+}, 512 scans for experiment with Ca^{2+}). Overall, the NMR results further confirmed that the reactivity of Mn oxides toward polyP hydrolysis was in the order of α-MnO_2 > δ-MnO_2 > birnessite > β-MnO_2.

Figure 2.2 Hydrolysis of various polyphosphate by Mn oxides (0.4 g L^{-1}) at pH 6.0 with or without 500 μM Ca^{2+}. Solid lines present the first-order kinetic model fitting results of P_{10} hydrolysis on α- and β-MnO_2 (a), P_{10} hydrolysis on δ-MnO_2 and birnessite (b), P_2 and P_3 hydrolysis on α-MnO_2 (c), and P_6 and P_{45} hydrolysis on α-MnO_2 (d), respectively, in the presence or absence of 500 μM Ca^{2+}.

Figure 2.3 [superscript]31[/superscript]P solution NMR spectra of liquid supernatants obtained after polyphosphate (P_{10}) reaction with α-MnO₂ (a), β-MnO₂ (b), δ-MnO₂ (c), and birnessite (d) at pH 6.0 with or without 500 μM Ca^{2+} for 5 d.

2.4.2 Effect of polyphosphate chain length

Previous studies have revealed a terminal-only hydrolysis pathway of polyP by proton (de Jager and Heyns, 1998) and enzymes (Huang et al., 2018b). Our recent study demonstrated that

polyP molecules with longer chain length had lower hydrolysis rate and extent during their interaction with phosphatase enzymes, due to higher molecular concentration of the shorter chained polyphosphates at the same total P concentration (Huang et al., 2018b). Here, the hydrolysis of polyP with varied chain length by α-MnO$_2$ was compared, where all polyP experiments started with the same total P concentration. The hydrolysis rate of longer chained polyP (P$_6$, P$_{10}$, and P$_{45}$) was relatively higher than that of short chained polyP (P$_2$ and P$_3$) (Figures 2.1a, 2.2, and 2.4, and rate data summarized in Table A.1). The hydrolysis rate of P$_{45}$ was the highest and the reaction completed within 3 h in the absence of Ca^{2+} and within 2 h in the presence of 500 μM Ca^{2+} (Figures 2.2d and 2.4, and Table A.1). Complete hydrolysis of P$_6$ and P$_{10}$ was achieved within ~5 h in the absence of Ca^{2+} (Figures 2.1a and 2.4c). The hydrolysis of P$_2$ and P$_3$ was relatively slow, taking 36 h (P$_2$) and 24 h (P$_3$) until completing hydrolysis (Figures 2.4a-b). The presence of 500 μM Ca^{2+} enhanced both the hydrolysis rate and extent of α-MnO$_2$ toward all five polyP species, consistent with previous results that the presence of Ca^{2+} enhanced the hydrolysis rate of triphosphate by MnO$_2$ in Lake Northam water (Inman et al., 2001). The supernatant P concentration during longer chained polyP reaction with α-MnO$_2$ at the first time point (10 min) was lower than that of shorter chained polyP (Figure A.6). These results may result from more phosphate groups of long chain polyP per molecule and Mn oxide mineral can fix much more total P for longer chained polyP adsorption in comparison with shorter chained species. The presence and adsorption of Ca^{2+} can also decrease total P concentration of five polyP species in the supernatant at initial reaction stage (Figure A.6).

Figure 2.4 Phosphate release as a function of time during the interaction of α-MnO₂ with polyphosphates with varied chain lengths: (a) P_2, (b) P_3, (c) P_6, and (d) P_{45}. Experiments were conducted at pH 6.0 with or without 500 μM Ca^{2+}.

A series of supernatants at different reaction time were selected to preform time-resolved solution ^{31}P NMR measurements (Figure 2.5) to reveal the evolution of P speciation during the hydrolysis of five polyP species by α-MnO₂. The NMR results indicated that the hydrolysis rates of longer chained polyP were higher than those of shorter chained ones, consistent with the results from batch experiments. The NMR signals of longer chained polyP completely disappeared within

1 h (Figures 2.5c-e). This period was shorter than that in batch experiments, likely due to the differences in polyP and Mn oxides concentration used. A closer examination of the NMR spectrum of P_{10} hydrolysis by α-MnO_2 after 10-min reaction showed the co-existence of signals for orthoP and polyP (Figure A.7). During the degradation of long chained polyP, their time-resolved NMR spectra did not show the chemical signals of shorten chain polyP (Figures 2.5c-e). In combination with the rapid and continuous orthoP release in batch experiments, we propose that mineral-catalyzed hydrolysis of polyP also proceeds with the terminal-only hydrolysis mechanism, similar to enzymatic hydrolysis of polyP (Huang et al., 2018b).

After 24 h reaction, some P_2 and P_3 signals still remained for the hydrolysis of P_2 and P_3 (short chain polyP), respectively (Figures 2.5a-b). The slow disappearance of P_2 or P_3 NMR signals in their reacted supernatant implied slow hydrolysis rate of these two polyPs over 24-hour reaction times. In time-resolved NMR spectra of P_3 hydrolysis, the intensity of the peaks at around -7.5 and -22.3 ppm rapidly decreased and the peak at -9.15 ppm (belonging to P_2) gradually increased and persisted even after 24 hours (Figure 2.5b). At 0.5 h, the peak at around -9.69 ppm showed a broad shoulder, which contained overlapping signals from both P_2 and P_3 end groups, possibly due to the close chemical shift of P_2 and P_3's terminal P groups or the potential magnetic interference from very fine α-MnO_2 particles that might have passed through the 0.2-µm syringe filter.

In order to explore the effect of Ca^{2+} and obtain better signals, we conducted a separate set of NMR experiment by reducing the concentration of α-MnO_2 by six-fold and adding 3 mM Ca^{2+}, and the results are shown in Figure A.8. With the lower concentration of α-MnO_2, the reaction time needed for complete hydrolysis of P_3 increased to 96 h. For this experimental set, time-resolved NMR spectra showed that the concentration of P_2 first increased then decreased as the reaction proceeded, and orthoP was continuously produced as the degradation product of P_3 and

P₂. Our NMR results are consistent with a previous study using anion exchange chromatography to identify the hydrolysis products of P_3 in Lake Northam water in the presence of amorphous MnO_2 (Figure A.8) (Inman et al., 2001). The hydrolysis pathway of P_3 thus is consistent with the above mentioned terminal-only hydrolysis mechanism, i.e., through the degradation of one P_3 molecule to produce one P_2 and one orthoP, followed by the degradation of the produced P_2 to two orthoP molecules.

Figure 2.5 Time-resolved ^{31}P solution NMR spectra of liquid supernatant after the reaction of polyphosphates of varied chain length with α-MnO₂ without Ca²⁺ addition at pH 6.0. Panels a–e are for P₂, P₃, P₆, P₁₀, and P₄₅, respectively.

2.4.3 Effect of metal cations

The promotion of Ca^{2+} on the hydrolysis process prompted us to further investigate the effects of varied Ca^{2+} concentrations and other divalent metal cations on the hydrolysis of polyP by Mn oxides (Figure 2.6). For this set of experiments, δ-MnO_2 was used and at a lower concentration of 0.1 g L^{-1} (compared to 0.4 g L^{-1} used for previous experiments), because α-MnO_2 or high concentration of δ-MnO_2 can lead to rapid degradation of polyP even without the presence of metal cations, making it difficult to differentiate the effect of Ca^{2+} and other divalent metal cations. Figure 2.6a showed that increasing concentration of Ca^{2+} increased the extent of polyP hydrolysis. In the presence of 500 µM Ca^{2+}, δ-MnO_2 hydrolyzed all polyP to orthoP within 48 h, while in the absence of Ca^{2+} only 8% polyP was hydrolyzed (Figure 2.6a). Dissolved total P concentration showed no obvious difference at different reaction time and/or Ca^{2+} concentration (Figure A.9a). This is likely due to the low concentration of δ-MnO_2 (0.1 g L^{-1}) and high concentration of total P (500 µM) in the suspension. The low surface coverage of P on δ-MnO_2 (~260 µM orthoP per g δ-MnO_2, Figure A.10) can hardly change the total P concentration in solution. Figure A.9b showed Ca^{2+} uptake by δ-MnO_2. At high Ca^{2+} loadings, there was still much Ca^{2+} remaining in solution. For example, at Ca^{2+} concentration below 10 µM, almost all Ca^{2+} was adsorbed onto the surface of δ-MnO_2; when Ca^{2+} was added to 500 µM, approximately 350 µM Ca^{2+} stayed in solution. The presence of surface adsorbed and/or free dissolved Ca^{2+} may enhance the formation of mineral-Ca-polyP ternary surface complex and/or Ca-polyP solution complexes. Figure 2.6b compared the effects of five common divalent metal cations (Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}) on the hydrolysis of polyP by δ-MnO_2. In general, all metal cations showed enhanced hydrolysis extent as compared to no-divalent metal controls (Figure 2.6a). At the same metal cation concentration, orthoP release from polyP hydrolysis followed the order of $Ca^{2+} \geq Mg^{2+} > Mn^{2+} > Zn^{2+} > Cu^{2+}$ (Figure 2.6b). Figure A.9d showed higher amount of Cu^{2+}/Zn^{2+}/Mn^{2+} uptake by δ-

MnO$_2$ surface. Total P uptake by δ-MnO$_2$ in the presence of Cu^{2+}, Zn^{2+}, and Mn^{2+} was also higher than that in the presence of Ca^{2+} and Mg^{2+} (Figure A.9c), possibly due to the stronger affinity of Cu^{2+}/Zn^{2+}/Mn^{2+} to phosphate groups (Maki et al., 2013; Sigel, 1993).

Figure 2.6 Effect of (a) varied Ca^{2+} concentration (0–500 µM) and (b) different metal cations at 500 µM on surface-catalyzed hydrolysis of polyphosphate P$_{10}$ by δ-MnO$_2$ at pH 6.0.

2.4.4. Hydrolysis of Ca-polyP solid granules

The effect of polyP phase (solid *vs* dissolved) on its hydrolysis was also investigated in the presence of α-MnO$_2$ (Figure 2.7), as previous studies have suggested the abundant presence of scattered micron-sized Ca-polyP particles in marine sediments (Diaz et al., 2008). The choice of α-MnO$_2$ was based on the fastest hydrolysis rate of polyP by α-MnO$_2$, which may make it easier to observe solid Ca-polyP transformation. The concentration of both total P and produced orthoP in solution increased as the reaction proceeded and reached steady state at around 24 h at pH 6.0, 7.5, and 9.0 (Figure 2.7). At steady state, the total P concentration in solution was ~1.7 times of orthoP concentration. The increase of total P concentration was resulted from the rapid and continuous dissolution of Ca-polyP solid granules. The resulting dissolved polyP could be adsorbed onto α-MnO$_2$ followed by hydrolysis and production of orthoP. Thus, at the initial stage,

the concentration of total P was very high with minor amount of orthoP in the supernatant, and a ratio of total P to orthoP in the supernatant was around 10 (Figure 2.7). Meanwhile, pH had obvious impacts on the concentrations of orthoP and total P in solution. The concentrations for both orthoP and total P were significantly higher at pH 6.0 than at pH 9.0 and pH 7.5, suggesting that the dissolution of Ca-polyP granules was dominated by proton-promoted mechanism. However, the larger amount of orthoP release at pH 6.0 cannot be solely correlated to proton hydrolysis of polyP, as at this pH the concentrations of total P and Ca^{2+} (Figure A.5c) were also higher than those at pH 9.0.

Figure 2.7 Change of phosphate (a) and total phosphorus concentration (b) as a function of time during hydrolysis of calcium polyphosphate granules catalyzed by α-MnO₂ at pH 6.0, 7.5, and 9.0.

Because total P concentration in the solution is higher than orthoP concentration, the solution should also contain unreacted polyP from the dissolution of Ca-polyP granules. Therefore, solution ^{31}P NMR analysis was conducted to investigate the change of P speciation in the supernatant (Figure A.11). When pH increased from 6.0 to 9.0, the signals of orthoP and polyP end groups shifted from 0.56 to 2.39 ppm for orthoP and from −7.66 to −5.77 ppm for polyP end

phosphate group. At 1 h reaction time, the signals for polyP were at around −7 and −22 ppm and the peak intensities were higher at pH 9.0 (compared to pH 6.0). ^{31}P solution NMR spectra revealed the presence of large amounts of orthoP in solution at all three pH values, consistent with the rapid dissolution Ca-polyP granules and hydrolysis of released polyP. The gradual increase of the intensity of polyP signals with increasing pH suggests the faster dissolution of Ca-polyP granules and/or slower hydrolysis of released polyP at lower pH. This is consistent with the result from batch experiments. After 5-d reaction, signals for polyP were very weak and hard to detect by NMR in all samples, likely due to the low concentration of total polyP molecules (10 PO_4 units per polyP molecule). Consistent with this, the NMR signal for orthoP dominated the whole spectra. These results indicated that only a small amount of polyP remained in the solution and the ratio of polyP to orthoP decreased significantly (in batch experiment, decreased from ~10 to ~1.7) after 5-day reaction. The results from both batch experiments and NMR spectroscopy suggest the rapid transformation of solid Ca-polyP into dissolved polyP and eventually dissolved orthoP.

Previous studies reported that the total dissolved Ca^{2+} and P concentrations required for supersaturation with respect to Ca-polyP glass was more than 3 orders of magnitude higher than those with respect to hydroxyapatite (Bunker et al., 1984). Due to the concurrent production of Ca^{2+} (from Ca-polyP dissolution) and orthoP (from the hydrolysis of polyP from Ca-polyP dissolution), the formation of new Ca-phosphate phase(s) is highly likely, especially at high pH values. P K-edge XANES spectroscopy was thus preformed to reveal the structural and mineralogical information of the solid products after 5-day reaction (Figure 2.8b) and compared to a suite of reference compounds (Figure 2.8a). As can be seen from Figure 2.8a, Ca-phosphate minerals exhibited a strong shoulder centered at ~2155 eV. For Ca phosphate minerals, highly crystalline compounds (such as octacalcium phosphate and hydroxyapatite) showed a secondary

peak at ~2164 eV, and lower crystallinity phases such as amorphous calcium phosphate (ACP) exhibited less distinct shoulder peaks (Figure 2.8a), consistent with spectra of previous P XANES standards (Ingall et al., 2011).

Figure 2.8 (a) P K-edge XANES spectra of phosphate reference compounds, including sodium polyphosphate salt (Na-polyP), synthesized calcium polyphosphate granules (Ca-polyP), α-MnO₂ adsorbed orthophosphate (α-MnO₂-orthoP), amorphous calcium phosphate (ACP), octacalcium phosphate (octa CaP), and hydroxyapatite. (b) Linear combination fitting of P XANES spectra of the solid reaction products from the hydrolysis experiments of Ca-polyP with α-MnO₂ at pH 6–9. Reaction condition: α-MnO₂ = 0.4 g L⁻¹; Ca-polyP granule = 0.02 g L⁻¹; NaCl = 0.1 M. Raw and fitted data are shown in dotted and solid lines, respectively. Fitting results are presented in Table A.2.

The P XANES spectra of the solids after 5-day reaction did not show well separated shoulder peaks at ~2164 eV, implying that high crystallinity calcium phosphate mineral phases did not contribute a significant fraction (Figure 2.8b). Linear combination fitting (LCF) of the XANES spectra indicated the absence of crystalline calcium phosphate phases in the solid products (Table A.2). With increasing solution pH, the relative percentage of ACP in the reacted solids increased

from 59.4±5.2% at pH 6.0 to 77.4±3.6 % at pH 9.0. Together with batch experiments and NMR results, LCF of P XANES suggested the overall transformation of Ca-polyP granules into amorphous calcium phosphate solids in the presence of α-MnO$_2$, and such transformation of solid phase was faster under alkaline pH conditions.

2.4.5 Mechanisms of polyP hydrolysis on Mn oxides

Due to the technical difficulties associated with obtaining/resolving spectroscopic signals, such as the magnetic property of Mn (difficulty to collect solid NMR spectra), low surface coverage of phosphate sorbed on MnO$_2$ (hard to achieve good signal to noise ratio), and band overlap of phosphate vibration with MnO$_2$ mineral in IR spectroscopy, direct spectroscopic evidence for the formation of surface inner-sphere complexes is still lacking. However, formation of surface inner-sphere complexes was generally considered to be the main mechanism for phosphate adsorption on Mn oxides (Ramstedt et al., 2005). For example, a considerable decrease of Mn oxide point of zero charge (PZC) upon phosphate adsorption suggested formation of inner-sphere phosphate complexes (Zaman et al., 2009). Using pair distribution function (PDF) analysis of X-ray total scattering, the PDF spectra of phosphate adsorbed on birnessite indicated that the peaks at ~1.54 Å can be ascribed to the P−O pairs in the PO$_4$ tetrahedron and the peaks at ~3.14 Å are most likely due to P−Mn pairs (Wang et al., 2016a), also suggesting the formation of inner-sphere complexes on birnessite. Our recent solid ^{31}P NMR results showed that, upon polyphosphate adsorption at the surface of γ-Al$_2$O$_3$, the main surface P species are phosphate groups in polyphosphate that formed direct bonds with the mineral surface as inner-sphere complexes (likely as bidentate binuclear complexes) and phosphate groups in polyphosphate that were not directly bonded to the mineral surfaces (Wan et al., 2019b). Due to similarity in P K-edge XANES spectra between α-MnO$_2$ adsorbed orthophosphate (α-MnO$_2$–orthoP) (Figure 2.8a) and

Fe/Al oxide-adsorbed orthophosphate samples (Prietzel et al., 2016; Wan et al., 2019b), surface inner-sphere phosphate complexes are possible to form upon phosphate adsorption onto Mn oxides.

During polyphosphate hydrolysis, PO_4 groups were activated via protonation of the double-bond oxygen to form 5-fold coordination intermediate, followed by a nucleophilic attack by water or hydroxyl molecules at acid pHs, or directly attacking by free hydroxyl ions at alkaline pHs (de Jager and Heyns, 1998; Kura, 1987a). During phosphate monoester hydrolysis by acid or alkaline phosphatase, an intermediate species was formed involving the oxygen atom of the terminal PO_4 group completely coordinated with two metal cations [e.g., Zn(II), Ca(II), Fe(II), Mn(II)] (Coleman, 1992; Schenk et al., 2008; Schenk et al., 2012). These metal complexes in phosphatase can initiate double Lewis acid activation for hydrolyzing phosphates by initially bridging the two metal centers with the two phosphoryl oxygen atoms (Williams et al., 1999). In combination with the above-mentioned mechanisms for phosphate ester or polyP hydrolysis by proton, hydroxyl, and phosphatases, we propose that Mn oxide-catalyzed polyP hydrolysis probably derives from the ability of Mn atoms to coordinate with phosphate groups via oxygen atoms (either bidentate or monodentate configuration), which can activate the P atom for a nucleophilic attack. Surface H_2O and $\equiv$Mn$-$OH groups are formed at Lewis acid Mn sites of Mn oxides (e.g., α-MnO_2 and δ-MnO_2), which are close to the neighboring Mn atoms coordinated with phosphoryl O atom (Allard and Gallard, 2013; Zhao et al., 2014), and serve as a nucleophilic agent attacking the P atom with a subsequent cleavage of the P$-$O$-$P bond. Surface Lewis acid sites were previously confirmed as reactive centers for α-MnO_2 catalytic ozonation and Fe/Ce (hydro)oxide catalytic dephosphorylation (Mäkie et al., 2013; Tan et al., 2008; Zhao et al., 2014).

In comparison with polyphosphate hydrolysis onto γ-Al$_2$O$_3$, Mn oxides showed several orders of magnitude higher activity than Fe/Al oxides.(Wan et al., 2019b) The main difference between Mn and Al oxides is that Mn oxides have a high vacancy content in their structure (Cheng et al., 2013; Hou et al., 2013; Hou et al., 2014). The presence of vacancy site at the surface of Mn oxides may be served as alternative active sites for dephosphorylation. Mn K-edge XANES of α-MnO$_2$ indicated the existence of ~10% Mn(III) in the structure of α-MnO$_2$ (Figure A.12). Our recent study also reported 15% and 6% Mn(III) in δ-MnO$_2$ and birnessite, respectively (Zhao et al., 2018). The presence of structural Mn(III) or vacancy sites may result in decreased stability of P–O–P bonds of terminal phosphate groups that are readily attacked by hydroxyl groups adsorbed on nearby sites or free reactive hydroxyl groups. The direct quantitative relationship between polyP hydrolysis and vacancy site density is beyond the scope of this study and warrants future studies.

2.4.6 Key factors influencing polyP hydrolysis on Mn oxides

Ca^{2+} can coordinate with the surface hydroxyl groups ($\equiv$Mn–OH) of Mn oxides, compensating the negative surface charge of Mn oxides (Liu et al., 2009), thus enhancing the adsorption of negatively charged species such as polyP (Inman et al., 2001). Also, Ca^{2+} may possibly form strong bond with phosphate groups to form surface ternary complexes and serve as a cation bridge (Maki et al., 2013). Due to the larger ionic radius of Ca atom (1.14 Å) compared to Mn (0.67 Å), we consider the formation of monodentate Ca-surface complexes to be more likely (as opposed to bidentate Ca surface complexes). Similar ternary surface complexes might also form in the presence of other divalent cations such as Zn^{2+}, Cu^{2+}, and Mn^{2+}, which can explain the enhanced hydrolysis of polyP in the presence of these cations. Compared to Mg^{2+} and Ca^{2+}, the relatively lower promotion effect of Zn^{2+}, Cu^{2+}, and Mn^{2+} on polyP hydrolysis may be attributed

to the different adsorption mechanisms/sites of Zn^{2+}, Cu^{2+} and Mn^{2+} on Mn oxides (Kwon et al., 2013; Zhao et al., 2016; Zhao et al., 2018), which might have affected the hydrolysis of polyP.

Comparing the hydrolysis efficiency of the four Mn oxides, it is clear that mineral structure, reactive site density, crystallinity, and surface properties (e.g., surface area) had distinct impacts on this mineral-catalyzed polyP hydrolysis. For example, the hydrolysis rates of all four Mn oxides followed the order of α-MnO_2 > δ-MnO_2 > birnessite > β-MnO_2 (Table A.1), which is consistent with their order of BET surface area: α-MnO_2 (146.43 ± 0.32 m^2 g^{-1}) > δ-MnO_2 (125.72 ± 0.6 m^2 g^{-1}) > birnessite (27.4 ± 0.6 m^2 g^{-1}) > β-MnO_2 (2.03 ± 0.07 m^2 g^{-1}). Comparing the hydrolysis of P_{10} by δ-MnO_2 and birnessite (which has similar crystal structure but different degrees of structure order), the less ordered (and poorly crystalline) δ-MnO_2 showed a higher hydrolysis rate as compared to the better ordered/crystalline birnessite. Due to the differences in crystallinity, δ-MnO_2 has higher surface area and higher site density of structural defects.(Zhao et al., 2018) PolyP hydrolysis rates on α-MnO_2 (BET surface area: 146.43 ± 0.32 m^2 g^{-1}) and δ-MnO_2 (BET surface area: 125.72 ± 0.6 m^2 g^{-1}) are 0.155 and 0.011 h^{-1}, respectively, when no Ca^{2+} added. α-MnO_2 presented much higher hydrolysis rate for polyP hydrolysis than δ-MnO_2. The highest reactivity of α-MnO_2 for polyP hydrolysis may have also resulted from its reactive facets that may have more Lewis acid sites and higher vacancy content at the surface. High reactive facets and surface Lewis acid sites were previously confirmed as reactive centers for α-MnO_2 catalytic ozonation and arsenite oxidation.(Luo et al., 2018; Zhao et al., 2014) The (100) face of α-MnO_2 can form more stable surface complexes with arsenate and arsenite than the (110) face, and the (100) face is more functional for the removal of arsenate and arsenite than the (110) face (Luo et al., 2018).

The terminal-only hydrolysis mechanism can explain the higher hydrolysis rate of long chained polyP, due to the stronger ability of long chain polyP molecules to compete with released

orthoP and/or short chained polyP molecules for adsorption onto Mn oxides. Additionally, only one terminal phosphate group of P_2 and P_3 could coordinate with the surface of Mn oxides, but another terminal phosphate group of longer chained poly P (P_6, P_{10} and P_{45}) might be able to form bond with the mineral surface, of course accompanied with some degrees of spatial constraints. For the hydrolysis of solid Ca-polyP granules, due to the release of Ca^{2+} and aqueous polyP from the dissolution of Ca-polyP granules, Ca^{2+} not only rapidly promoted the hydrolysis of aqueous polyP by α-MnO_2, but also served as a complexing cation with the orthoP produced from the hydrolysis of aqueous polyP, eventually leading to the precipitation of calcium phosphate solid(s), which might be an important pathway for P burial in marine sediments.

2.5 Conclusions

Our results revealed the rapid hydrolysis and transformation (within hours or days) of polyP at the Mn oxide-water interface with the rate order of α-MnO_2 > δ-MnO_2 > birnessite > β-MnO_2. The presence of common divalent metal cations (Ca^{2+} and Mg^{2+}) can remarkably enhance the hydrolysis of polyP by Mn oxides. The presence of other common metal cations (Mg^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}) also has positive impacts on polyP hydrolysis by δ-MnO_2. Although the concentration of metals in our experiments is slightly higher than their environment concentrations, our results can be inferred for some natural systems. For example, microbial Mn/Fe reduction in soils and sediments may lead to high metal (e.g., Cu, Zn, and Mn) concentrations in the pore-water. (Cooper et al., 2006; Müller et al., 2002) Calcium polyphosphate granules can be hydrolyzed by α-MnO_2 and led to the formation of amorphous calcium phosphate solid(s), which are often considered as precursors for apatite mineralization. The hydrolysis rates for longer chain polyP were relatively higher than those of shorter chain ones. PolyP with shorter chain lengths showed relatively slower hydrolysis rates, which might help explain the presence of high fractions of

pyrophosphate in natural sediments and soils (Worsfold et al., 2008). These results lay the foundation for better understanding the interfacial geochemical processes governing polyP transformation in soil and sediment environments and show the significance for the interpretation of P bioavailability in aquatic environments. Future studies are warranted to explore the effects of other common environmental minerals and solution conditions (e.g., freshwater *vs* seawater), as well as comparison between abiotic (e.g., mineral catalyzed) *vs.* biotic (e.g., phosphatase enzyme) mediated polyP hydrolysis rates, in order to better understand the processes governing the fate of polyP under varied and complicated environmental settings.

Supplementary Information for Chapter 2 can be found at **APPENDIX A. MANGANESE OXIDE CATALYZED HYDROLYSIS OF POLYPHOSPHATE**.

CHAPTER 3. POLYPHOSPHATE ADSORPTION AND HYDROLYSIS ON ALUMINUM OXIDES

3.1 Abstract

The geochemical behaviors of phosphate-containing species at mineral-water interfaces are of fundamental importance for controlling phosphorus mobility, fate, and bioavailability. This study investigated the sorption and hydrolysis of polyphosphate (a group of important long-chained phosphate molecules) on aluminum oxides in the presence of divalent metal cations (Ca^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+}) at pH 6–8. γ-Al_2O_3 with three particle sizes (5, 35, and 70 nm) was used as an analog of natural aluminum oxides to investigate the particle size effect. All metal cations enhanced polyphosphate hydrolysis at different levels, with Ca^{2+} showing the most significant enhancement, and the difference in the enhancement might be due to the intrinsic affinity of metal cations to polyphosphate. In the presence of Ca^{2+}, the hydrolysis rate decreased with increasing mineral particle size. Solid-state ^{31}P nuclear magnetic resonance spectroscopy revealed the main surface P species to be amorphous calcium phosphate precipitates, phosphate groups in polyphosphate that formed direct bonds with the mineral surface as inner-sphere complexes, and phosphate groups in polyphosphate that were not directly bonded to the mineral surfaces. Our results reveal the critical roles of mineral-water interface processes and divalent metal cations on controlling polyphosphate speciation and transformation.

3.2 Introduction

Phosphorus (P) is as an essential nutrient for all living organisms and commonly exists as diverse phosphate-containing molecules in natural environments (Paytan and McLaughlin, 2007). Polyphosphate is a group of important phosphate-containing species, composed of at least three phosphate ions joint by phosphoanhydride ($P-O-P$) bonds. Polyphosphates are synthesized by all living organisms and serve many important biological functions, such as ATP substitute, energy source, regulator for P stress and survival, and life evolution (Kornberg et al., 1999; Rao et al., 2009). Polyphosphates can be extracellular or intracellular. During common cell events, such as extracellular release, lysis, death, and burial of microorganisms, polyphosphates are released into various natural environments (Diaz et al., 2008). For example, polyphosphates are found to represent 1–13% of total P in planktonic organisms (Diaz and Ingall, 2010; Rao et al., 2009), the dissolved and particulate pools of seawater,(Martin et al., 2014; Paytan et al., 2003a) and marine sediments (Sannigrahi and Ingall, 2005), as well as 0.4–7% of total P in soils (Ebuele et al., 2016). Polyphosphates are also important industrial chemicals, frequently used as reagents for water treatment, medicine, fertilizers, flame retardants, and food additives (Kulakovskaya et al., 2012). The widespread anthropogenic application of polyphosphates can ultimately result in their release into soils, water bodies, and sediments. Thus, understanding polyphosphate transport and transformation in natural environments has important implications for both geological and anthropogenic P cycles.

In soils and sediments, phosphate groups can strongly adsorb onto metal (hydr)oxides, and the sorption behavior depends on phosphate concentration, mineral type, pH, ionic strength, and the presence of competing or complexing ions (Li et al., 2013a; Ruttenberg, 2014; Ruttenberg and Sulak, 2011). Since the sorption of phosphate groups at mineral-water interfaces can significantly

impact P distribution, mobility, transformation, and bioavailability, detailed understanding on the interaction between phosphate group-containing molecules and common minerals is needed to evaluate the interfacial behavior and bioavailability of P in aquatic and terrestrial environments (Arai and Sparks, 2007). Yet, very few studies have examined the chemical behaviors of polyphosphate at the mineral-water interface. Tripolyphosphate was reported to adsorb on aluminum (Al) hydroxide by forming monodentate binuclear inner-sphere complexes (Guan et al., 2005). Tripolyphosphate was also found to hydrolyze on the surface of manganese oxides, which was enhanced in the presence of Ca^{2+} and Mg^{2+} ions (Inman et al., 2001). Thus, it is likely that both adsorption and hydrolysis of polyphosphate on mineral surfaces can significantly affect its fate and transport at the mineral-water interface.

Al (oxyhydr)oxides, such as amorphous Al hydroxides, boehmite, and gibbsite, are among the most abundant and reactive minerals found in natural environments, and are commonly considered as a critical metal oxide group (along with Fe and Mn oxides) that can significantly affect the environmental behaviors of numerous trace elements and contaminants (Li et al., 2011; Li et al., 2012a; Yan et al., 2015). It is commonly accepted that mineral surface properties can significantly affect their reactivity during interfacial biogeochemical reactions. Thus, during the interaction of phosphate-containing molecules (such as polyphosphate) with minerals (via processes such as sorption and hydrolysis), mineral surface properties such as surface reactive sites, surface area, and particle size are likely to play key roles in determining the surface speciation and hydrolysis rate and extent.

In this study, we systematically characterized the uptake and hydrolysis kinetics and mechanisms of polyphosphate on Al oxide minerals under varied solution chemistry, such as pH and metal cation presence. γ-Al_2O_3 is an analog to naturally occurring aluminum (oxy)hydroxides

and Al-rich clay minerals and a well characterized phase that has been used to represent natural Al oxide minerals for numerous studies on the sorption of nutrients and metals (e.g., P, Zn, and other metals) (Li et al., 2011; Li et al., 2012a; Li et al., 2013b; Ren et al., 2012; Yan et al., 2015). It is commercially available in different particle sizes (Yan et al., 2015), allowing examination of the particle size effect. Compared to environmental Al oxide minerals (e.g., corundum, boehmite, gibbsite, and bayerite), γ-Al_2O_3 has higher surface area and reactivity for P adsorption (Li et al., 2012a; Li et al., 2013b; Yan et al., 2015). Additionally, since γ-Al_2O_3 is not a paramagnetic mineral substrate (as compared to Fe/Mn minerals), solid-state ^{31}P nuclear magnetic resonance (NMR) spectroscopy have been successfully applied to systematically characterize surface P species and reaction mechanisms during their interaction with aluminum (oxy)hydroxides (Li et al., 2013b; Yan et al., 2015). Batch experiments were conducted to determine the hydrolysis rate and extent of polyphosphate by γ-Al_2O_3 with three different sizes (5, 35, and 70 nm), at varied pH (6 and 8), and in the presence of common divalent metal cations (Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}). Solution and solid-state ^{31}P NMR spectroscopy analyses were conducted to reveal the hydrolysis mechanism and reaction products in both solid and solution phases. P K-edge X-ray absorption near edge structure (XANES) spectroscopy was used to identify the phase composition of the solid reaction products. Results from this study provide new insights for the abiotic transformation of polyphosphate and the formation mechanisms of calcium phosphate minerals at environmental interfaces. The results also lay the foundation for better understanding the geochemical processes controlling phosphorus transport and transformation in natural environments.

3.3 Experimental Methods

3.3.1 Materials and characterization

Polyphosphate sodium salt with an average chain length of 10 (hereafter referred to as P_{10}) was purchased from Sigma-Aldrich. Three γ-Al_2O_3 samples with different average particle sizes were used, including a 5 nm sample (Sky Spring Nanomaterials Inc., Cat No. 1328QI), 35 nm sample (Sigma-Aldrich, Cat No. 544833), and 70 nm sample (Alfa Aesar, Cat No. 43266). Details on sodium polyphosphate salt and γ-Al_2O_3 samples are available in Appendix B. *Text S1-S2*.

3.3.2 Hydrolysis of polyphosphate by aluminum oxides

Two sets of experiments were designed to explore the effects of γ-Al_2O_3 particle size, pH, and metal cations on polyphosphate hydrolysis at room temperature, as detailed below and summarized in Table B.1.

Experiment Set I examined the effects of γ-Al_2O_3 particle size (5, 35, 70 nm), pH (6.0 *vs* 8.0), and presence/absence of Ca^{2+}. This set of experiments used polyphosphate concentration of 2 mM (as total P), with/without 1 mM Ca^{2+}, and 0.4 g L^{-1} γ-Al_2O_3. Prior to the adsorption and hydrolysis experiments, 0.04 g γ-Al_2O_3 (5, 35, 70 nm) and 0.58 g NaCl (to achieve final background electrolyte strength of 0.1 M) were mixed in 95 mL deionized water in a glass bottle and equilibrated overnight under magnetic stirring at 7 Hz. For experiments exploring the effects of Ca^{2+} on polyphosphate adsorption and hydrolysis, 2 mL stock solution of $CaCl_2$ (50 mM) was first added to 93 mL deionized water before the addition of γ-Al_2O_3 and NaCl solids, in order to achieve a final Ca^{2+} concentration of 1 mM. pH of the suspension was manually adjusted to 6±0.05 or 8.0±0.05 using 0.05 M HCl and NaOH. After overnight equilibration, 5 mL of polyphosphate stock solution (with ~40 mM in total P concentration) was added into the suspension, in order to achieve a final polyphosphate concentration of 2 mM (as total P). The suspension pH was

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immediately re-adjusted. After this initiation of reaction, the pH of the reaction suspension was adjusted several times within the first 2 h, then at 3, 5, 7, 10, 24, 48, 72, 96, 168, and 216 h. At specific time points, aliquots (2 mL) of the suspension was filtered (0.22-μm Millipore membrane) and the supernatant analyzed for the concentrations of orthophosphate and total P. For total P analysis, all P in the supernatant was hydrolyzed to inorganic orthophosphate via potassium persulfate autoclave digestion (Das et al., 2014), and orthophosphate concentration was determined using the phosphomolybdate colorimetric assay (Murphy and Riley, 1962) on an UV–vis spectrometer (Carey 60, Agilent).

Experiment Set II was designed to compare the effects of different divalent metal cations (Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}) on polyphosphate hydrolysis. This set of experiments used decreased concentrations of polyphosphate (1 mM as total P), metal cations (0.5 mM), and 5 nm γ-Al_2O_3 (0.1 g L^{-1}), and the purpose was to minimize direct precipitation of metal-polyphosphate solids, which may affect the mineral-catalyzed hydrolysis process. Specifically, calculated amount (1 mL) of metal chloride stock solutions (50 mM) was added into 96.5 mL deionized water to reach the targeted final metal concentration of 0.5 mM, then 0.01 g 5 nm γ-Al_2O_3 was mixed with the solution to prepare the dispersed suspensions. After overnight equilibration, 2.5 mL of polyphosphate stock solution was added into the prepared suspension. The concentration of metal cations in the filtered supernatant was measured using inductively coupled plasma-mass spectroscopy (ICP-MS). All experiments were performed in duplicate. In order to collect good NMR spectra (both solution and solid) with acceptable signal to noise ratio, polyphosphate concentrations was set up to 2 mM in total P concentration. This concentration is also similar to the one used in our recent study on enzymatic hydrolysis of polyphosphate (Huang et al., 2018b), which allows comparison between enzymatic and mineral-catalyzed polyphosphate hydrolysis.

High metal concentration was used to clearly demonstrate their promotion effects on polyphosphate hydrolysis at the γ-Al$_2$O$_3$ surface.

3.3.3 Spectroscopy analyses

At the end of 216 h (9 d) reaction, the reaction suspensions were centrifuged to separate the solid and supernatant. The supernatant samples were directly used for solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy analysis. The wet pastes were freeze-dried for solid state ^{31}P NMR spectroscopy analyses, including single pulse magic angle spinning (SP/MAS), ^{31}P\{^{1}H\} cross-polarization MAS (CP/MAS), and 2-dimensional (2D) ^{31}P\{^{1}H\} heteronuclear correlation (HetCor), and ^{27}Al SP/MAS NMR analyses. P K-edge X-ray absorption near edge structure (XANES) spectroscopy analysis was also conducted on the reacted solids following our previous procedure (Huang et al., 2018b). Details of NMR and XANES data collection and analyses are available in Appendix B. *Text S3-S5*.

3.4 Results and Discussion

3.4.1 Polyphosphate adsorption and hydrolysis on aluminum oxides

Previous study showed that γ-Al$_2$O$_3$ with different particle sizes had different reactivity for phytate and orthophosphate sorption, with the sorption capacity for phytate being an order of magnitude higher than that for orthophosphate (Yan et al., 2015). Here, we investigated size-dependent adsorption and hydrolysis of polyphosphate by different sized γ-Al$_2$O$_3$ in the absence or presence of 1 mM Ca^{2+} (Experimental Set I; Figures 3.1 and B.4). During the experimental duration, orthophosphate was continuously produced and released into the solution from polyphosphate hydrolysis (Figure 3.1), and the production rate can be fitted by first-order kinetic model (Figure B.4 and Table 3.1). In the absence of Ca^{2+} and at both pH 6.0 and 8.0, the production

rate and extent of orthophosphate was slightly higher for 5 nm γ-Al$_2$O$_3$ (0.19–0.197 10^{-3} h^{-1}) as compared to 35 and 70 nm γ-Al$_2$O$_3$ (0.146– 0.174 10^{-3} h^{-1}). In the presence of 1 mM Ca^{2+}, the hydrolysis rate of polyphosphate was obviously improved for all three sized γ-Al$_2$O$_3$ (Figure 3.1 and B.4), roughly following the order of 5 > 35 > 70 nm γ-Al$_2$O$_3$, with 3.5−4.1 times increase at pH 6.0 and 6.5−10.8 times increase at pH 8.0 (Table 3.1).

The evolution of total dissolved P concentration in the solution (which is the sum of dissolved polyphosphate and orthophosphate) (Figure B.3) is different from the typical features for orthophosphate or organic phosphate adsorption on Fe and Al (hydr)oxides (Ruttenberg and Sulak, 2011; Yan et al., 2014b). In general, phosphate adsorption on Fe/Al (oxyhydr)oxides can experience a rapid initial uptake followed by slow uptake, reaching steady state within approximate 5 h (Ruttenberg and Sulak, 2011; Yan et al., 2014b). In this study, total P concentration in solution decreased quickly within the first few hours, then gradually increased (Figure B.3). The concentration of solution Ca^{2+} at day 9 was significantly lower than that at the beginning of experiments (Figure B.5), suggesting a large degree of Ca^{2+} immobilization during polyphosphate hydrolysis. We hypothesize that the initial rapid decrease was likely due to the rapid adsorption of polyphosphate onto γ-Al$_2$O$_3$, and that the gradual increase of total P concentration over time was likely resulted from the hydrolysis of polyphosphate and production of dissolved orthophosphate. We further hypothesize that the change in solution Ca^{2+} concentration was due to the complexation and precipitation of Ca^{2+} with polyphosphate and/or produced orthophosphate. These hypotheses were further investigated using solution and solid state ^{31}P NMR, as detailed below.

Figure 3.1 Dynamics of orthophosphate release to solution during the hydrolysis of polyphosphate (Experiment Set I) by 5 nm (a, b), 35 nm (c, d), and 70 nm (e, f) γ-Al₂O₃. Reaction condition: polyphosphate concentration 2 mM (as total P), pH 6.0 or 8.0, with/without 1 mM CaCl₂.

3.4.2 Solution ^{31}P NMR

Since distinctive chemical shifts (δ) can be identified for the NMR spectra of orthophosphate ($\delta_P = \sim 1$ ppm) and polyphosphate ($\delta_P = \sim -9$ ppm and -21 ppm for end and middle phosphate groups, respectively), solution ^{31}P NMR spectroscopy can be used to evaluate aqueous P speciation during the hydrolysis of polyphosphate (Huang et al., 2018b). Solution ^{31}P NMR spectra of polyphosphate supernatant after 9-day reaction with three γ-Al$_2$O$_3$ phases are shown in Figure 3.2. The spectra of the supernatant showed distinctive chemical shifts of orthophosphate and polyphosphate. It also demonstrated the significant enhancement of Ca^{2+} on the hydrolysis of polyphosphate by all three γ-Al$_2$O$_3$ at both pH 6.0 and 8.0. In the absence of Ca^{2+}, the NMR spectra were dominated by the signal from polyphosphate middle groups, centered at -21.71 ppm (Figure 3.2). However, in the presence of Ca^{2+}, the peak intensity of orthophosphate became a significant component and was similar to that of the polyphosphate end group (Figure 3.2) or sometimes even stronger, such as during the reaction of polyphosphate with 5 nm γ-Al$_2$O$_3$ at pH 8.0 (Figure 3.2a).

Table 3.1 First-order kinetics fitting parameters from batch experiments in Figure 3.1.

Experimental condition			Reaction time (h)	k (10^{-3} h^{-1})	R^2
γ-Al$_2$O$_3$ particle size (nm)	pH	Ca^{2+} concentration			
5	6.0	–	216	0.19	0.939
5	6.0	1 mM	216	0.776	0.969
35	6.0	–	216	0.169	0.919
35	6.0	1 mM	216	0.644	0.995
70	6.0	–	216	0.174	0.969
70	6.0	1 mM	216	0.605	0.990
5	8.0	–	216	0.197	0.921
5	8.0	1 mM	216	1.89	0.958
35	8.0	–	216	0.146	0.888
35	8.0	1 mM	216	1.58	0.948
70	8.0	–	216	0.167	0.890
70	8.0	1 mM	216	1.08	0.972

Additionally, NMR spectra did not show the random production of shorter chained polyphosphate, which would have resulted in multiple peaks from middle phosphate groups with different chain length, as discussed in detail in our recent study.(Huang et al., 2018b) This, in combination with the continuous orthophosphate production during batch experiments (Figure 3.1), suggests that orthophosphate was released one by one from the terminal phosphate groups of polyphosphate molecules, similar to the case of enzyme catalyzed polyphosphate hydrolysis (Huang et al., 2018b).

Figure 3.2 ^{31}P solution NMR spectra of the liquid supernatant from polyphosphate hydrolysis (Experiment Set I) by 5 nm (a), 35 nm (b), and 70 nm (c) γ-Al₂O₃. Reaction condition: polyphosphate concentration 2 mM (as total P), pH 6.0 or 8.0, with/without 1 mM CaCl₂, 9 d reaction time.

To determine the relative percentage of orthophosphate and polyphosphate in the supernatant, their NMR peaks were integrated and compared. The obtained relative percentage information (based on the ratio of integrated peak areas) was compared to that obtained from wet chemistry analysis (using orthophosphate concentration in Figure 3.1 and total P concentration in

Figure B.3). The calculated results were presented in Table B.2. Without Ca^{2+}, polyphosphate was the main P species (> 90%) in solution, with 93.6, 94.56, and 94.83% polyphosphate at pH 6.0 and 93.46, 95.07, and 95.31% polyphosphate at pH 8.0 for the supernatants of the 5, 35, 70 nm γ-Al_2O_3 experiments, respectively (Table B.2). In the presence of Ca^{2+}, orthophosphate became another main P species and reached 19.33, 16.72, and 15.19% at pH 6.0 and 47.49, 35.37, and 26.78% at pH 8.0 for 5, 35, 70 nm γ-Al_2O_3 supernatants, respectively (Table B.2). The presence of Ca^{2+} increased the relative percentage of orthophosphate by ~3.5 and 8.5 times for pH 6.0 and 8.0, respectively. Such enhancement is similar to the enhancement observed in kinetic fitting results (Tables 3.1 and B.2). Overall, these results consistently indicated the ability of Ca^{2+} in enhancing polyphosphate hydrolysis by γ-Al_2O_3.

3.4.3 Solid State ^{31}P SP/MAS NMR

To further elucidate the structure of the solid reaction products, solid state ^{31}P NMR analysis was conducted on the freeze-dried solid reaction products. ^{31}P SP/MAS solid state NMR spectra of polyphosphate reacted with γ-Al_2O_3 in the absence/presence of Ca^{2+} for 9 days at pH 6.0 and 8.0 were shown in Figure 3.3. At pH 6.0 and in the absence of Ca^{2+}, spectra of samples with different sized γ-Al_2O_3 yielded two similar peaks at chemical shifts of around −12 and −21 ppm (Figure 3.3a). This suggests the presence of similar phosphorus species and similar interaction mechanism(s) between polyphosphate and different sized γ-Al_2O_3. In the presence of Ca^{2+} at pH 6.0, the two peaks at chemical shifts of around −12 and −21 ppm still dominated the NMR spectra but showed different relative intensity as compared to the samples in the absence of Ca^{2+} (Figure 3.3b). Previous studies showed two NMR peaks at 0 and −6 ppm for orthophosphate adsorption on boehmite (Li et al., 2013a) and two NMR peaks at −1 and −6 ppm for phytate adsorption on γ-Al_2O_3 (Yan et al., 2015). These peaks were typically attributed to inner-sphere phosphate surface

complexes on Al (oxyhydr)oxides (Li et al., 2013a; Yan et al., 2015). Specifically, the peaks at ~ −1 and −6 ppm were assigned to deprotonated bridging bidentate and singly protonated bridging bidentate surface complexes, respectively (Li et al., 2013a). In this study, we tentatively assign the phosphate groups in polyphosphate into two categories: those forming direct bonds with the mineral surface (i.e., inner-sphere surface complexes), and those not directly bonded to the mineral surfaces, as schematically illustrated in Figure B.2. These two categories of phosphate groups in polyphosphate molecular are later referred to as polyP-P$_{bonded}$ and polyP-P$_{unbonded}$. In our study, the chemical shift at ~ −21 ppm can be assigned to polyP-P$_{unbonded}$ due to its similarity to the solution NMR signal of middle phosphate groups at ~ −21 ppm (Figure 3.2) (Huang and Tang, 2015b). The peak at ~ −12 ppm, close to that of phytate and orthophosphate adsorption at ~ −6 ppm (Li et al., 2013a; Yan et al., 2014a), might be attributed to (1) inner-sphere surface complexes between phosphate groups in polyphosphate and mineral surface (i.e., polyP-P$_{bonded}$), (2) formation of Al polyphosphate/phosphate precipitates, or (3) formation of Ca polyphosphate/phosphate precipitates. Factors (2) and (3) can be eliminated, as discussed below.

For factor (2), the formation of Al phosphate precipitates can result in ^{31}P chemical shift at high field such as aluminum phytate at δ_P = −11.2 ppm (Yan et al., 2014a) and aluminum orthophosphate at δ_P = ~−10 ppm (Kim and Kirkpatrick, 2004). To rule out the possible formation of aluminum polyphosphate/phosphate precipitates, we measured both solid state ^{31}P and ^{27}Al NMR spectra of these samples as well as a synthetic aluminum polyphosphate standard (Figure B.7−8). The ^{31}P chemical shift of aluminum polyphosphate precipitates was at δ_P = ~ −23.55 ppm (Figure B.7), far away from the −12 ppm chemical shift observed for reacted samples. In addition, the ^{27}Al NMR spectrum of aluminum polyphosphate showed one main peak at δ_{Al} = −0.29 ppm and one shoulder peak at δ_{Al} = 29.54 ppm (Figure B.7a). The ^{27}Al NMR spectra of the reacted

samples did not show these peaks at both pH 6.0 and 8.0 (Figure B.8), similar to unreacted γ-Al$_2$O$_3$ samples (with two chemical shifts at δ_{Al} = ~65 and ~8 ppm) (Yan et al., 2015). By combining ^{31}P and ^{27}Al NMR results, the formation of aluminum phosphate or polyphosphate precipitates can be eliminated as dominant species for the presence of ^{31}P chemical shift at δ_P = ~ −12 ppm.

For factor (3), the formation of calcium polyphosphate can result in ^{31}P NMR spectra with two peaks at δ_P = −9.09 and −24.13 ppm (Figure B.7b), different from the reacted samples (δ_P at around −12 and −21 ppm). Moreover, after 9-day reaction at pH 6.0, most polyphosphate and orthophosphate remained in the solution (Figure B.3) and were not likely to result in the formation of large amounts of calcium polyphosphate precipitates on the mineral surface. In combination with the similarity of chemical shifts with/without the presence of Ca^{2+} in Figure 3.3a–b, the formation of calcium polyphosphate should not be the main factor resulting in the ^{31}P NMR spectra shape.

Based on the discussion above, the chemical shift at δ_P = ~ −12 ppm can be assigned to factor (1), the formation of polyP-P$_{bonded}$, i.e., inner-sphere surface complexes (likely as bidentate binuclear complexes) between phosphate groups of polyphosphate and γ-Al$_2$O$_3$ surface (Li et al., 2013a; Li et al., 2012b). The difference in chemical shift (−12 ppm) as compared to orthophosphate or phytate adsorption on aluminum (hydro)oxides (around −6 ppm) (Li et al., 2013a; Yan et al., 2014a) is likely due to the effect of neighboring P atoms in polyphosphate. The bond of bidentate surface complexes with the neighbor phosphate group might lead to the movement of ^{31}P chemical shift toward high field in comparison with the bond with hydrogen atoms. A previous study also indicated that the phosphate tetrahedra in apatite associated with chlorine resulted in the movement of chemical shift toward high field relative to those associated with fluorine (Vaughn et al., 2018).

Figure 3.3 ^{31}P solid-state NMR spectra of γ-Al$_2$O$_3$ reacting with polyphosphate (Experiment Set I). Reaction condition: polyphosphate concentration 2 mM (as total P), pH 6.0 or 8.0, with/without 1 mM CaCl$_2$, 9 d reaction time. Asterisks denote spinning side bands.

At pH 8.0 and without Ca^{2+}, the ^{31}P NMR spectra of polyphosphate reacted with γ-Al$_2$O$_3$ displayed the same shape as those at pH 6.0 (Figures 3.3a and c), indicating the presence of similar species at pH 6.0 and 8.0. However, when Ca^{2+} was present at pH 8.0, the ^{31}P NMR spectra showed obvious difference from pH 6.0 samples (Figures 3.3b and d), with one main peak at around −9 ppm and two shoulder peaks at around 2.67 and −20.5 ppm. The chemical shift at ~2.67 ppm belongs to calcium phosphate precipitates, and their crystallinity [amorphous *vs.* crystalline (Li et

al., 2012b)] will be addressed below in CP dynamic experiments. The chemical shift at ~ -9 ppm can be attributed to phosphate groups of polyphosphate as inner-sphere surface complexes on γ-Al_2O_3 (i.e., polyP-P$_{bonded}$). The shift of δ_P at around -12 to -9 ppm might be due to the strong chelation between Ca^{2+} ions and these inner surface complexes (i.e., potential formation of ternary surface complexes and/or precipitates) at high pH (Figure B.5). Figure B.9 showed the Gaussian peak deconvolutions of ^{31}P NMR spectra from Figure 3.3d, and the relative intensities for each resonance line (or corresponding P species) were presented at the bottom of the figures. The relative percentage of calcium phosphate precipitates, phosphate group as inner-sphere surface complexes (i.e., polyP-P$_{bonded}$), and phosphate group in polyphosphate that were not associated with the mineral surface (i.e., polyP-P$_{unbonded}$) were at ~10, 70, and 20%, respectively (Figure B.10).

3.4.4 Two-dimensional $^{31}P\{^{1}H\}$ CP-HetCor spectra

As discussed above, the reacted solids contained phosphate groups in polyphosphate as adsorbed surface complex (i.e., polyP-P$_{bonded}$), phosphate groups in polyphosphate that were not associated with the mineral surface (i.e., polyP-P$_{unbonded}$), as well as Ca phosphate precipitates. To further elucidate the protonation and binding state of the phosphate groups, as well as the crystallinity of the Ca phosphate precipitates, 2-D solid state ^{31}P NMR measurement was performed.

The $^{31}P\{^{1}H\}$ CP/MAS NMR spectra (data not shown) of the samples of Figure 3.3 were similar in chemical shift with their single pulse spectra. Since the ^{31}P signal in CP/MAS spectra is transferred from the closest ^{1}H through the ^{1}H–^{31}P dipolar coupling, the ^{31}P signal intensity depends on the ^{31}P–^{1}H distance and numbers of neighboring protons (Li et al., 2012b). To reveal the protonation of phosphate groups as well as the solid P products (amorphous vs crystalline calcium phosphate precipitates) (Figure 3.3), a series of CP/MAS solid state ^{31}P NMR experiments

with variable τ_{CP} values were conducted for three samples (sample information in Figure B.10 caption, fitting methods in Appendix B. *Text S4*). The CP kinetics can be described using the classical biexponential equation (Appendix B. *Text S4*), in which intensity increases at short contact times with a time constant T_{PH} and then decreases at longer times with a time constant $T_{1\rho,H}$ (Kolodziejski and Klinowski, 2002; Mason et al., 2011). T_{PH} relates to the spatial proximity of P to H, and $T_{1\rho,H}$ to the relaxation of the ^{1}H nuclei (Mason et al., 2011). For a system with two 1/2 nucleus (in this case ^{31}P and ^{1}H), the heteronuclear dipolar-dipolar interaction is inversely proportional to the internuclear distance, and longer internuclear distance is related to longer T_{PH} and lower CP efficiency (Xu et al., 2007). The mobility of the proton sources (e.g., water molecules, surface P–OH and Al–OH groups) also affects the CP efficiency (Xu et al., 2007).

The buildup of signals at $-16.5 < \delta_P < -2$ ppm and $-30 < \delta_P < -16.5$ ppm in the CP/MAS spectra kinetics for all three samples (polyphosphate reacted with 5 nm γ-Al$_2$O$_3$ at pH 6.0 and 8.0, with or without 1 mM Ca^{2+}) shows highest CP efficiency, indicating that the main hydrogen species may be P–OH groups derived from the polyP-P$_{bonded}$ and polyP-P$_{unbonded}$ species (Figures E10.a–c) (Xu et al., 2007). The signal buildup at $-2 < \delta_P < 3.9$ ppm in Figure B.10c–d indicated that the formed precipitates of calcium phosphate were amorphous phase, since (1) amorphous calcium phosphate has shorter T_{PH} time (0.54 ± 0.15 ms) with our calculate result of 0.3 ms when compared to crystalline hydroxyapatite (a crystalline Ca phosphate phase) (1.52 ± 0.08 ms);(Mason et al., 2011; Mathew et al., 2011) (2) hydroxyapatite would show a significantly slower ^{31}P NMR signal buildup compared to its amorphous counterparts and the signal area intensity for hydroxyapatite would decay much slowly with the relaxation time $T_{1\rho,H}$ close to infinity (Klimavicius et al., 2014; Li et al., 2012b; Mathew et al., 2011).

Figure 3.4 2D ^{31}P {^{1}H} CP-HetCor spectra of the solid reaction products from 5 nm γ-Al$_2$O$_3$ reacting with polyphosphate (Experiment Set I) at 2 mM polyphosphate (as total P), pH 6.0 or 8.0, with or without 1 mM Ca^{2+}, and 9 d reaction time. (a–c) Contour plots for polyphosphate hydrolysis with Ca^{2+} at pH 6.0 (a), without Ca^{2+} at pH 8.0 (b), with Ca^{2+} at pH 8.0 (c), respectively. Spectra on the top and left of the 2D contour plots are sum projections of the ^{31}P and ^{1}H dimensions, respectively. (d) Spectra of ^{31}P cross-sections (the F2 dimension) taken at ^{1}H chemical shifts of 5.14 ppm for the 2D contour maps in (a–c). (e) Spectra of ^{1}H cross-sections (the F1 dimension) taken at ^{31}P chemical shifts of 1.28, 10.01, and 20.27 ppm for the 2D contour map in (c).

We further collected 2-D ^{31}P{^{1}H} HetCor spectra of these three samples in Figure B.10 to

distinguish phosphate sites by the nature of associated hydrogen environments, and their 2-D

spectra for 3 ms CP time are shown in Figure 3.4. The spectra in the center of Figures 3.4a–c were

typical 2-D contour plots with the sum projection of the ^{31}P dimension on the top and the ^{1}H sum

projection on the left. The ^{31}P projection closely resembled their corresponding SP/MAS spectra

(Figure 3.3), and the ^{1}H projection contained signal only from H atoms near phosphate. The

similarity of the ^{1}H projection in Figure 3.4a–c indicated no significant difference of overall

hydrogen environment. Therefore, the analysis for ^{1}H cross-section spectra at different P chemical

shifts was needed. Within the 2-D spectrum of 5 nm γ-Al$_2$O$_3$ sample (reacted with polyphosphate

in the presence of Ca^{2+} at pH 6.0) shown in Figure 4.4a, six different domains were identified.

Four domains contained relatively high contour density for the pairs of shifts (δ_H, δ_P) centered at

around (1.5 ppm, –14.2 ppm), (1.5 ppm, –22.1 ppm), (5.1 ppm, –14.2 ppm), and (5.1 ppm, –22.1

ppm). Two domains had lower contour density correlating a peak at δ_H = ~ 7.7 ppm in the ^{1}H sum

projection with peaks at δ_P = ~ –14.2 and – 22.1 ppm in the ^{31}P projection. The six domains of 5

nm γ-Al$_2$O$_3$ sample prepared without Ca^{2+} addition at pH 8.0 (Figure 4.4b) showed similar

positions as the sample in the presence of Ca^{2+} at pH 6.0 and only showed different contour density

(Figure 4.4a). No contour at δ_P = ~ 1.5 ppm was observed for these two samples. These results

indicated that the same coordination environment of hydrogen and phosphorus for surface

complexes dominated upon polyphosphate adsorption, due to the lower capability of

orthophosphate (produced from polyphosphate hydrolysis, carrying less negative charge as

compared to polyphosphate) to compete with polyphosphate for surface adsorption/complexation.

However, the 2-D spectrum of 5 nm γ-Al$_2$O$_3$ sample, reacted with polyphosphate in the

presence of Ca^{2+} at pH 8.0, displayed different contour shape. The domains at around (1.5 ppm, –

9.6 ppm), (5.1 ppm, –9.6 ppm), and (7.7 ppm, –9.6 ppm) dominated the spectrum. For the ^{31}P peak

at δ_P = 1.58 ppm, only the ^{1}H peak at near δ_H = 5.1 ppm appeared. The appearance of a ^{1}H peak at

5.1 ppm in the cross-section of the 1.58 ppm ^{31}P peak primarily originated from water molecules and hydroxyl group of amorphous calcium phosphate since the existence of crystalline hydroxyapatite is eliminated by CP dynamic results in Figure D.10 (Klimavicius et al., 2014; Li et al., 2012b; Mathew et al., 2011). Li et al (2012) for the first time revealed that boehmite surface catalyzed the crystallization of hydroxyapatite based on the observation of the 2-D HetCor domain in the ^{31}P peak at $\delta_P = 2.65$ ppm and the ^{1}H peak at near $\delta_H = 0.2$ ppm (Li et al., 2012b), which did not appear in our 2-D HerCor spectra (Figure 3.4). The ^{1}H peak correlated to the surface-adsorbed orthophosphate dominated by restrictedly mobile water in the surface fluid layer could give a relatively narrow peak at $\delta_H = \sim 5$ ppm (Li et al., 2010). If the adsorption of orthophosphate (produced from polyphosphate hydrolysis) could lead to the contour plot in (5.1 ppm, 1.58 ppm), we should observe it in the samples of γ-Al$_2$O$_3$ reacted with polyphosphate at pH 6.0 with Ca^{2+} (Figure 4.4a) and at pH 8.0 without Ca^{2+} (Figure 4.4b). However, the contour plot in (5.1 ppm, 1.58 ppm) did not appear in Figures 4.4a and b. After 9-day reaction, approximately half of total dissolved phosphate remained in the solution as polyphosphate (Table B.2), which strongly competed for adsorption sites with orthophosphate. Due to the higher negative charge in polyphosphate molecules as compared to orthophosphate, the adsorbed orthophosphate ($\delta_P = \sim -3.0$ ppm) (Li et al., 2013a), which was not observed in ^{31}P CP/MAS NMR spectra in ^{31}P cross-sections (the F2 dimension) taken at ^{1}H chemical shifts of 5.14 ppm (Figure 4.4d), should not be a main surface phosphorus species on γ-Al$_2$O$_3$. Due to the possible existence of three surface phosphorus species, we chose the spectra of ^{1}H cross-sections (the F1 dimension) taken at ^{31}P chemical shifts of 1.58, –10.01, and –20.27 ppm in the sample of Figure 4.4c and displayed in Figure 4.4e. Their ^{1}H NMR spectra did not show obvious difference, indicating that the proton environments mainly came from water molecules.

3.4.5 Effect of metal ions on polyphosphate hydrolysis

The effects of common divalent metal cations (Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}) on polyphosphate hydrolysis were further investigated using 5 nm γ-Al_2O_3 at pH 6.0 and 8.0 (Experimental Set II of Table B.1) (Figure B.11). Due to the lowered concentration of polyphosphate, metal cations, and γ-Al_2O_3 (details in Experimental Set II) compared to those used in the Experimental Set I (Table B.1), the hydrolysis extent (Figures B.11a and c) was lower than that those shown in Figure 3.1 (hydrolysis experiments with/without 1 mM Ca^{2+}). Compared to the control experiment (where 4.1±0.7% polyphosphate was hydrolyzed in the absence of metal cations after 9-day reaction, data not shown), the presence of divalent metal cations all facilitated the hydrolysis of polyphosphate at different degrees. At the same concentration of metal cations (0.5 mM), orthophosphate production followed the order of $Cu^{2+} > Zn^{2+} \approx Ca^{2+} \approx Mn^{2+} > Mg^{2+}$ at pH 6.0, and $Ca^{2+} > Cu^{2+} > Mn^{2+} \geq Zn^{2+} > Mg^{2+}$ at pH 8.0 (Figures B.11a and c). For total P uptake, no significant differences were observed for all metal cations at pH 6.0 (Figure B.11b). At pH 8.0, total P uptake followed the order of $Cu^{2+} > Zn^{2+} \approx Mn^{2+} > Ca^{2+} \approx Mg^{2+}$ (Figure B.11d). Additionally, the uptake of Cu^{2+} and Zn^{2+} was always higher than that of Mn^{2+}, Ca^{2+}, and Mg^{2+} at both pH values (Figure B.12). The simultaneous decrease of Cu^{2+}/Zn^{2+} and total P concentration suggested the potential formation of metal phosphate/polyphosphate precipitates at pH 8.0. Formation of Zn-Al layer double hydroxides at pH 8.0 might be another reason leading to the lowest concentration of Zn^{2+} in the supernatant (Wan et al., 2019c).

To investigate P speciation in these systems (5 nm γ-Al_2O_3, presence of five different metal cations), P K-edge XANES and ^{31}P solid-state NMR spectroscopy analyses were performed on the freeze-dried reacted solids (Figures 3.5 and B.13–14). P K-edge XANES spectra of samples reacted in the presence of different metal cations did not show significant difference (Figure B.14).

The small peak at 2157.1 eV is likely due to the residual or recrystallized Na-polyphosphate during freeze drying (Figure B.14). To facilitate P XANES analysis, a suite of reference compounds (Figure B.14c, details in Appendix B. *Text S5*) was used to preform linear combination fitting (LCF) to reveal the possible P species only in Ca^{2+} system and their relative contributions. For Ca^{2+} system (Figures 3.5a–b), fitting results indicated that the main P species in the reacted solids are γ-Al_2O_3-adsorbed polyphosphate and/or orthophosphate, as well as amorphous calcium phosphate. Here, due to the spectra similarity of γ-Al_2O_3-adsorbed orthophosphate with γ-Al_2O_3-adsorbed polyphosphate (Figure B.14a), the relative percentages of the former from LCF can contain large uncertainty, especially consider the fact that NMR results did not show significant contribution of γ-Al_2O_3-adsorbed orthophosphate. The percentage of amorphous calcium phosphate became higher at pH 8.0, consistent with the result of solid state NMR spectra (Figure 3.3).

The solid-state ^{31}P NMR spectra of samples reacted in the presence of different metal cations are shown in Figures 5.5c–e and B.13. Due to the paramagnetic nature of Cu and Mn, NMR measurements of Cu/Mn-containing samples were very challenging and resulted in poor NMR spectra (Figure B.13). In Mn^{2+} system, no phosphorus chemical shift was observed (Figure B.14b).

For the Ca^{2+} system at pH 8.0, similar to the Ca^{2+} system in Figure 3.3, the main P species included phosphate groups as inner-sphere surface complexes (i.e., polyP-P_{bonded}) at $\delta_P = -10.78$ ppm, phosphate groups in polyphosphate that were not with mineral surface (i.e., polyP-$P_{unbonded}$) at $\delta_P = -20.2$ ppm, and calcium phosphate precipitates ($\delta_P = 1.41$ ppm) (Figure 3.5c). At pH 6.0, it only contained the first two species (Figure 3.5c).

For the Mg^{2+} system (Figure 3.5d), we observed phosphate group as inner-sphere surface complexes (i.e., polyP-P_{bonded}) at $\delta_P = -11.98$ ppm, as well as phosphate groups in polyphosphate

that were not associated mineral surface (i.e., polyP-P$_{unbonded}$) at $\delta_P = -22.58$ ppm. At pH 8.0, surface complexed phosphate species (i.e., polyP-P$_{bonded}$) became dominant, likely due to Mg^{2+} facilitated surface adsorption/complexation of phosphates.

Fig. 5 (a–b) Linear combination fitting (LCF) results of P K-edge XANES spectra of the 9-day reaction products (Experiment Set II) from hydrolysis of polyphosphate (1 mM as total P) by 0.1 g/L Al$_2$O$_3$ in the presence of 0.5 mM Ca^{2+} at pH 6.0 (a) and 8.0 (b). LCF reference compounds are ACP (amorphous calcium polyphosphate), octa Ca-orthoP (octacalcium phosphate), Na-polyP (sodium polyphosphate salt), Ca-polyP (calcium polyphosphate precipitates, Al$_2$O$_3$_polyP (Al$_2$O$_3$-adsorbed polyphosphate), and Al$_2$O$_3$_orthoP (Al$_2$O$_3$-adsorbed orthophosphate). (c–e) are ^{31}P solid-state NMR spectra of γ-Al$_2$O$_3$ (Experiment Set II) reacting with polyphosphate (1 mM as total P) at pH 6.0 or 8.0 in the presence of 0.5 mM Ca^{2+} (c), Mg^{2+} (d), or Zn^{2+} (e).

For the Zn^{2+} system (Figure 3.5e), we observed the presence of zinc phosphate precipitates (δ_P = 2.78 ppm) (Roming et al., 2008) besides inner-sphere surface phosphate complexes (polyP-P_{bonded}, δ_P = −10.21 ppm) and phosphate group not associate with mineral surfaces (polyP-$P_{unbonded}$, δ_P = −20.99 ppm). We also monitored the structure alteration of γ-Al_2O_3 using ^{27}Al solid-state NMR spectroscopy (Figure B.15). No changes were observed for γ-Al_2O_3 structure after 9-day reaction in the presence of Ca^{2+}, Mg^{2+}, Cu^{2+}, and Mn^{2+}. However, a new shoulder peak at δ_{Al} = 12.8 ppm was observed for the Zn^{2+} system (Figure B.15f), suggesting the formation of Zn-Al layer double hydroxide precipitates (Li et al., 2012a; Wan et al., 2019c).

3.5.6 Reaction mechanisms

During the hydrolysis of phosphate monoester by acid or alkaline phosphatase, at the enzyme active site, intermediate species was formed involving the oxygen atom of the terminal PO_4 group completely coordinated with two metal cations [e.g. Zn(II), Ca(II), Fe(II), Mn(II)] (Coleman, 1992; Schenk et al., 2008; Schenk et al., 2012). These metal complexes in phosphatase can initiate double Lewis acid activation for hydrolyzing phosphates by initially bridging the two metal centers with the two phosphoryl oxygen atoms (Williams et al., 1999). Purple acid phosphatases can catalyze the hydrolysis of phosphate esters and condense phosphate through a binuclear metal center (di-iron Fe-Fe or Fe-Mn/Zn) and produce orthophosphate due to the net transfer of the phosphoryl group to water (Huang, 2018a; Schenk et al., 2013). Based on our results, the phosphatase-mimetic ability of γ-Al_2O_3 toward polyphosphate degradation/hydrolysis is likely derives from the ability of Al atoms coordinating with phosphate groups in polyphosphate probably via the formation of bidentate binuclear surface complexes, which activates the P atom for a nucleophilic attack. Surface H_2O and $\equiv$Al−OH, close to the neighboring Al atoms coordinated with phosphoryl O atom (Mäkie et al., 2013; Olsson et al., 2010), might serve as a nucleophilic

agent attacking the P atom with a subsequent cleavage of the P−O−P bond and this ≡Al−OH can be viewed as reactive centers for activating the dissociation of the P−O bond onto γ-Al$_2$O$_3$ (Olsson et al., 2010; Tan et al., 2008).

Our observed terminal-only hydrolysis mechanism indicated that one or two terminal phosphate group(s) of polyphosphates can associate with Al atoms at the surface of γ-Al$_2$O$_3$ and form bidentate binuclear surface complexes (as schematically illustrated in Figure B.6). Ca^{2+} and other divalent metal cations can likely coordinate with the surface hydroxyls (≡Al–OH) of γ-Al$_2$O$_3$ and/or polyphosphate molecules, forming ternary surface complexes (as previously observed in the presence of phosphate, which can eventually enhance the surface coverage of phosphate) (Inman et al., 2001; Li et al., 2013b; Yan et al., 2018). The difference in hydrolysis extent of five divalent metal cations might be due to their intrinsic affinity to polyphosphate. The order of stability constants of divalent metal cations (except for Mn^{2+}, which is unavailable in the literature) and tripolyphosphate complexes follows Cu^{2+} ($10^{8.01)}$) > Zn^{2+} ($10^{6.55}$) > Ca^{2+} ($10^{4.98}$) > Mg^{2+} ($10^{4.58}$) (Maki et al., 2013). This is similar to the order of metal cation promotion on polyphosphate hydrolysis at pH 6.0 (Figure B.11a). However, this order was not followed at pH 8.0, likely due to the formation of copper hydroxide precipitates (for Cu^{2+}) or Zn-Al layer double hydroxides (for Zn^{2+}). This was supported by the observation of significant removal of Cu^{2+} and Zn^{2+} from solution. Thus, if not considering Cu^{2+} and Zn^{2+}, at pH 8.0, Ca^{2+} has stronger affinity than Mg^{2+} for polyphosphate complexation and promotion for polyphosphate hydrolysis.

3.5 Environmental Implications

Polyphosphate adsorption and degradation at the mineral-water interface is of great relevance to the cycling of polyphosphate of natural origin and from human activities. Our

laboratory experiments on the hydrolysis of polyphosphate hydrolysis at the mineral-water interface suggested that varied-size γ-Al$_2$O$_3$ can promote the hydrolysis of polyphosphate, which is further enhanced in the presence of divalent metal cations. Additionally, at pH 8.0 (similar to seawater pH) and in the presence of Ca^{2+}, continuous hydrolysis of polyphosphate on the surface of γ-Al$_2$O$_3$ can lead to the formation of amorphous calcium phosphate precipitates (possibly within days). If the reaction was allowed to proceed for longer term, this might lead to the eventual formation of hydroxyapatite, as was previously observed during long time co-sorption of orthophosphate and calcium on boehmite at pH 7.0 (Li et al., 2012b). Li et al., (2012) observed the immediate formation of amorphous calcium phosphate precipitates upon mixing orthophosphate and Ca^{2+} solutions at pH 9.0 in the absence of boehmite, which gradually transformed into hydroxyapatite after 30-day aging (Li et al., 2012b). In our case, further hydrolysis of polyphosphate may eventually lead to the formation of hydroxyapatite or other crystalline calcium phosphate phases within months at pH 8.0, due to the co-sorption of Ca^{2+} and orthophosphate (produced from polyphosphate hydrolysis) on the surface of γ-Al$_2$O$_3$ and aging of the produced amorphous calcium phosphate precipitates.

Overall, our results revealed the critical roles of mineral-water interface reaction and divalent metal cations on controlling polyphosphate degradation and transformation, and laid the foundation for better understanding the interfacial geochemical processes governing phosphorus cycling in sediments, soils, and water bodies. Such abiotically mediated polyphosphate transformation process might offer additional insights for explaining marine sedimentary phosphorus burial via the precipitation of fine-grained apatite particles from exogenous polyphosphate intermediates as previously observed (Diaz et al., 2008; Goldhammer et al., 2010; Schulz and Schulz, 2005). Although the concentrations of polyphosphate and metals used in this

study are much higher than those in common natural environments, the concentration of Ca^{2+} and Mg^{2+} in seawater (~10 and 50 mM, respectively) are much higher than the concentration used in this study. In marine sediments, calcium polyphosphate granules can also provide local environments with high Ca^{2+} and polyphosphate concentrations (Diaz et al., 2008). Additionally, microbial Mn/Fe reduction in soils and sediments may lead to high metal (e.g., Cu, Zn, and Mn) concentrations in the pore-water (Cooper et al., 2006; Müller et al., 2002). Future studies might consider exploring the effects of other common environmental minerals (e.g., Fe oxides) and solution conditions (e.g., freshwater *vs* seawater), as well as comparisons between abiotically (e.g., mineral catalyzed) and biotically (e.g., enzyme) mediated polyphosphate hydrolyses, in order to fully understand the processes affecting the fate of polyphosphate under varied and complex environmental settings.

Supplementary Information for Chapter 3 can be found at **APPENDIX B. POLYPHOSPHATE ADSORPTION AND HYDROLYSIS ON ALUMINUM OXIDES**.

CHAPTER 4. IRON OXIDE-CATALYZED HYDROLYSIS OF POLYPHOSPHATE AND THE PRECIPITATION OF CALCIUM PHOSHATE MINERALS

4.1 Abstract

Interfacial chemistry of phosphorus (P) is important for understanding P sequestration and bioavailability in nature. Polyphosphate is a group of important phosphate species in aquatic environments. The geochemical behaviors of polyphosphate at the mineral-water interface play critical roles in mediating aquatic P transformation, yet remain poorly constrained. This study investigated the hydrolysis of polyphosphate in the presence of common iron (Fe) oxide minerals (ferrihydrite, hematite, goethite, lepidocrocite) and the subsequent precipitation of calcium phosphate minerals. Batch studies and microscopic and spectroscopic characterizations were conducted to reveal P speciation and complexation state under varied solution chemistry. All four Fe oxides can hydrolyze polyphosphate and the presence of calcium cations (Ca^{2+}) enhanced both the rate and extent of the hydrolysis. The apparent hydrolysis rate followed the order of lepidocrocite > hematite > ferrihydrite > goethite in the presence of Ca^{2+}. A terminal-only pathway via one-by-one cleavage of terminal phosphate groups was the dominant hydrolysis mechanism. At alkaline pH conditions, amorphous calcium phosphate formation was observed in the presence of Ca^{2+}, which transformed to crystalline hydroxyapatite upon long-term aging. This is the first study directly demonstrating the roles of naturally abundant minerals in controlling polyphosphate transformation into crystalline calcium phosphate minerals. This study provides also new insights

for understanding interfacial processes controlling polyphosphate transformation and calcium phosphate mineral formation and broadens our current knowledge in aquatic P cycling.

4.2 Introduction

Phosphorus (P) is an essential nutrient for all life and often viewed as a limiting macronutrient for primary production in estuarine and marine environments (Paytan and McLaughlin, 2007). Among all biological P-containing molecules, polyphosphate is an important phosphate-containing polymer of at least three phosphate ions joined by phosphoanhydride (P−O−P) bonds (Wan et al., 2019a, b). Polyphosphate widely occurs in natural systems such as soils, sediments, and aquatic environments (Omelon and Grynpas, 2008), and can be synthesized by a wide range of microorganisms such as bacteria and plankton (Diaz et al., 2008; Martin et al., 2014; Orchard et al., 2010; Zhang et al., 2015). It serves many biological functions such as being an ATP substitute, energy source, and regulator for P stress and survival, as well as for life evolution (Kornberg et al., 1999; Rao et al., 2009). It is also an important industrial chemical and frequently used in water treatment, fertilizers, and food additives (Kulakovskaya et al., 2012), resulting in its common release into the aquatic environments (Diaz et al., 2008; Kulakovskaya et al., 2012) and potential subsequent contamination of water bodies.

In aquatic environments, especially marine settings, abundant diatom-derived polyphosphate may play key roles in geologic P sequestration and sedimentary P burial (Diaz et al., 2008). It is generally accepted that the main mechanism leading to P removal from the oceans is via the formation of apatite mineral in sediments, yet the formation processes are kinetically inhibited under oceanic conditions and not fully understood (Diaz et al., 2008). A possible mechanism for explaining sedimentary P burial is the precipitation of fine-grained apatite mineral

particles from microbially released polyphosphate intermediates (Diaz et al., 2008; Goldhammer et al., 2010; Omelon and Grynpas, 2008; Schulz and Schulz, 2005). Thus investigating the interfacial behaviors of polyphosphate at the sediment/soil-water interface is critical for understanding polyphosphate transformation and fate for better constraint on global P cycling (Hupfer et al., 2004; Sharpley et al., 1994) as well as potential P contamination due to the anthropogenic release of polyphosphate.

Extensive studies have investigated the cycling of different P species (e.g., orthophosphate and organic phosphates) and their interfacial behaviors such as adsorption and precipitation at the mineral-water interface (Abdala et al., 2015b; Ruttenberg and Sulak, 2011; Wang et al., 2013a; Yan et al., 2014b). However, very few studies have explored polyphosphate transformation at the mineral-water interface and the roles of abiotic factors in controlling its stability and degradation. Triphosphate hydrolysis is facilitated by amorphous manganese (Mn) oxides, and the reaction is further enhanced by the presence of calcium (Ca^{2+}) and magnesium (Mg^{2+}) cations (Inman et al., 2001). Tripolyphosphate adsorption on goethite is an important step for the subsequent hydrolysis, and drying of the adsorbed tripolyphosphate in the presence of Ca^{2+} resulted in the formation of a Ca-trimetaphosphate surface complex (Hamilton et al., 2017). In aquatic environments, polyphosphate transformation at the sediment-water interface was found to contribute to a significant portion of soluble reactive phosphorus, but details of the transformation process and the related formation of calcium phosphate minerals remain poorly understood (Diaz et al., 2012; Omelon and Grynpas, 2008). We hypothesize that orthophosphate released from polyphosphate hydrolytic degradation is a key step for the nucleation and precipitation of calcium phosphate minerals. Our recent study on the enzymatic hydrolysis of polyphosphate indicated that orthophosphate released from the enzymatic degradation of polyphosphate can precipitate with

Ca^{2+} to form amorphous calcium phosphate (ACP) solids (Huang et al., 2018b). Our recent abiotic studies also showed that polyphosphate can be hydrolyzed by aluminum (Al) and Mn oxides, and the hydrolysis was promoted by the presence of divalent cations such as Ca^{2+} and copper (Cu^{2+}) cations (Wan et al., 2019a, b). However, research on the roles of common iron (Fe) (oxyhydr)oxide (hereafter Fe oxide) minerals in polyphosphate transformation under environmentally relevant conditions is still lacking.

Iron oxides are widely present in many surface and subsurface environments and can strongly regulate the interfacial chemistry of nutrients and contaminants (Hochella et al., 2008; Jambor and Dutrizac, 1998). They typically have high affinities for P via surface adsorption or precipitation due to their highly positive surface charge, large surface area, and strong reactivity (Wang et al., 2015; Wang et al., 2013b). These interfacial behaviors are affected by mineral structure and solution chemistry (Barreto et al., 2020; Ruttenberg and Sulak, 2011; Wan et al., 2017b; Yan et al., 2014b). In this study, we investigate the kinetics and mechanisms of polyphosphate adsorption and hydrolysis on four environmentally representative Fe oxides (ferrihydrite, hematite, goethite, and lepidocrocite) under varied solution conditions [pH, with or without Ca^{2+}, deionized (DI) water *vs.* artificial seawater]. Batch experiments were combined with P K-edge X-ray absorption near edge structure (XANES) spectroscopy, solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM) with electron energy loss spectroscopy (EELS) to characterize the reaction products. Results from this study provide new insights for the abiotic transformation of polyphosphate on mineral surfaces under environmentally relevant conditions, as well as the formation mechanisms of calcium phosphate minerals upon polyphosphate hydrolysis in the presence of Ca^{2+}.

4.3 Materials and Methods

4.3.1 Materials

Details on polyphosphate sodium salt (P_{10}, Sigma-Aldrich) and Fe oxide mineral (ferrihydrite, goethite, hematite, and lepidocrocite) synthesis (Cornell and Schwertmann, 2004; Lanzl et al., 2012) and characterization are in Appendix C. *Text S1-S2* and Figures A.1d and C.1. Artificial seawater (ASW) was prepared according to D1141-98 standard (ASTM D1141-98, 2013; Nguyen Dang et al., 2017) and contains 420 mM NaCl, 28.8 mM Na_2SO_4, 10.5 mM $CaCl_2$, 54.6 mM $MgCl_2$, 9.32 mM KCl, and 2.79 mM $NaHCO_3$.

4.3.2 Hydrolysis of polyphosphate on Fe oxide minerals

Three parallel sets of batch experiments were conducted to investigate polyphosphate adsorption and hydrolysis on Fe oxide minerals under three solution chemistry conditions: (1) DI water with 0.1 M NaCl at pH 6, 7.5, and 9 (Experimental Set 1); (2) DI water with 0.1 M NaCl and 1 mM Ca^{2+} at pH 6, 7.5, and 9 (Experimental Set 2); and (3) artificial seawater (ASW) at pH 8 (Experimental Set 3), as detailed below. All experiments were performed in duplicate.

For Experimental Set 1 (DI experiments), 0.04 g Fe oxides and 0.58 g NaCl (0.1 M) were mixed in 95 mL DI water in a glass bottle and equilibrated for 18 h under magnetic stirring. Experimental Set 2 (Ca^{2+} experiments) was conducted to explore the effect of Ca^{2+} on polyphosphate hydrolysis and transformation. 2 mL stock solution of $CaCl_2$ (50 mM) was first added into 93 mL DI water before the addition of 0.04 Fe oxides and 0.58 g NaCl. For these two experiment sets, the pH value of the suspension was constantly monitored and manually maintained at 6 ± 0.05, 7.5 ± 0.05, or 9 ± 0.05 using 0.05 M HCl or 0.05 M NaOH. For Experimental Set 3 (ASW experiments), the ASW has an initial pH of 8 ± 0.05. Prior to the experiment, 0.04 g Fe oxides were added in 95 mL ASW. The pH of the ASW-mineral suspension stayed stable at ~8

due to its strong buffering capacity and was adjusted using 0.05 M HCl and 0.05 M NaOH only if needed. For all experimental sets, after overnight dispersion, 5 mL of polyphosphate stock solution (40 mM as total P) was added into the suspensions to achieve a final polyphosphate concentration of 2 mM as total P. To collect good ^{31}P solution NMR spectra with acceptable signal to noise ratio, total P concentration was here set up to 2 mM, which is also consistent with our recent studies (Huang et al., 2018b; Wan et al., 2019b).

For all three sets of experiments, after the addition of polyphosphate stock solution to the Fe oxide suspension, pH of the suspension was immediately measured and adjusted to the desired value. After that, the pH value of the reaction suspension was measured and adjusted several times within the first 2 h and at 3, 5, 7, 10, 24, 48, 72, 96, 168, and 216 h (9 d) before the aliquot of the suspension was taken. At specific time points, 2 mL aliquot of the suspension was taken and immediately filtered through a 0.22-µm Millipore membrane. The supernatant was analyzed for orthophosphate and total P concentrations. For total P analysis, all P in the supernatant was hydrolyzed to inorganic orthophosphate via potassium persulfate autoclave digestion (Das et al., 2014). Orthophosphate concentration was determined using the phosphomolybdate colorimetric assay (Murphy and Riley, 1962) on an UV–vis spectrometer (Carey 60, Agilent). The concentration of Ca^{2+} in the supernatant was measured by inductively coupled plasma-optical emission spectrometry (ICP-OES).

To investigate the potential formation of apatite upon aging, long-term experiments were conducted on hematite and lepidocrocite with polyphosphate (as total P) and Ca^{2+} at 1 or 2 mM at pH 9. Three aging experiments were conducted: Sample 1 has 0.4 g L^{-1} hematite, 1 mM Ca^{2+}, 2 mM polyphosphate as total P, and 150 d reaction; Sample 2 has 0.4 g L^{-1} hematite, 2 mM Ca^{2+}, 1 mM polyphosphate as total P, and 70 d reaction; Sample 3 has 0.4 g L^{-1} lepidocrocite, 1 mM Ca^{2+},

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2 mM polyphosphate as total P, and 150 d reaction. The preparation process was the same as Experimental Set 2 and details are in Table C.2.

4.3.3 Solution and solid phase analyses

At the end of reaction, the suspensions were centrifuged to separate the solid and supernatant. The supernatants were further filtered by 0.22-μm Millipore membrane for solution ^{31}P NMR spectroscopy analysis (details in Appendix C. *Text S3*). The centrifugation-separated wet pastes were washed twice with DI water and freeze-dried for analysis by X-ray diffraction (XRD), Fourier transformed infrared (FTIR) spectroscopy, high resolution transmission electron microscopy (HRTEM), and P K-edge XANES spectroscopy, as detailed in Appendix C. *Text S3*. P K-edge XANES analysis was conducted at Beamline 14-3 at the Stanford Synchrotron Radiation Lightsource (SSRL), Menlo Park, CA. A list of P reference compounds (details in Appendix C. *Text S2*) were used for linear combination fitting (LCF) analysis of the sample spectra.

4.4 Results and Discussion

4.4.1 Polyphosphate adsorption and hydrolysis

Adsorption and hydrolysis of polyphosphate on Fe oxides in the absence/presence of 1 mM Ca^{2+} are shown in Figures 4.1a and C.2-4. Figure 4.1a showed a typical kinetic curve of polyphosphate hydrolysis on Fe oxides (here using hematite as an example, see Figure C.2 for all Fe oxides), similar to our previous observations on polyphosphate hydrolysis on Al and Mn oxides (Wan et al., 2019a, b). Solution pH, Ca^{2+}, and mineral type all influenced the release of orthophosphate from polyphosphate hydrolysis (Figure C.2). To directly compare the hydrolysis on different Fe oxides, efforts were taken to fit the hydrolysis rate. Polyphosphate degradation is a complex process, involving one-by-one cleavage of the terminal phosphate groups (details in

Section 4.3.2) and production of orthophosphate, the resulting gradual shortening of polyphosphate chain length, and the subsequent reaction of orthophosphate with other dissolved ions (e.g., complexation/precipitation with Ca^{2+} if it is present) (Wan et al., 2019a, b). A first-order kinetic model showed the best fitting results for apparent hydrolysis rate (Figure C.3 and Table C.1), and the R^2 values were 0.906–0.998 for pH 6 and 7.5 and 0.842–0.966 for pH 9 (Table C.1). The relatively lower R^2 values for pH 9 experiments in the presence of Ca^{2+} is likely due to the formation of Ca-phosphate ternary surface complexes and/or precipitates (Li et al., 2012b; Wan et al., 2016; Wan et al., 2017b), which decreased orthophosphate concentration in the supernatants and thus the goodness of fit.

In the absence of Ca^{2+} (Figure C.3), the apparent hydrolysis rates were ~0.25×10^{-3} h^{-1} for ferrihydrite, hematite, and goethite. Lepidocrocite showed a high hydrolysis rate, which increased from 0.35 10^{-3} h^{-1} at pH 6 to 1.14 10^{-3} h^{-1} at pH 9. This is similar to previously observed higher reactivity of lepidocrocite than other Fe or metal oxides for degrading tris(1,3-dichloro-2-propyl) phosphate (a typical organophosphate flame retardant and phosphate triester) (Fang et al., 2018).

The presence of Ca^{2+} significantly increased orthophosphate production for all four Fe oxides and the highest level of orthophosphate release was observed at pH 7.5 within 9-day reaction (Figures 5.1a and C.2). In the presence of 1 mM Ca^{2+}, the hydrolysis extent roughly followed the order of lepidocrocite > hematite > ferrihydrite > goethite (Figure C.2). For example, with Ca^{2+} at pH 7.5, orthophosphate release reached 884 µM (46.4% of total P loading), 670 µM (40.4%), 489 µM (38.5%), and 324 µM (15.8%) for lepidocrocite, hematite, ferrihydrite, and goethite, respectively (Figures C.2 and C.4). Meanwhile, the apparent hydrolysis rates also increased for all four Fe oxides in the presence of Ca^{2+} (Figures 5.1b-f and Table C.1), which was affected by solution pH. At pH 6 and 7.5, the enhancement of polyphosphate apparent hydrolysis

rates increased relative to those at pH 9. The low hydrolysis at pH 9 might be attributed to the potential formation of calcium phosphate minerals and/or surface ternary complexes that reduced the release of orthophosphate into the supernatants and thus the observation overall apparent hydrolysis rates.

We also monitored the dynamics of total P concentration in the supernatants (Figure C.4). At pH 6 and 7.5, polyphosphate rapidly adsorbed on ferrihydrite, hematite, goethite, and lepidocrocite during the first 10 h. As the reaction proceeded, subsequent polyphosphate hydrolysis and orthophosphate release led to the increase of total P concentration in the supernatant (Figure C.4). This trend in total P concentration was consistent with our previous studies (Wan et al., 2019a, b). Interestingly, in ferrihydrite suspension, total P concentration continuously decreased at all three pH values within the 9-day reaction, likely due to a large amount of orthophosphate adsorption on ferrihydrite that has large surface area and high reactivity toward P adsorption (Wang et al., 2013a; Wang et al., 2017). At pH 6 and 7.5, the presence of Ca^{2+} decreased the total P concentration in the supernatant, likely due to the formation of surface ternary complex(es) (Li et al., 2012b; Wan et al., 2019b), which subsequently facilitated polyphosphate/orthophosphate uptake by Fe oxide surfaces (Figure C.4). At pH 9, a different trend of total P concentration was observed: the presence of 1 mM Ca^{2+} significantly reduced total P concentration at 10–216 h (Figure C.4). Within 9-day reaction, the Ca^{2+} concentration gradually decreased, reaching ~400 µM at pH 9 (Figure C.5). At this pH, the simultaneous decrease of Ca^{2+} and total P concentrations suggests the potential formation of calcium phosphate complexes/precipitates, as discussed in *Section 4.3.2*.

Figure 4.1. Polyphosphate hydrolysis on Fe oxides under different solution conditions. (a) Dynamics of orthophosphate production from polyphosphate hydrolysis on hematite in the absence/presence of 1 mM Ca^{2+} at pH 6, 7.5, and 9. Panel (b-e) are fitting results of the hydrolysis kinetics (b: ferrihydrite, c: hematite, d: goethite, and e: lepidocrocite) in the absence/presence of 1 mM Ca^{2+} at pH 6, 7.5, and 9.

4.4.2 Polyphosphate transformation on Fe oxide surface

To reveal the hydrolysis mechanism, we employed solution ^{31}P NMR to characterize aqueous P speciation in the 9-day reaction supernatants (Figure 4.2) and P K-edge XANES to determine the complexation state and phase of different P species in the solid products (Figures 4.3 and C.7). In the absence of Ca^{2+}, the chemical shifts associated with polyphosphate end P groups (at around −8 ppm) and middle P groups (at around −21.7 ppm) were observed (Figures 4.2a-c). The chemical shift at ~1 ppm is attributed to orthophosphate in the supernatant. Since the increase of solution pH decreases the protonation of orthophosphate and polyphosphate end P groups, the chemical shifts of these two P environments gradually shifted to the left as pH increased from 6 to 9. Specifically, the chemical shifts shifted from 0.43 to 2.46 ppm for orthophosphate and from −9.03 to −5.86 ppm for polyphosphate (Figures 4.2a-c). However, in the supernatants of lepidocrocite reaction suspension, the intensity of chemical shift of orthophosphate increased significantly as pH increased from 6 to 9, suggesting the gradual increase of orthophosphate production due to polyphosphate hydrolysis. The chemical shift of orthophosphate at 2.46 ppm dominated solution ^{31}P NMR spectrum for reaction on lepidocrocite at pH 9 (Figure 4.2c), suggesting the high reactivity of lepidocrocite toward polyphosphate hydrolysis, which increased with increasing pH, and is consistent with the observed trend in batch experiments (Figure 4.1e).

Figure 4.2 ^{31}P solution NMR spectra of the liquid supernatants from polyphosphate hydrolysis on Fe oxides at pH 6 (a, d), 7.5 (b, e), and 9 (c, e) in the absence (a-c) or presence (d-f) of 1 mM Ca^{2+} for 9 days. Ferr: ferrihydrite; Hem: hematite; Goet: goethite; and Lep: lepidocrocite.

In the presence of Ca^{2+}, solution ^{31}P NMR showed consistent result as batch experiments in which Ca^{2+} significantly promoted polyphosphate hydrolysis (Figures 4.2d-f). For example, in the presence of 1 mM Ca^{2+}, the chemical shift of orthophosphate became dominant in the NMR spectra of supernatants for all Fe oxides. For hematite and lepidocrocite at pH 7.5 and 9, the intensity of orthophosphate chemical shift at ~1 ppm was much stronger than that of polyphosphate middle P groups. This indicates that the concentration of orthophosphate was higher than

polyphosphate in the supernatants and most of polyphosphate was hydrolyzed after 9-day reaction in the presence of Ca^{2+}. Solution ^{31}P NMR spectra of all supernatants in Figure 4.2 did not show the random appearance of shorter chained polyphosphates, which would have led to the occurrence of multiple chemical shifts at around -21 ppm belonging to the middle groups of polyphosphates with varied chain lengths, as we have previously discussed in details (Huang et al., 2018b; Wan et al., 2019a, b). Combined with the continuous production of orthophosphate in the reaction suspensions (Figure C.2), ^{31}P NMR results suggest that a terminal-only hydrolysis pathway was the dominant mechanism for polyphosphate hydrolysis on Fe oxides, where orthophosphate was produced via one-by-one cleavage of terminal P−O−P bonds in polyphosphate molecules, similar to the cases of enzyme-, Al oxide-, and Mn oxide-catalyzed polyphosphate hydrolysis (Huang et al., 2018b; Wan et al., 2019a, b).

Since P K-edge XANES spectra of phosphates adsorbed on Fe oxides have different features compared to calcium phosphate minerals (Figure C.6), we used P XANES to identify and quantify surface P species in the solid products. P XANES spectra of calcium phosphate mineral or polyphosphate salt references showed post-edge characteristics that are different from varied P reference compounds (Figure C.6a). P K-edge XANES spectra of orthophosphate or polyphosphate adsorbed on Fe oxides showed a minor pre-edge feature at -5 to -1 eV (relative to edge position) and lacked the post-edge characteristics (Figure C.6b), due to the electronic transition of a P 1s electron into an Fe(3d)-O(2p)-P(3p) antibonding molecular orbital (Khare et al., 2005). Therefore, P K-edge XANES can be useful in identifying solid P species especially for systems containing both Fe and Ca phosphate phases, either through a semi-quantitatively analysis using LCF or a visual comparison of the sample spectra to reference compounds (Huang and Tang, 2016). Surface P speciation in 9-day reaction products was revealed based on their characteristics

in the P XANES spectra (Figures 4.3 and C.7), as detailed below.

Figure 4.3 Results of linear combination fitting analysis of P XANES spectra of the 9-day reaction products from polyphosphate hydrolysis on ferrihydrite (a, Ferr), hematite (b, Hem), goethite (c, Goet), and lepidocrocite (d, Lep) in the presence of 1 mM Ca^{2+} at pH 9. Raw and fitted data are in black solid and red dotted lines, respectively. Panel (e) is a zoomed view of the post-edge features in comparison with the P XANES spectrum of polyphosphate adsorption on ferrihydrite (9-day reaction time). Red arrow indicates the post-edge features of amorphous calcium phosphate (ACP).

In the absence of Ca^{2+}, P XANES spectra of polyphosphate reacted with Fe oxides was similar to their corresponding polyphosphate or orthophosphate-adsorbed references (Figures C.6

and C.7), suggesting that the main P species in these products were adsorbed phosphates via inner-sphere complexation on Fe oxide surface (Abdala et al., 2015a; Abdala et al., 2015b). However, in the presence of Ca^{2+}, the main peak at 2155.8 eV broadened (Figure 4.3e) and a small shoulder peak at ~2164 eV appeared in the spectra of solid reaction products at pH 9 (Figures 4.3a-d, with zoomed view of the post-edge region in Figure 4.3e). These characteristics suggest the presence of newly formed calcium phosphate minerals. Additionally, P XANES spectra did not show well-separated shoulder peaks at ~2164 eV, suggesting that the formed calcium phosphate minerals were amorphous phase and not crystalline (Huang et al., 2018b; Wan et al., 2019b). We then conducted LCF analysis of the sample XANES spectra using several representative reference compounds, including ACP, calcium polyphosphate, sodium polyphosphate, and polyphosphate or orthophosphate adsorbed on Fe oxides. LCF results showed that, as pH increased from 6 to 9, ACP contents increased from 22.6% to 56% for ferrihydrite, from 8.3% to 26.6% for hematite, from 0 to 17.1% for goethite, and from 3.9% to 45.5% for lepidocrocite (Figures C.7). At pH 9, P XANES spectra of the solid products showed the highest ACP contents (Figure 4.3). Thus, ^{31}P NMR and P XANES analyses suggest that the short term transformation pathway of polyphosphate on Fe oxides includes polyphosphate adsorption as inner-sphere surface complexes, polyphosphate hydrolysis and orthophosphate release, and the precipitation of orthophosphate with Ca^{2+} to form ACP phase, consistent with our previous studies (Wan et al., 2019a, b).

4.4.3 Formation of crystalline calcium phosphate minerals upon aging

Although the formation of ACP was observed during 9-day reaction in the presence of Ca^{2+}, whether ACP can transform into crystalline calcium phosphate minerals (e.g., hydroxyapatite) in this system remains unclear. Our recent study proposed that the long-time aging of the produced ACP solids might lead to the eventual formation of crystalline calcium phosphate phases, which

can help explain the wide occurrence of crystalline apatite in aquatic environment (Wan et al., 2019b). In this study, we employed multiple complementary techniques (P K-edge XANES, FTIR, XRD, and HRTEM) to characterize the solid products upon long-term aging. We prepared three aged samples with 70- or 150-day reaction time (Sample 1–3; details in Experimental section and Table C.2), and their P XANES spectra showed the formation of hydroxyapatite upon aging (Figure 4.4a). For the hematite sample (Sample 2) aged for 70 days with 2 mM Ca^{2+} and 1 mM polyphosphate (as total P), we observed the obvious separation in peaks at ~ 2164 eV that can be attributed to crystalline calcium phosphate minerals (Figure 4.4a). P XANES spectra of calcium phosphate reference compounds usually have two distinct post-edge shoulders (+2 eV and +11 eV relative to edge position) which become less distinctive with decreasing crystallinity (Figure C.6a) (Brandes et al., 2007; Franke and Hormes, 1995). LCF results showed that the percentages of hydroxyapatite were 11.2% for Sample 1, 83.9% for Sample 2, and 30.3% for Sample 3 (Table C.2). Meanwhile, these samples contained surface adsorbed phosphate and ACP. After aging at 70 days or longer time, the hydrolysis of polyphosphate was expected to be close to complete (as supported by FTIR data discussed below), although LCF showed minor adsorbed polyphosphate.

Laboratory XRD patterns of the three aged samples did not show diffraction peaks of crystalline calcium phosphate minerals (Figure C.8a), likely due to the low crystallinity of newly formed hydroxyapatite and the relatively small amount of produced hydroxyapatite as compared to the background signal from highly crystalline hematite and lepidocrocite. Thus we further conducted synchrotron XRD analysis of these samples and found that Sample 2 (hematite, 1 mM polyphosphate as total P, 2 mM Ca^{2+}, 70-day aged) showed weak diffraction peaks belonging to hydroxyapatite (002) and (211) planes at 0.34 and 0.28 nm d-spacing (Figure 4.4b). These peaks were not observed for the other two samples containing high percentages of ACP and the low

percentages of hydroxyapatite (Table C.2).

Figure 4.4 P K-edge XANES spectra (a), synchrotron XRD patterns (b), and FTIR spectra (c) of the aged products of polyphosphate hydrolysis on hematite (Hem) and lepidocrocite (Lep) in the presence of Ca^{2+} (1 mM or 2 mM) at pH 9. For P XANES linear combination fitting analysis, raw and fitted data are in grey solid and blue dotted lines, respectively. Panel (d) is TEM image of Hem_Ca:P(2:1)_pH 9.0 sample, with HRTEM image of the selected

region (e) and its electron diffraction pattern (f). Panel (i) is RBG EELS map of Ca (g) and Fe (h), indicating the co-existence of hematite and hydroxyapatite, which is further validated by synchrotron XRD analysis. Ca:P (1:2) indicates 1 mM Ca^{2+} and 2 mM polyphosphate as total P; Ca:P(2:1) indicates 2 mM Ca^{2+} and 1 mM polyphosphate as total P.

FTIR spectroscopy was applied to further reveal surface P speciation and to distinguish adsorbed P species and solid P minerals in the IR region of 1450–600 cm^{-1}, which originates primarily from the stretching vibrations of surface phosphate groups (Figure 4.4c) (Wan et al., 2016). Compared with the corresponding IR spectra of unreacted Fe oxides, the abundant P absorbance bands below 1200 cm^{-1} belong to orthophosphate asymmetrical and symmetrical vibrations (Elzinga and Sparks, 2007; Wan et al., 2016). We did not observe the feature IR bands of P−O−P vibration in polyphosphate molecules beyond 1220 cm^{-1} (Michelmore et al., 2000; Wan et al., 2017a), suggesting the complete hydrolysis of polyphosphate after long-time aging. For Sample 2 (hematite, Ca:P ratio of 1:2, 70-day aging), four IR absorbance bands at 1112, 1026, 961, and 871 cm^{-1} appeared, and the band positions and spectrum shape were similar to those of hydroxyapatite (Wan et al., 2016). For other two samples, in addition to IR bands of hydroxyapatite or ACP, we observed two main IR peaks at 1101 and 913 cm^{-1}, which can be assigned to the vibrations of inner-sphere orthophosphate complexes on Fe oxide surface (Elzinga and Sparks, 2007). FTIR results indicated the presence of hydroxyapatite and orthophosphate surface complexes on Fe oxides as the two main surface P species and the complete hydrolysis of polyphosphate on Fe oxides upon long-time aging. ACP has a single IR peak at ~1026 cm^{-1} that overlaps with the IR bands of hydroxyapatite (Skrtic et al., 2002) and thus cannot be distinguished here (Figure 4.4c). A shorter aging time and a higher Ca:P ratio (2:1) of Sample 2 as compared to the other two samples suggest that aging is a necessary step for ACP transformation to hydroxyapatite and that excess phosphate ions might inhibit the transformation.

TEM was applied to visualize the presence and distribution of hydroxyapatite in Sample 2 that is dominated by hydroxyapatite and hematite. TEM images showed large aggregated particles in the reaction products (Figure 4.4d). In selected region (Figure 4.4e), HRTEM showed the presence of two different mineral phases with lattice fringes at 0.37 nm d-spacing that belongs to the (012) plane of hematite, as well as 0.34 nm and 0.28 nm that belong to the (002) and (211) planes of hydroxyapatite. Electron diffraction (ED) pattern of this selected area showed the diffraction rings for hematite (012) and (110), as well as hydroxyapatite (002) and (211) (Figure 4.4f). The distribution of hydroxyapatite and hematite was characterized by electron energy loss spectroscopy (EELS) elemental maps of Ca (Figure 4.4g) and Fe (Figure 4.4h). Fe and Ca maps can be used to infer the distribution of hematite and hydroxyapatite, since these were the main mineral phases determined by synchrotron XRD, FTIR, and P XANES. The Ca map showed non-uniform distribution and abundance (reflected by color intensity) of hydroxyapatite (Figure 4.4g). The Fe map showed more uniform distribution of hematite (Figure 4.4h). RBG color maps of Ca and Fe showed the presence of hydroxyapatite on hematite surface (Figure 4.4i). Due to the strong aggregation of hematite particles, the image region presented in Figure 4.4d might contain relatively few hematite particles, leading to a low intensity of Fe signal (Figure 4.4h). To prove this, we selected another region to collect EELS elemental maps of Ca and Fe (Figures C.8b-f) and collected high-angle annular dark field-scanning transmission electron microscopy (HAADF-STEM) image in the larger region and energy dispersive X-ray spectroscopy (EDS) spectrum for three selected points (Figure C.9). In the EELS maps (Figures C.8d and e), we found aggregated particles containing high abundance of hematite with minor hydroxyapatite distributed on hematite surface (Figure C.8f).

4.4.4 Polyphosphate hydrolysis in artificial seawater

Polyphosphate transformation plays a key role in geologic P sequestration and marine P burial (Diaz et al., 2008; Huang et al., 2018b; Omelon and Grynpas, 2008). Natural water bodies (fresh water and seawater) typically have high concentrations of Ca^{2+} (~0.8 mM for fresh water and ~10.5 mM for seawater) (Nguyen Dang et al., 2017; Yuan et al., 2019), which might enhance polyphosphate hydrolysis and transformation on environmental minerals (Wan et al., 2019a, b). We conducted polyphosphate hydrolysis experiments on Fe oxides in artificial seawater (ASW) (Figure 4.5) in order to investigate polyphosphate transformation under marine conditions. The results showed that polyphosphate was gradually hydrolyzed on four Fe oxides in ASW (Figure 4.5a) and the hydrolysis can be fitted using first-order kinetic model (Figures 4.5b and Table C.3). Additionally, in lepidocrocite/hematite/goethite systems, total P concentrations decreased at the beginning, due to the hydrolysis of polyphosphate, and gradually increased at the late stage of polyphosphate hydrolysis, while total P concentration in ferrihydrite system was gone down during 9-day hydrolysis (Figure C.10). These were consistent with the trends of total P concentrations for polyphosphate hydrolysis by four Fe oxides in the presence of 1 mM Ca^{2+} (Figure C.4).

The extent and rate of hydrolysis followed the order of lepidocrocite > hematite > ferrihydrite ≥ goethite (Figures 4.5a and b). Although the mineral reactivity order for polyphosphate hydrolysis in ASW was in line with the order in the presence of 1 mM Ca^{2+} in DI water, the absolute values of apparent hydrolysis rates and extents (pH 8) were smaller than those at pH 7.5 or 9 (Figure 4.1, Tables C.1 and C.3). In ASW, the significant decrease of apparent hydrolysis rate could be attributed to the high salinity of ASW. Polyphosphate hydrolysis by alkaline phosphatase was found to decrease in seawater (Huang et al., 2018b) and the high salinity of seawater led to the strong aggregation of metal (hydr)oxides (Keller et al., 2010), which may

decrease surface area for polyphosphate adsorption and hydrolysis. P XANES (Figure 4.5c-f) indicated that the main P species in ASW experiments are Fe oxide-adsorbed orthophosphate and polyphosphate and ACP solids, similar to the products of polyphosphate hydrolysis in the presence of 1 mM Ca^{2+} at pH 9 (Figure 4.3).

Figure 4.5 Kinetics (a) and first-order fitting results (b) of polyphosphate hydrolysis on ferrihydrite (Ferr), hematite (Hem), goethite (Geot), and lepidocrocite (Lep) in artificial seawater. Panels (c–f) are linear combination fitting analysis of P XANES spectra of 9-day reaction products from polyphosphate hydrolysis on ferrihydrite (c), hematite (d), goethite (e), and lepidocrocite (f) in artificial seawater. Raw and fitted data are in black solid and red

dotted lines, respectively. Red arrow indicates the post-edge features of amorphous calcium phosphate (ACP).

However, in the solid products, the amount percentage of ACP (11.4–22.5%) in ASW experiments is relatively less than those (1.9–37.4% at pH 7.5 and 17.1–56% at pH 9) in DI water experiments in the presence of 1 mM Ca^{2+}, despite the higher concentration of total Ca^{2+} (10.5 mM) in ASW. Our recent studies found that both Mg^{2+} and Ca^{2+} can enhance the hydrolysis of polyphosphate on Al and Mn oxides in DI water and the enhancement increased with increasing concentrations of Ca^{2+} and Mg^{2+} (Wan et al., 2019a, b). The low content of ACP formed in ASW is possibly due to the increased aggregation of Fe oxides in high salinity ASW, which delayed/prevented the hydrolysis and transformation of polyphosphate into ACP and subsequent transformation to crystalline apatite. The presence of concentrated Mg^{2+} (54.6 mM in ASW) might have stabilized ACP via poisoning of crystal growth propagation for hydroxyapatite and inhibits the transformation of ACP to hydroxyapatite (Hilger et al., 2020).

4.4.5 Mechanisms of polyphosphate hydrolysis and calcium phosphate mineral formation

Fe oxide surface contains some μ-(hydr)oxo bridges that can complex with phosphate ion via bidentate binding (Huang, 2018a; Kim et al., 2011). Previous studies showed that the coordination structure of phosphate groups on ferrihydrite, hematite, goethite, and lepidocrocite is dominantly binuclear bidentate (Elzinga and Sparks, 2007; Kim et al., 2011; Wan et al., 2017b; Wang et al., 2017; Wang et al., 2013b). This coordination structure is similar to the organic phosphate complexation with metal cofactors (e.g., Mn, Fe, Zn, and Ca) in acid and alkaline phosphatase proteins (Huang, 2018a; Rodriguez et al., 2014b; Yong et al., 2014). Such structure makes the coordinated phosphate group prone to attack by proximally surface hydroxyl groups, and Fe oxides were thus reported to mimic the behavior of phosphatase (Huang, 2018a; Wan et al.,

2019b). The presence of Ca^{2+} might perform as a promoted chelating factor that enhances the adsorption density of polyphosphate on Fe oxides (Li et al., 2012b) and as a metal co-factor that complexes with terminal phosphate groups (Yong et al., 2014). These two functions of Ca^{2+} can further increase the hydrolysis reactivity of Fe oxides for polyphosphate. Previous research on alkaline phosphatase showed that two alkaline phosphatases have active sites containing one Fe(III) and two Ca(II) ions for PhoD (Rodriguez et al., 2014b) and two Fe(III) and three Ca(II) ions for PhoX (Yong et al., 2014), and the water molecules bound to Ca(II) could plausibly be activated to catalyze the dephosphorylation of organic phosphate monoesters. Additionally, the competitive adsorption of undegraded/shortened polyphosphate and produced orthophosphate is another key factor leading to the rapid hydrolysis of polyphosphate on Fe oxides, due to more phosphate groups and higher negative charge of polyphosphate molecules. Polyphosphate can be thus viewed as a dissociation ligand to replace the produced orthophosphate from hydrolysis reactive sites, which can further drive the continuous hydrolysis reaction of polyphosphate on Fe oxides. Dissociation of the produced orthophosphate is further demonstrated by the first formation of ACP solids at the beginning of polyphosphate hydrolysis in the presence of Ca^{2+}. The previous studies showed that the homogenous precipitation of Ca^{2+} and orthophosphate preferred to form ACP solids, whereas the co-sorption of Ca^{2+} and orthophosphate on boehmite facilitated the formation of hydroxyapatite (Huang et al., 2018; Li et al., 2012).

The continuously produced orthophosphate can complex with Ca^{2+} to form ternary surface complexes and/or precipitates (Li et al., 2012b; Wan et al., 2016), which decreases the concentrations of both Ca^{2+} and total P in the solution. We thus calculated the saturation state of reaction suspensions using the software PHREEQC (Parkhurst and Appelo, 2013). The saturation indices with respect to hydroxyapatite in four Fe oxide systems after 9-day reaction were at –3.97

to –3.08 for pH 6, 4.03–5.38 for pH 7.5, and 8.48–0.53 for pH 9. This suggests that the reaction suspensions were oversaturated at pH 7.5 and 9 and prone to the precipitation of Ca phosphate minerals. This is consistent with our P XANES results showing high ACP percentages at pH 7.5 and 9 (Figure C.7). For experiments conducted in ASW, the saturation indices for hydroxyapatite are 6.59–8.17, again suggesting the oversaturation for calcium phosphate minerals and can explain the observed ACP phase in the solid products (Figure 4.5).

As hydrolysis and orthophosphate production proceeds, precipitated ACP may gradually transform into crystalline calcium phosphate minerals upon long-term aging. A previous study indicated that the time frame for amorphous to crystalline transformation is depend on solution chemistry (Eanes, 1998). High pH and temperature facilitate the transformation of ACP to crystalline Ca-P phases, whereas the inclusion of inorganic ions (e.g., Mg^{2+}, carbonate, and pyrophosphate) in ACP can delay this transformation (Eanes, 1998). In our study, hydroxyapatite was the main crystalline Ca phosphate formed. Thus we propose that the primary pathway for polyphosphate transformation into crystalline hydroxyapatite at mineral-water interface involves the following steps: 1) Fe oxides have the function as phosphatase enzyme mimics to hydrolyze polyphosphate via one-by-one cleavage of terminal P groups, which is further enhanced in the presence of Ca^{2+}; 2) the produced orthophosphate complexes with Ca^{2+} to rapidly form ACP solids; 3) the formed ACP solids gradually transform into crystalline hydroxyapatite at room temperature and pressure.

The reactivity of Fe oxides for hydrolyzing polyphosphate relates to the surface area and exposed facet for phosphate complexation and hydrolysis. Ferrihydrite and hematite have higher surface areas than goethite and show relatively higher apparent hydrolysis rates. Lepidocrocite has layered structure and may have more reactive sites on the (100) and (001) planes that can catalyze

polyphosphate hydrolysis (Kim et al., 2011). Another possibility is related to the synthesis of lepidocrocite (via oxygenation of Fe(II) solution at neutral pH) that leads to the potential presence of Fe(II) in the structure for the enhanced polyphosphate hydrolysis (Cornell and Schwertmann, 2004). The molecule scale mechanisms of polyphosphate hydrolysis on different Fe oxide phases is beyond the scope of this study and warrants future investigation.

4.5 Geochemical Perspectives

This study reveals the interfacial behaviors (adsorption, hydrolysis, and precipitation) of polyphosphate on environmentally abundant Fe oxides, which is of great relevance to understanding P cycling in aquatic environments. Our findings fill the knowledge gap between polyphosphate transformation and apatite formation, and help explain the rapid diagenesis of polyphosphate and P burial at sediment-water interface (Diaz et al., 2008; Hupfer et al., 2004). Previous research showed that polyphosphate was a key intermediate for regulating the precipitation of fine-grained apatite and P burial at sediment-water interface, though the mechanisms were unclear and microbial activities were proposed to be a possible factor for polyphosphate transformation into crystalline apatite (Diaz et al., 2008; Goldhammer et al., 2010; Schulz and Schulz, 2005). Our results show that abiotic processes at the mineral-water interface could also strongly mediate polyphosphate transformation, providing a new angle for explaining sedimentary P sequestration in geological environments. To our knowledge, this is the first study demonstrating the direct influences of natural minerals in controlling polyphosphate transformation into crystalline calcium phosphate minerals. Future studies are warranted to explore polyphosphate transformation in different geological environments (e.g., fresh water *vs* seawater, presence of organic matter, sedimentary incubation) and further compare the relative contributions of abiotic (e.g., mineral-catalyzed) *vs.* biotic (e.g., enzymatic) processes in mediating

polyphosphate hydrolysis and transformation, in order to further understand the processes influencing the transformation, fate, and bioavailability of complex phosphate-containing molecules in geological environments.

Supplementary Information for Chapter 4 can be found at **APPENDIX C. IRON OXIDE-CATALYZED HYDROLYSIS OF POLYPHOSPHATE AND THE PRECIPITATION OF CALCIUM PHOSHATE MINERLS**.

CHAPTER 5. REVISITING THE ROLES OF MINERALS IN THE PHOSPHORUS CYCLE

5.1 Abstract

Phosphorus (P) is an essential macronutrient for all living organisms. Despite a diversity of P species in natural and biological systems, orthophosphate is the most bioavailable form of P. Mineralization of complex P species (e.g., organic and condense P) into orthophosphate is thus necessary for their cycling and is traditionally considered to be carried out primarily by enzymes. Recent studies suggest that natural minerals can act as abiotic catalysts to facilitate the mineralization of complex P species. However, quantitative comparison between the biotic and abiotic pathways of complex P mineralization is still missing, impeding our capability to assess P cycling in the environment. This study compares the hydrolysis rates of six organic and three condensed phosphates by representative phosphatases (acid and alkaline phosphatases) and metal oxide minerals (hematite, birnessite, and boehmite). Results show that enzymes and minerals have different substrate preferences, as alkaline phosphatase hydrolyzes phosphate monoesters at a faster rate than condense phosphates, whereas minerals show faster rates toward condensed phosphates than phosphate monoesters. Although hydrolysis rates by enzymes (~µM/d) are orders of magnitude higher than that of minerals (~µM/d), normalization considering the natural abundance of enzymes and minerals points to similar contributions of both processes in soil and sediment environments. The results reveal the significance of natural minerals in complex P

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mineralization, one that is traditionally overlooked but with important implications for constraining the biogeochemical cycling of P in the environment.

5.2 Introduction

Phosphorus (P) is an essential element for all life as a structural and functional component of organisms (Karl, 2014; Paytan and McLaughlin, 2007), and is generally viewed as a limiting macronutrient in aquatic environments (Ruttenberg, 2014; Thingstad et al., 2005). As one of the most abundant element on Earth, P presents an average crustal abundance of 0.1% by weight (Canfield et al., 2005). Total P concentration was estimated to be 170–21,000 mg kg^{-1} in surface and subsurface environments (e.g., soils and sediments) (Klein et al., 2019). Different P-containing compounds are found in natural environments, including orthophosphate, organic phosphate esters, condensed phosphates, phosphite, and phosphonates, etc. (Figueroa and Coates, 2017; Paytan and McLaughlin, 2007; Van Mooy et al., 2015). Orthophosphate is generally considered as the most common P species and the only P species that can be directly and effectively used by most living organisms, though recent studies showed that some microorganisms can also uptake low weight dissolved organic phosphates and reduced forms of P (Karl, 2014; Van Mooy et al., 2015). Beside orthophosphate, soils and sediments also contain a lot of other common and important P forms, such as organic phosphates (9.3–69.2% of total P) and condensed phosphates (0.4–13.0% of total P) (Diaz et al., 2008; Liu et al., 2018; Recena et al., 2018; Wan et al., 2019b). The main forms of organic and condensed phosphates are phosphate monoesters (e.g., glycerophosphate, glucose 6-phosphate, adenosine 5'-monophosphate, and inositol phosphates), phosphate diesters (e.g., nicotinamide adenine dinucleotide), and phosphoanhydrides (e.g., adenosine 5'-triphosphate, pyrophosphate, tripolyphosphate, and long chained polyphosphate) (Baldwin, 2013; Huang et al., 2017; Wan et al., 2019a). The cycling and bioavailability of these P compounds have significant

impacts on global biogeochemical processes, marine primary productivity, terrestrial biological productivity, and agricultural production (Ruttenberg, 2014; Van Mooy et al., 2015).

In natural ecosystems, the movement of P among different reservoirs is regulated by various biotic (e.g., mineralization, uptake, release, and assimilation) and abiotic processes (e.g., adsorption, desorption, dissolution, and precipitation) (Defforey and Paytan, 2018; Ruttenberg, 2014). For example, in open oceans, ~90% of gross primary production is supported by local P recycling from organic and condensed phosphates, and microorganisms are primarily responsible for P assimilation and remineralization (Karl, 2014). In soils, biological agents, such as fine roots, bacteria, and fungi, determine the strict retention of P within surface soils, whereas geological agents such as iron (Fe) and aluminum (Al) oxide minerals control the movement and transport of dissolved inorganic and organic phosphates in subsoils and eventually to stream waters (Wood et al., 1984). To predict P bioavailability and understand global P cycling, we need to understand the main abiotic and biotic processes controlling the transformation of different P compounds into orthophosphate and to quantify the relative contribution of these processes under various environmental settings.

Due to the high specificity and high reaction rates, enzymatic hydrolysis of phosphate esters and condensed phosphates – a biotic process – is traditionally viewed as the main mechanism for the degradation of complex P molecules to produce bioavailable orthophosphate (Huang et al., 2018b; Olsson et al., 2012). Although natural oxide minerals are often considered as an important abiotic factor controlling global P cycling, their specific roles are yet to be fully understood (Klein et al., 2019; Wan et al., 2019b; Wood et al., 1984). Iron and Al oxide minerals have large surface areas and highly positive surface charges, and are able to uptake a large amount of phosphates on their surfaces via adsorption, incorporation, and/or surface precipitation. Thus these minerals are

traditionally viewed as a sink of P in aquatic environments and a fixation of P in soil environments (Ruttenberg and Sulak, 2011; Wang et al., 2016b; Yan et al., 2014a). Manganese (Mn) oxides are negatively charged at circumneutral pHs and thus generally have a low affinity for phosphate adsorption. Recently, a few studies showed that natural Fe, Al, and Mn oxides can act as abiotic catalysts and enable the rapid hydrolysis of phosphate esters and phosphoanhydrides into orthophosphate (Huang, 2018b; Klein et al., 2019; Wan et al., 2019a), and the rate of Mn oxide-catalyzed hydrolysis of organic phosphate ester is an order of magnitude higher than Fe and Al oxides (Baldwin et al., 2001). These studies provide a new angle for evaluating the roles of natural minerals in regulating the P cycle. We hypothesize that, in addition to the traditional view of being a *sink* for phosphate, natural minerals can provide a *source* of bioavailable orthophosphate by serving as abiotic catalysts to facilitate the breakdown of complex P compounds. However, despite the previous studies on phosphatase- and mineral-catalyzed hydrolysis of organic phosphate esters and condensed phosphates (Huang et al., 2018b; Huang, 2018b; Wan et al., 2019b), direct quantification and comparison of P mineralization rates via these biotic (enzyme) and abiotic (mineral) pathways are still missing.

This study systematically compares and evaluates the hydrolysis of nine representative P compounds in the presence of two common P enzymes (acid and alkaline phosphatases) and the most common phases of Fe, Al, and Mn oxide minerals (hematite, boehmite, and birnessite, respectively). Selected P-containing compounds (details in Tables 1) include six organic phosphates: β-glycerophosphate (GP), D-glucose 6-phosphate (G6P), adenosine 5′-monophosphate (AMP), adenosine 5′-triphosphate (ATP), β-nicotinamide adenine dinucleotide (NP), and *myo*-inositol hexakisphosphate (IHP), as well as three condensed phosphates: pyrophosphate (P_2), tripolyphosphate (P_3), and polyphosphate (with 45 P unit, P_{45}). To our

knowledge, the results provide the first quantitative comparison of biotic (enzyme) and abiotic (mineral) hydrolysis rates of these phosphate compounds. ^{31}P nuclear magnetic resonance (NMR) spectroscopy was conducted to determine the preferential hydrolysis of organic phosphate monoester and polyphosphate by enzymes and minerals. By estimating the environmental distribution and abundance of these phosphates and oxide minerals in soils and sediments, we provide an initial assessment of the relative contributions of these biotic and abiotic pathways in governing P transformation and mineralization in different environmental settings.

5.3 Materials and Methods

5.3.1 Materials and reagents

All P-containing compounds were obtained from Sigma-Aldrich, including six organic phosphates: β-glycerophosphate (GP), D-glucose 6-phosphate (G6P), adenosine 5′-monophosphate (AMP), adenosine 5′-triphosphate (ATP), β-nicotinamide adenine dinucleotide (NP), and *myo*-inositol hexakisphosphate (IHP), and three condensed phosphates: pyrophosphate (P_2), tripolyphosphate (P_3), and polyphosphate (P_{45}). Enzyme and mineral-catalyzed hydrolysis experiments were conducted using two common enzymes (acid and alkaline phosphatases, both from Sigma-Aldrich) and three most common Fe, Mn, and Al oxide minerals (hematite, birnessite, and boehmite, respectively). Information on the source, synthesis, and characterization of all materials are in Appendix D. *Text S1* and Table 5.1.

Table 5.1 Label, type, chemical formula, structure, and stock solution concentrations of the organic and condensed phosphate compounds used in this study.

Label	Type	Compound and chemical formula	Structure [a]	Concentration of stock solution [b]
GP	Phosphomonoester	β-Glycerophosphate disodium salt hydrate $(HOCH_2)_2CHOP(O)(ONa)_2 \cdot x H_2O$		50 mM in total P (0.047±0.001%)
G6P	Phosphomonoester	D-Glucose 6-phosphate disodium salt hydrate $C_6H_{11}Na_2O_9P \cdot xH_2O$		50 mM in total P (0.717±0.003%)
NP	Phosphodiester	β-Nicotinamide adenine dinucleotide $C_{21}H_{27}N_7O_{14}P_2 \cdot xH_2O$		40 mM in total P (0.027±0.005%)
AMP	Phosphomonoester	Adenosine 5′-monophosphate monohydrate $C_{10}H_{14}N_5O_7P \cdot H_2O$		50 mM in total P (0.044±0.002%)
ATP	Phosphomonoester	Adenosine 5′-triphosphate disodium salt hydrate $C_{10}H_{14}N_5Na_2O_{13}P_3 \cdot xH_2O$		20 mM in total P (1.245±0.026%)
IHP	Phosphomonoester	Phytic acid sodium salt hydrate $C_6H_6O_{24}P_6 \cdot 12Na$		60 mM in total P (3.325±0.041%) [c]
P_2	Condensed phosphate	Sodium pyrophosphate decahydrate $Na_4P_2O_7$		40 mM in total P (0.394±0.133%)
P_3	Condensed phosphate	Sodium tripolyphosphate $Na_5P_3O_{10}$		20 mM in total P (0.629±0.057%)
P_{45}	Condensed phosphate	Sodium phosphate glass, type 45		40 mg L^{-1} (~45 mM) in total P (0.528±0.051%)

Notes: [a] Structures of GP, G6P, NP, AMP, ATP, IHP, P_2 and P_3 are from manufacturer (*Sigma-Aldrich*).

[b] Percent values in the parentheses indicate the amount of hydrolyzed P in the stock solution after 120-day storage.

[c] 3.325% hydrolysis attributes to pre-existed orthophosphate in IHP salts with 97% purity.

5.3.2 Hydrolysis experiments

For enzymatic hydrolysis of P compounds, stock enzyme solutions were prepared at 2 units mL^{-1} for acid phosphatase in a MOPS buffer (0.01 M) at pH 6 and for alkaline phosphatase in a HEPES buffer (0.01 M) at pH 8. In 15 mL centrifuge tubes, 8.3 mL deionized (DI) water containing 0.01 M MOPS buffer (pH 6, for acid phosphatase) or 0.01 M HEPES buffer (pH 8, for alkaline phosphatase) was mixed with varied-volume phosphate stock solution (~ 0.2 mL; details in Table 1), 0.5 mL of 2 M NaCl solution, and 1 mL phosphatase stock solution to achieve a total P concentration of ~1 mM and enzyme concentration of 200 units L^{-1}. The two enzymatic experiments were conducted at different pH values (acid phosphatase at pH 6 and alkaline phosphatase at pH 8) for optimal enzymatic activity as suggested by the manufacturer. Both pH values are representative of environment conditions.

To explore the effects of divalent metal cations (Ca^{2+}, Mg^{2+}, Zn^{2+}, and Mn^{2+}), parallel experiments were conducted by adding 0.1 mL of 50 mM metal stock solution to the centrifuge tubes to achieve a metal concentration of 0.5 mM. All experiments were performed in a shaking incubator (225 rpm) at 37 °C. At specific time points, 50 μL solution was sampled and immediately analyzed for orthophosphate production using the phosphomolybdate colorimetric assay (Murphy and Riley, 1962) on an UV–vis spectrometer (Carey 60, Agilent). The observed orthophosphate production is higher than 1000 μM for P_{45} hydrolysis by acid phosphatase, likely because the chemical formula of P_{45} is unknown thus making it difficult to accurately calculate P_{45} concentration as total P. However, this does not affect the reaction trends and conclusions.

For mineral-catalyzed hydrolysis experiments, 500 mL of 2.4 g L^{-1} hematite, birnessite, and boehmite stock suspensions were prepared and equilibrated overnight under magnetic stirring. Then, 22.45 mL DI water containing 0.01 M MOPS buffer or 0.01 M HEPES buffer was mixed

with P stock solution (~ 0.75 mL), 0.3 mL of 50 mM Ca^{2+} stock solution, 1.5 mL of 2 M NaCl stock solution, and 5.0 mL of 2.4 g L^{-1} mineral stock suspension in a 50 mL glass bottle to achieve a total P concentration of ~1 mM and mineral concentration of 0.4 g L^{-1}. To explore the effects of metal cations [including control (no metal addition), Mg^{2+}, Zn^{2+}, and Mn^{2+}], parallel experiments were conducted by adding 0.3 mL of 50 mM metal stock solutions to the 50 mL glass bottles to achieve a metal concentration of 0.5 mM (the amount of added DI water was adjusted accordingly to maintain the same total volume of reaction suspension). All reaction suspensions were constantly agitated. At specific time points, 0.5 mL suspension was taken, immediately centrifuged, and filtered through a 0.22-µm Millipore membrane, and 50 µL of the supernatant was used to analyze orthophosphate concentration. To prevent microbial growth, 10 µL of 5 mM sodium azide (NaN$_3$) solution was added to the reaction suspensions at varied time points. The spontaneous hydrolysis of nine phosphates in their stock solution (without any enzyme or mineral addition) over 120-day storage was monitored to evaluate the stability of these compounds (results in Table 5.1) and were considered as no-enzyme/mineral controls. All experiments were performed in duplicate.

5.3.3 Solution ^{31}P NMR Analysis

To compare the preferential hydrolysis of organic and condensed phosphates by phosphatases and minerals, the hydrolysis kinetics of GP and P$_{45}$ were monitored using time-resolved ^{31}P solution NMR spectroscopy. GP and P$_{45}$ were chosen as representative organic phosphate monoester and polyphosphate in nature.

For enzymatic hydrolysis of co-present GP and P$_{45}$, 498.5 µL DI water containing 0.01 M MOPS buffer (pH 6, for acid phosphatase) or 0.01 M HEPES buffer (pH 8, for alkaline phosphatase) was mixed with 17.5 µL P$_{45}$ stock solution, 14 µL GP stock solution, 100 µL D$_2$O,

and 70 µL phosphatase stock solution in the NMR sample tubes to achieve a total P concentration of 2 mM with ~1 mM P_{45} (as total P) and ~1 mM GP. NMR spectra were collected immediately after the addition of phosphatase stock solution and at varied time points at room temperature (22 °C).

For mineral-catalyzed hydrolysis of co-present GP and P_{45}, 12.9 mL DI water containing 0.01 M MOPS buffer or 0.01 M HEPES buffer was mixed with 0.5 mL P_{45} stock solution, 0.4 mL GP stock solution, 0.2 mL of 50 mM Ca^{2+} stock solution, 1 mL of 2 M NaCl stock solution, and 5.0 mL of 1.6 g L^{-1} mineral stock suspension in a 50 mL glass bottle to achieve a mineral concentration of 0.4 g L^{-1} and total P concentration of 2 mM with 1 mM P_{45} (as total P) and 1 mM GP. At specific time points, 1.5 mL suspension was taken, immediately centrifuged, and filtered through a 0.22-µm Millipore membrane, and the supernatant was analyzed by solution ^{31}P NMR spectroscopy.

Solution ^{31}P NMR spectra were collected on a Bruker AMX 400 MHz spectrometer operated at 162 MHz and 297 K. Parameters of 90° pulse width, 6.5k data points (TD) over an acquisition time of 0.51 s, and relaxation delay of 15 s were applied. Chemical shift was calibrated using 85% H_3PO_4 as the external standard. At least 256 scans (~0.5 h) were collected for each sample.

5.4 Results and Discussion

5.4.1 Overall hydrolysis trends

All enzymes and minerals were able to hydrolyze the studied P compounds to different extents (Figure D.1). Due to the large matrix of P compounds, enzymes, and minerals studied, we first show the representative kinetic curves of polyphosphate (P_{45}) hydrolysis by acid and alkaline phosphatases, hematite, boehmite, and birnessite (Figure 5.1). Acid phosphatase shows a higher

rate for P_{45} hydrolysis than alkaline phosphatase, and both enzymes show much higher rates than hematite, boehmite, and birnessite. For the three minerals, hematite shows a high rate and extent for P_{45} hydrolysis at pH 8.0 after 17–24 days, followed by birnessite and boehmite.

Figure 5.1 *Hydrolysis of polyphosphate (P_{45}) by phosphatases and minerals.* **The concentrations of polyphosphate (as total P), enzymes, and minerals are ~1000 µM, 200 unit L^{-1}, and 0.4 g L^{-1}, respectively. Note that the two enzymatic experiments were conducted at different pH values (acid phosphatase at pH 6 and alkaline phosphatase at pH 8) for optimal enzymatic activity, but pH values are representatively of environmental conditions.**

We then further compare the hydrolysis of six organic phosphates (GP, G6P, NP, AMP, ATP, and IHP) and three condensed phosphates (P_2, P_3, and P_{45}) by phosphatases and minerals (Figure D.1) at pH 6.0 and 8.0. Acid and alkaline phosphatases can rapidly hydrolyze organic phosphate monoesters and condensed phosphates, except for IHP (a phosphate monoester) (Figures D.1a-f). Due to the steric structure, IHP resists rapid hydrolysis and a previous study indicated that IHP hydrolysis is limited to enzymatic reactions (Jarosch et al., 2019). Acid phosphatase can slowly hydrolyze NP (a typical phosphodiester), which cannot be hydrolyzed by alkaline phosphatase (Figure D.1a and d). For the three mineral systems, all phosphates are slowly hydrolyzed, and after 17–24 days the hydrolysis extents of phosphoanhydrides (ATP, P_2, P_3 and

P$_{45}$) are much higher than those of phosphate monoesters (GP, G6P, AMP, and IHP) and diester (NP) (Figures D.1g-o). For example, the hydrolysis extents of phosphoanhydrides and phosphate esters are in the range of 11.5–93.1% and 1.5–30.1%, respectively.

5.4.2 Kinetic fitting of hydrolysis rates

To quantitatively compare the hydrolysis rates of different experiments, we performed kinetic fitting for orthophosphate production during enzyme and mineral-catalyzed hydrolyses of organic and condensed phosphates. Mineral-catalyzed hydrolysis curves were previously fitted as first order kinetic reaction (Huang et al., 2018b; Wan et al., 2019b) and enzymatic hydrolysis was fitted using the Michaelis–Menten equation (Jarosch et al., 2019). Here we use linear fitting of the initial reaction stage to avoid complicated mechanism discussions and provide an initial direct comparison of these systems. The fitted hydrolysis rates are summarized in Figure 5.2 and presented in Figure D.2. The linear fitting parameters (Table D.1) show that NP and IHP have very low hydrolysis rates (a few $\mu M\ hr^{-1}$) by both acid and alkaline phosphatases, as compared to the higher rates (hundreds of $\mu M\ hr^{-1}$) of other seven phosphates. Acid phosphatase can hydrolyze three condensed phosphates at higher rates than alkaline phosphatase (Figure 5.2a). For example, the hydrolysis rate of P$_2$ by acid phosphatase (1281.12 ± 129.0 $\mu M\ hr^{-1}$) is much higher than that by alkaline phosphatase (203.72 ± 15.2 $\mu M\ hr^{-1}$) (Table D.1). In contrast, for organic phosphate monoesters, alkaline phosphatase has a higher hydrolysis rate than acid phosphatase (Figure 5.2a).

As shown in Figure D.2, the three minerals have low hydrolysis rates (a few $\mu M\ d^{-1}$) for five organic phosphate esters, except for ATP which contains both P−O−P (phosphoanhydride) and P−O−C (phosphate ester) bonds (Figures 5.2b-d; Table D.1). ATP can be viewed as an organic condensed phosphate (its structure in Table 5.1). The hydrolysis rates of three condensed phosphates are an order of magnitude higher than those of phosphate monoesters and are affected

by mineral type and solution pH. For the hydrolysis of condensed phosphate, birnessite has a higher rate at pH 6 than 8, while hematite and boehmite have higher rates at pH 8.0 than pH 6.0 (Figures 5.2b-d). The hydrolysis rates roughly follow the order of birnessite > hematite > boehmite at pH 6.0 and hematite > birnessite > boehmite at pH 8.0 (Table D.1). Regardless of solution pH, birnessite and hematite displays an order of magnitude higher hydrolysis rates (tens of μM d^{-1}) than boehmite (a few μM d^{-1}). Specifically, birnessite, hematite, and boehmite can hydrolyze P_{45} at 65.63, 19.17, and 4.07 μM d^{-1} at pH 6.0, and 45.12, 59.93, and, 4.99 μM d^{-1} at pH 8.0, respectively (Table D.1).

Figure 5.2 *Fitted rates of the initial linear range for enzyme and mineral-catalyzed hydrolyses of organic and condensed phosphates.* **(a) Hydrolysis rates by acid and alkaline phosphatase**

at pH 6 and 8, respectively. (b-d) are the hydrolysis rates on hematite, birnessite, and boehmite, respectively, at both pH 6 and 8. Error bars indicate standard deviation (SD) of the replicate experiments.

5.4.3 Preferential hydrolysis of phosphates by enzymes and minerals

Based on the differences in hydrolysis rates of organic and condensed phosphates, we hypothesize that phosphatases and minerals might have different preferences to hydrolyze phosphate monoesters and condensed phosphates. To test this hypothesis, we performed time-resolved solution ^{31}P NMR experiments to investigate the hydrolysis of co-represent GP and P_{45} by enzymes or minerals. GP and P_{45} were chosen as representative organic phosphate monoester and polyphosphate in natural environments.

In the presence of acid phosphatase at pH 6, we observed the simultaneous decrease in intensity for the chemical shifts of GP (at 1.68 ppm) and P_{45} middle P groups (at -21.8 ppm), as well as an accompanied increase in the intensity of orthophosphate chemical shift (at 0.44 ppm) (Figure 5.3a), suggesting no obvious preference of acid phosphatase in hydrolyzing organic and condensed phosphates. In contrast, a different trend was observed for alkaline phosphatase (Fig. 3b). During the first 4 hours of the hydrolysis, the chemical shift of GP ($\delta_P = 3.76$ ppm) gradually disappears as the chemical shift of orthophosphate ($\delta_P = 2.25$ ppm) increases to become a dominate P species, while the chemical shift of P_{45} middle P groups ($\delta_P = -21.66$ ppm) shows limited change within this time frame. After the complete disappearance of GP chemical shift (at 8.5 hours), P_{45} was rapidly hydrolyzed to produce orthophosphate (Figure 5.3b). These results indicate that alkaline phosphatase mainly hydrolyzes organic phosphate monoester in the co-presence of condensed phosphate (Figure 5.3b). This is also consistent with the result that alkaline phosphatase

has a higher hydrolysis rate for organic phosphate monoesters than phosphoanhydrides (Figure 5.2a).

Figure 5.2 *Time-resolved ^{31}P solution NMR spectra showing P speciation dynamics during the hydrolysis of co-present glycerophosphate (GP) and polyphosphate (P_{45}) by phosphatases and minerals.* **(a) acid phosphatase at pH 6, (b-e) alkaline phosphatase, birnessite, hematite, and boehmite at pH 8, respectively. Purple, blue, and yellow vertical bars indicate the characteristic chemical shifts of GP, orthophosphate, and polyphosphate terminal P groups, respectively. The exact position of these peaks are affected by solution pH.**

In the presence of minerals, as the reaction proceeds, the intensity of GP chemical shift show very limited change, and the chemical shift of orthophosphate gradually appears and dominates the NMR spectra due to P_{45} hydrolysis (Figures 5.3c-e and D.3). As shown in the batch experiments (Figures D.1g-o), the three minerals present limited reactivity and very slow rates toward hydrolyzing organic phosphate monoesters. Although it is difficult to quantify the amount of GP hydrolyzed into orthophosphate using NMR spectroscopy, we hypothesize minimal GP hydrolysis by minerals in the co-presence of P_{45} and that the three minerals prefer to hydrolyze P_{45}, due to the high negative charge of P_{45} and its abundant phosphate groups that can compete to occupy the adsorption sites on mineral surface. This result is consistent with the observation that hematite, birnessite, and boehmite show higher activity in hydrolyzing phosphoanhydrides than organic phosphate esters (Figures 5.2c-d). Overall, these results indicate the different preferences of phosphatases and minerals toward hydrolyzing organic phosphate monoesters and condensed phosphates in nature.

5.4.4 Extrapolation to soils and sediments

With the normalized rate data (Table D.2), we can then evaluate the relative contributions of phosphatase and mineral catalyzed processes in different environmental settings. We compiled literature data on the contents and distribution of phosphatases and oxide minerals in soils and sediments (Appendxi *ref 6-31*) (Figure D.4 and Table D.3). We found that alkaline phosphatase activity distributes in a wide range of 0.00005–12.02 unit g^{-1} in sediments and 0.00018–2.27 unit g^{-1} in soils (Figure D.4a and Table D.3). Acid phosphatase activity is relatively lower and is in the range of 0.00004–1.17 unit g^{-1} in soils and sediments. The content distribution of Fe/Al/Mn oxides is around 0.0001–0.7364 g g^{-1} in soils/sediments, and the contents of Fe/Al/Mn oxides in soils are much higher than those in sediments (Figure D.4b and Table D.3). By multiplying the

environmental contents of phosphatases (unit g^{-1}) and minerals (g g^{-1}) by the hydrolysis rates (μmol d^{-1} $unit^{-1}$ for phosphatases or μmol d^{-1} g^{-1} for minerals) for each studied P species, we obtained the hydrolysis contributions for each phosphate-enzyme/mineral system in soils and sediments with an uniform unit of μmol d^{-1} g^{-1}. This normalization allows us to directly compare the relative significance of organic and condensed phosphate hydrolysis by phosphatases and minerals in soils and sediments (Figure 5.4).

Using this approach, alkaline phosphatase is estimated to significantly contribute to the hydrolysis of organic phosphate monoesters (GP, G6P, and AMP) and the associated hydrolysis rates can reach hundreds of μmol d^{-1} g^{-1} (Figure 5.4). Due to the high stability of NP and IHP, their hydrolysis rates by phosphatases and minerals are relatively low at a few μmol d^{-1} g^{-1} (Figures 5.4g and h). The hydrolysis rates of other seven P compounds by acid phosphatase, birnessite, and hematite are similar and estimated to be tens of μmol d^{-1} g^{-1} (Figure 5.4). Meanwhile, we note that the contribution of alkaline phosphatase to the hydrolysis of three condensed phosphate is similar to or even lower than that of acid phosphatase, birnessite, and hematite (Figures 5.4g-i) under different conditions. Especially for long chain polyphosphate (P_{45}), hematite and birnessite may dominate their hydrolysis in soils with hydrolysis rates of 110.3 and 32.9 μmol d^{-1} g^{-1} at pH 8.0, respectively (Figure 5.4i). Regardless of the P species considered, boehmite consistently shows the lowest activity toward hydrolyzing organic and condensed phosphates (Figure 5.4). To our knowledge, this is the first direct quantitative comparison of the relative contributions of environmental phosphatases and minerals on P mineralization and provides the basis for further exploration on the critical roles of natural minerals in global P cycling.

Figure 5.4 *Estimated range of hydrolysis rates for organic and condensed phosphates by acid/alkaline phosphatases and Mn/Fe/Al oxide minerals in soils and sediments.* **(a-i) GP, G6P,**

ATP, P₂, P₃, AMP, NP, IHP, and P₄₅, respectively. The contents and distribution of these phosphatases and minerals are based on literature data (Appendix D. *ref 6-31*) and summarized in Table D.3 and Figure D.4. Error bars are calculated based on the standard deviation (SD) from the replicate hydrolysis experiments.

5.4.5 Hydrolysis mechanisms

The phosphorylation activity of acid and alkaline phosphatases is attributed to the metal cofactors in the structure of enzyme proteins that can form bidentate binding to terminal phosphate groups of the substrate P molecules (Lassila et al., 2011; Yong et al., 2014). For instance, acid phosphatase has an active site containing one Fe(III) and one Mn(II) (Rodriguez et al., 2014a) and alkaline phosphatase (PhoA) active site contains two Zn(II) ions (Sunden et al., 2017). Recent studies on the crystal structure of two new alkaline phosphatases (PhoD and PhoX) revealed that their active hydrolysis sites contain one Fe(III) and two Ca(II) ions in PhoD (Rodriguez et al., 2014a) and two Fe(III) and three Ca(II) ions in PhoX (Yong et al., 2014). Interestingly, mineral surfaces also contain large amounts of surface metal atoms that might serve similar functions as metal cofactors in phosphatase proteins via the formation of inner-sphere complexes with the terminal phosphate groups of the substrate P molecules (Baldwin et al., 1995; Huang, 2018b; Wan et al., 2019b). The presumed transition state geometry of bidentate binuclear complexes at the mineral-water interface makes adsorbed phosphate easily attacked by proximally coordinated hydroxyl groups [e.g., μ-(hydr)oxo bridges], leading to the hydrolysis of phosphate esters and phosphoanhydrides (Baldwin et al., 1995; Huang, 2018b; Wan et al., 2019b).

For the enzymatic dephosphorylation, the biological molecules in the phosphatase structure can promote the dissociation of newly formed orthophosphate from the metal cofactor-phosphate complex (Coleman, 1992). However, in mineral system, competitive adsorption onto the mineral surfaces exist between the initial organic/condensed phosphate molecules and produced

orthophosphate. The weak competitive ability of organic phosphate monoesters determines that they cannot replace the formed and coordinated orthophosphate. Although IHP has a high negative charge and high adsorption capacity, the coordination geometry of surface IHP complexes may resist to be attacked by a nucleophile. We note that the rate of mineral-catalyzed hydrolysis for condensed phosphates is negatively correlated to the chain length (Figures 5.2b-d). The high hydrolysis rate of long chained condensed phosphates can be attributed to their higher negative charge and stronger competitive adsorption ability, which promote the dissociation of the produced orthophosphate from the mineral surface and allows continuous proceeding of the hydrolysis reaction (Wan et al., 2019a). The hydrolysis extents and rates of organic and condensed phosphates at the mineral-water interface is thus determined by the competitive adsorption and dissociation of newly formed orthophosphate. These results reveal the similarity and differences of enzyme- and mineral-catalyzed pathways toward the hydrolysis of organic phosphate esters and condensed phosphates (Klein et al., 2019; Olsson et al., 2012; Wan et al., 2019b).

To further explore the effects of extrinsic metal ions on phosphatase and mineral hydrolysis, we conducted hydrolysis experiments of ATP by acid and alkaline phosphatases, hematite, birnessite, and boehmite with or without the presence of 0.5 mM metal cations (Ca^{2+}, Mg^{2+}, Zn^{2+}, and Mn^{2+}) at pH 6 and 8 (Figure D.5). ATP has both phosphoanhydride and phosphate ester and can be readily hydrolyzed by phosphatases and minerals (Figure 5.2). Metal cations have limited impacts on ATP hydrolysis by acid phosphatase at pH 6 but inhibition effects were observed for alkaline phosphatase at pH 8 (Figure D.5a-b), consistent with previous studies (Hoehamer et al., 2005; Huang et al., 2018b; Wu et al., 2013). Thus these extrinsic metal cations cannot directly improve the hydrolysis reactivity of metal co-factors in phosphatase active sites. However, at pH 8, metal cations may strongly complex with the phosphate groups of ATP molecules and inhibit

the coupling of ATP terminal phosphate group with the active sites of alkaline phosphatase, subsequently reducing the enzymatic catalysis reaction (Huang et al., 2018b). Meanwhile, we observed a promotion effect of metal cations on ATP hydrolysis by hematite and birnessite at different levels and such effect is more obvious at pH 8 than pH 6 (Figures D.5c-f), as the stronger co-sorption and/or complexation of metal cations and phosphate groups on minerals at pH 8 increases the chance of hydrolysis reactions for these phosphate molecules (Li et al., 2012b; Wan et al., 2019b; Wan et al., 2017b). In boehmite suspensions with 0.5 mM metal cations, the slight enhancement in ATP hydrolysis rate is likely due to the already low reactivity of boehmite for ATP hydrolysis (Figures D.5g-h). The results of metal cation experiments indicate the different effects of metal cations on ATP hydrolysis by phosphatases and minerals, and such effects are influenced by the types of enzymes, minerals, metal type, and solution pH, which warrant future research.

5.4.6 Abiotic and biotic pathways in P cycling

It is generally accepted that P is biologically conserved in the surface soils by close coupling between biological decomposition and uptake, and that natural minerals (especially the abundant and high reactivity Fe- and Al-oxides) function as geochemical media that regulate the constant and low-level losses of dissolved P from subsurface soils (Wood et al., 1984). Specifically, microbial activities significantly contribute to the biological decomposition of complex phosphate molecules (Nannipieri et al., 2011). For example, as the typical extracellular enzymes, acid and alkaline phosphatases rapidly catalyze the breakage of phosphate ester bonds in organic phosphate monoesters and terminal phosphate groups in phosphoanhydrides, leading to the hydrolysis of organic and condensed phosphates and production of orthophosphate (Huang et al., 2018b; Jarosch et al., 2019). At the solid/water interfaces in soils and sediments, Fe and Al oxides are commonly

considered to be a main P fixation pathway via surface adsorption or surface precipitation of dissolved phosphates (Wood et al., 1984; Yan et al., 2014a). Thus, it is commonly accepted that, in the global P cycle, microbes or plants undertake a biological role by regulating the *degradation* (e.g., hydrolysis) of complex P compounds and *production* of orthophosphate (e.g., mineralization), whereas natural minerals play a geochemical role in controlling the *sequestration* and *fixation* (e.g., adsorption, precipitation) of environmental phosphates (Defforey and Paytan, 2018). However, the results from this study suggest that natural minerals can play significant roles in catalyzing the *degradation* of complex P compounds and *production* of orthophosphate, thus we further evaluate the relative contributions of enzymes vs minerals on these specific roles in environmental settings (Fig. 4 and below).

Phosphatases and minerals possess different hydrolysis abilities toward organic phosphate esters and condensed phosphates (Figures 5.1-2) and selective reactivity for different phosphate substrates (Figure 5.3). Acid and alkaline phosphatases can hydrolyze phosphate monoesters (except for IHP) and condensed phosphates at high rates (Figure 5.1a). A possible explanation is that the unit concentration of the enzymes used in this study is much higher than that in natural environments. By normalizing the hydrolysis rates of phosphatase and mineral processes to include the environmental contents and activity of these phosphatases and minerals, the contribution of mineral-catalyzed phosphoanhydride hydrolysis is estimated to be comparable to enzymatic processes in soils and sediments with highly mineral contents (Figure 5.4). Thus mineral-catalyzed hydrolysis as an abiotic process might play significant yet previously overlooked roles in the transformation of phosphoanhydride compounds (e.g., ATP, pyrophosphate, and polyphosphate) in the environments (especially soils). Specifically, birnessite and hematite show higher hydrolysis activity for phosphoanhydrides (ATP, P_2, P_3, and P_{45}) than organic phosphate esters (Figure 5.4).

Boehmite consistently shows low hydrolysis reactivity for all nine phosphates tested, which may be attributed to its low interfacial reactivity. The difference in mineral crystal structure might affect the reactivity of oxide minerals toward hydrolyzing different phosphates, and future studies should explore the specific structure of varied phosphate compounds at the mineral-water interface to provide detailed mechanistic explanations.

In this study, we reveal the critical roles and significant contributions of environmental metal oxide minerals in the degradation and mineralization of organic and condensed phosphates. This provides a new pathway for environmental P transformation and new insights for understanding global P cycle. For instance, in the surface layers of lake and marine sediments where the rapid transformation of condensed phosphate into orthophosphate occurs during diagenesis, mineral-catalyzed hydrolysis may play an important role in the mineralization and transformation of condensed phosphates or other phosphate species (Diaz et al., 2008; Hupfer et al., 2004). Using isotopic dilution approaches, a previous study indicated that typical basal gross organic P mineralization rates range between 0.003 and 0.08 μmol P d^{-1} g^{-1} in arable soils, but the rates can be up to 0.4 μmol P d^{-1} g^{-1} in grassland and forest soils (Bünemann, 2015). The author also pointed out that soil organic P mineralization rates determined by phosphomonoesterase activity measurements are one to two orders of magnitude greater than those determined by isotopic dilution. Our calculated values based on laboratory-controlled experiments are slightly larger than these observed in soils and sediments, likely due to the influences of natural complex matrices (e.g., pH variation, different solid composition, the presence of competitive anions, organic matters, and contaminants). Nonetheless, this study provides a first quantitative comparison of biotic and abiotic contributions to the transformation and mineralization of organic and condensed phosphates. Our results are of significance to broaden the current knowledge on

the roles of enzymes and minerals in P transformation and cycling via biotic and abiotic controls in natural systems. Related research should be conducted in the future to provide a mechanistic explanation for surface-catalyzed phosphate hydrolysis/degradation at molecular level and further explore the critical roles of natural minerals in P remineralization and cycling in the environments.

Supplementary Information for Chapter 5 can be found at **APPENDIX D. REVISITING THE ROLES OF MINERALS IN THE PHOSPHORUS CYCLE**.

CHAPTER 6. MINERAL AND ENZYME FACILITATED POLYPHOSPHATE TRANSFORMATION AND PHOSPHORUS SEQUESTRATION IN MARINE SEDIMENT

6.1 Abstract

Despite the critical roles of oceanic phosphorus (P) cycle in marine primary productivity, the mechanisms leading to P burial as authigenic apatite are not completely understood. Authigenic apatite formation is kinetically inhibited under oceanic conditions. A possible explanantion for apatite sedimentary burial flux is that apatite minerals are precipitated from exogenous polyphosphate intermediates as fine-grained particles. This study investigated mineral- and enzyme-catalyzed polyphosphate mineralization and transformation into calcium (Ca)-phosphate minerals in mesocosm incubations of sediments amended with different minerals and enzymes. The evolution of P speciation is analyzed by a variety of state-of-the-art techniques, such as P K-edge X-ray absorption near edge structure (XANES) spectroscopy, synchrotron X-ray diffraction (XRD), and scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDX). The extents and rates of polyphosphate hydrolysis in sediment incubation followed the order of alkaline phosphatase > acid phosphatase ≥ birnessite > hematite > boehmite ≈ raw sediment. Additionally, regardless of sediment sterilization state and method, the trends and extents of orthophosphate production were similar among the five treatments (no treatment, autoclaved, high temperature, UV-light, and NaN_3), suggesting that microbial activity had limited

impacts on polyphosphate hydrolysis under the experimental condition and duration. Upon polyphosphate hydrolysis in sediment incubations, amorphous calcium phosphate (ACP) solids formed and did not transform into crystalline Ca-phosphate minerals due to the high Mg^{2+} content. For parallel experiments where Mg^{2+} was removed from the reacting suspensions, equilibrium P concentrations were significantly lower and the formed ACP solids eventually transformed into hydroxyapatite during the late stage of sediment incubations. Comparing the incubations of surficial and deep sediments, no obvious difference in solid P speciation (mainly as adsorbed P species and ACP solids) was observed in the products. The rates of mineral-catalyzed polyphosphate hydrolysis in deep sediments were relatively higher than those in surficial sediments and the difference was mainly attributed to mineralogical difference of these two sediments. This study advances our current understanding of marine P burial under the influences of mineralogical and biological controls and may help explain the widespread occurrence of crystalline Ca-phosphate minerals in marine sedimentary environments.

6.2 Introduction

Phosphorus (P) is an essential but limiting macronutrient for most living (micro)organisms (Paytan and McLaughlin, 2007; Ruttenberg, 2014). In the oceans, P limitation significantly impacts primary productivity across vast geographical areas over both modern and geological timescales, thus controlling biogeochemical cycles of other major elements and influencing global climate (Benitez-Nelson, 2000). Despite the critical roles of the marine P cycle in global biogeochemical processes, the mechanisms leading to P removal as authigenic apatite from the oceans are still not fully understood. A variety of calcium (Ca)-phosphate minerals are produced by geological (igneous apatite), geochemical/geomicrobiological (phosphorite), and biological (biological apatite) processes in various environments (Omelon and Grynpas, 2011; Wang and

Nancollas, 2008). Marine P sequestration via authigenic apatite formation in the sediments is a major pathway for P removal from the oceans (Diaz et al., 2008). However, because authigenic apatite formation is kinetically inhibited under oceanic conditions, the abundant occurrence of apatite minerals in marine sediments has been a mystery for more than a century (Brandes et al., 2007; Diaz et al., 2008).

A possible explanation for apatite marine burial flux is that apatite minerals are precipitated from exogenous polyphosphate intermediates as fine-grained particles (Diaz et al., 2008; Omelon and Grynpas, 2008). Polyphosphate is a polymer of P with at least three phosphate groups jointed by phosphoanhydride (O–P–O) bonds and commonly occurs in chain structure in natural environments (Kornberg et al., 1999; Rao et al., 2009). Polyphosphate is widely found in nature and represents 1–13% of total P in planktonic organisms (Diaz and Ingall, 2010; Rao et al., 2009), the dissolved and particulate pools of seawater (Martin et al., 2014; Paytan et al., 2003b) and marine sediments (Sannigrahi and Ingall, 2005), 1.5–11.4 % of total P in lake sediments (Hupfer et al., 2004), and 0.4–7% of total P in soils (Ebuele et al., 2016). A portion of the biologically internal polyphosphate is released to aquatic environments during common cell events such as extracellular release, lysis, and death, thereby becoming biologically external (Diaz et al., 2008). As a consequence, substantial levels of polyphosphate are routinely detected in both dissolved and particulate fractions in freshwater and seawater columns, and lake and marine sediments (Brandes et al., 2007; Diaz et al., 2008; Hupfer et al., 2004; Sannigrahi and Ingall, 2005). Subsequent release of this P reservoir into local environments is considered as a potential P source to induce Ca-phosphate nucleation (Omelon and Grynpas, 2008).

Rapid transformation and mineralization of polyphosphate was found to widely exist in lake and marine surface sediments (0–2 cm) (Diaz et al., 2008; Hupfer et al., 2004). Surficial

sediments contain high contents of natural organic matter (NOM) and environmental oxide minerals, which may facilitate local microbial and mineral activities for aquatic P cycling (Ruttenberg, 2014). Our recent studies revealed the critical roles of enzymes and minerals in mediating polyphosphate mineralization toward the formation of Ca-phosphate minerals (Huang et al., 2018b; Wan et al., 2019a, b; Wan et al., 2020). In the presence of high Ca^{2+} contents (10 and 20 mM), amorphous calcium phosphate (ACP, a precursor of crystalline Ca-phosphate minerals) precipitates were observed during polyphosphate hydrolysis by enzymes, likely due to the increasing concentration of orthophosphate from polyphosphate degradation and the subsequent oversaturation with respect to Ca-phosphate solids (Huang et al., 2018b). Mineral-catalyzed hydrolysis of polyphosphate was observed in the presence of Fe, Al, and Mn oxides, and the hydrolysis extents and rates were promoted in the presence of Ca^{2+}. Ca^{2+} also complexed with the orthophosphate produced from polyphosphate hydrolysis and induced the precipitation of ACP solids (Wan et al., 2019a, b; Wan et al., 2020), which eventually transformed into crystalline hydroxyapatite upon long-term aging (Chapter 4) (Wan et al., 2020). These results from controlled laboratory studies indicate that both abiotic (mineralogical control) and biotic (enzymatic control) factors play critical roles in polyphosphate mineralization and transformation into authigenic Ca-phosphate minerals in marine sediments.

To further explore the effects of enzyme and mineral controls on polyphosphate mineralization and transformation into Ca-phosphate solids under environmental conditions, this study investigates mineral- and enzyme-catalyzed polyphosphate hydrolysis in mesocosm incubations using marine sediments from two different depths: 0.5–2 cm (surficial) and 10–12 cm (deep). Solid phase transformation during the sediment incubation experiments was characterized using P K-edge X-ray absorption near edge structure (XANES) spectroscopy, synchrotron X-ray

diffraction (XRD), and scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy (EDX). Results from this study provide new insights for understanding abiotic and biotic contribution on polyphosphate mineralization at the sediment-water interface, as well as mechanistic explanations for Ca-phosphate mineral formation upon polyphosphate hydrolysis with Ca^{2+} in marine environments.

6.3 Experimental Methods

6.3.1 Materials

Polyphosphate sodium salt (P_{10}) was purchased from Sigma-Aldrich. Hematite, boehmite, and birnessite were chosen as representative Fe, Al, and Mn oxide minerals. Boehmite was purchased from Sasol Germany GmbH. Characterization of P_{10}, as well as hematite and birnessite synthesis and characterization are detailed in Appendix E. *Text S1* and Figure A.1d. Alkaline phosphatase from *Escherichia coli* and acid phosphatase from potato were purchased from Sigma-Aldrich. Artificial seawater (ASW) was prepared according to D1141-98 standard (ASTM D1141-98, 2013; Nguyen Dang et al., 2017) and contains 420 mM NaCl, 28.8 mM Na_2SO_4, 10.5 mM $CaCl_2$, 54.6 mM $MgCl_2$, 9.32 mM KCl, and 2.79 mM $NaHCO_3$.

Sediment samples (Core_Z) were collected at the Saltmarsh Ecosystem Research Facility (SERF) on Skidaway Island, GA on June 26, 2014. Core_Z is located at 31°58'32.19"N, 81°1'55.56"W, on the unvegetated bank of a small intertidal creek and was collected using a hand corer. Sediments collected from the mixtures of 0.5–2 cm (surficial; #1) and 10–12 cm (deep; #2) layers in Core_Z were used to perform mesocosm incubation experiments. Surficial and deep sediment samples were sealed in 50 mL centrifugal tubes and preserved at –20 °C before incubation experiments. Details on sediment collection and P characterizations can be found in Appendix E. *Text S2*.

6.2.2 Sediment incubation experiments

All sediment incubations were conducted in duplicates. Stock sediment slurries were prepared by mixing 2 g of homogenized wet surficial (#1) or deep (#2) sediments with 50 mL artificial seawater. The slurries were dispersed via continuous magnetic stirring (10 Hz) for 2 hours. pH value of the stock slurries was measured using a pH meter (Orion Star A111) and, if needed, manually adjusted to 8.0 ± 0.05 with either 0.05 M HCl or 0.05 M NaOH. The stock slurries of surficial and deep sediments were either unamended (labelled as no treatment) or sterilized using different methods to inhibit microbial activity. Sterilization methods include autoclaving at wet cycle for 20 minutes (labelled as autoclave), heat kill at 100 °C for 20 minutes (labelled as high temperature or HT), UV-light illumination for 1 hour (labelled as UV-light), or chemically treated using 0.1 mL of 2 g L^{-1} NaN$_3$ solution (labelled as NaN$_3$). Mineral and enzyme stock solutions were prepared at 4 g L^{-1} for birnessite, hematite, and boehmite, and 10 unit L^{-1} for acid and alkaline phosphatases, by following our previous methods (Huang et al., 2018b; Wan et al., 2019a). Four Experimental Sets were designed to explore the effects of minerals and enzymes on polyphosphate hydrolysis and transformation in sediment incubation, as detailed below and summarized in Table E.1.

For Experimental Set 1 of mineral- and enzyme-catalyzed polyphosphate hydrolysis, the incubation slurries were prepared by pipetting 5 mL of homogenized stock sediment slurry to 43.4 mL (for enzyme experiments) or 39.4 mL ASW (for mineral experiments), followed by the addition of 1 mL phosphatase stock solution or 5 mL of mineral suspension. After magnetic stirring at 10 Hz for 30 minutes, 0.625 mL of polyphosphate stock solution (~40 mM as total P concentration) was added to the slurry to achieve a polyphosphate concentration of ~500 μM as total P. The final concentrations of minerals and enzymes are 0.4 g L^{-1} and 0.2 unit L^{-1},

respectively. Surficial and deep sediments contain 66.7 and 64.8 wt% total solids, respectively, measured after freeze-drying. Thus the final concentrations of surficial and deep sediments were estimated to be approximate 2.67 and 2.59 g L^{-1}, respectively, in the incubation slurries.

Previous studies have shown that high concentration of Mg^{2+} (0.84-3.34 mM) can inhibit ACP transformation into hydroxyapatite (Hilger et al., 2020). Given that the Mg^{2+} concentration in ASW is 54.6 mM, we designed Experimental Set II to investigate the effect of Mg^{2+} on ACP transformation and apatite formation. In these experiments, polyphosphate hydrolysis was investigated using surficial sediments amended with birnessite, hematite, acid phosphatase, or alkaline phosphatase in an ASW matrix that contains no Mg^{2+} (Table E.1).

Experimental Set III focused on mineral-catalyzed polyphosphate hydrolysis in deep sediments (10–12 cm) in comparison to the surficial sediments (0.5–2 cm). We only tested polyphosphate hydrolysis in the presence of minerals (birnessite, hematite, and boehmite) since mineral-catalyzed hydrolysis is comparable to enzymatic ones in surficial sediment incubations (Explanations in *Section 6.3.2*).

Control Experiments were designed to study polyphosphate hydrolysis by unamended raw sediments with no treatment or treated by high temperature and 0.1 mL of 2 g L^{-1} NaN_3 ($HT+NaN_3$) sterilization (Table E.1).

The preparation of Experimental Set II/III and control experiments were the same as Experimental Set I. 10 µL of NaN_3 solution was periodically added to the pre-treated incubation suspensions to inhibit potential microbial growth over the 150-day incubation. At certain time points, 1 mL aliquot of the suspension was taken, immediately centrifuged, and filtered through a 0.22-µm Millipore membrane. Orthophosphate concentration in the supernatants was determined using the phosphomolybdate colorimetric assay (Murphy and Riley, 1962) on an UV–vis

spectrometer (Carey 60, Agilent). At a few selected time points, 10 mL of the suspensions were centrifuged, and the obtained wet pastes were washed twice with deionized (DI) water and freeze-dried for XRD, SEM-EDX, and P K-edge XANES analyses.

6.3.3 Characterizations of reaction products

Powder XRD measurement was conducted on a Panalytical Empyrean diffractometer (Cu Kα radiation, λ = 1.5418 Å) using zero-background holders (MTI corp.). Scan parameters were 0.04° step size and 9 second/step at 5–80° 2θ. Synchrotron XRD data were collected at Beamline 11-ID-B at the Advanced Photon Source (APS) at 58.6491 keV (λ = 0.2114 Å) and sample-to-detector distances of 100 cm.

SEM-EDX analysis was conducted using a Hitachi SU8230 SEM coupled with Oxford X-Max^N EDX operated at an accelerating voltage of 3 kV. Elemental mappings were performed at 15 kV, 30 μA, and 200 ms dell time.

P K-edge XANES analysis was conducted at Beamline 14-3 at the Stanford Synchrotron Radiation Lightsource (SSRL), Menlo Park, CA. Sample powders were brushed evenly onto P-free Kapton tape and mounted to a 4-slot solid sample plate maintained under He atmosphere. P XANES spectra were collected in fluorescence mode using a PIPS detector at 2100–2485 eV. Energy calibration used $AlPO_4$ (edge position at 2152.8 eV). XANES data analysis used the software Ifeffit (Ravel and Newville, 2005). All spectra were carefully examined for energy calibration, merged, and normalized. Linear combination fitting (LCF) was performed on XANES spectra at energy range of –15 to 50 eV relative to the edge energy. The goodness of fit was evaluated using the residual factor (R-factor) and the fit with smallest R-factor was reported. Details of the P reference compounds for LCF are in Appendix E. *Text S3*.

6.4 Results

6.4.1 Sediment characterization

Representative depth profiles of dissolved O_2, Mn^{2+}, Fe^{2+}, organic-Fe(III) complex [org-Fe(III)], and dissolved FeS_{aq} in the sediment Core_Z are shown in Figure E.1. Sediment profiles showed low concentrations of O_2, Fe^{2+}, and organic-Fe(III) at both 0.5–2 and 10–12 cm depths. However, surficial sediments contained high concentration of Mn^{2+} at 3.5–554 µM and the deep sediments had very low FeS_{aq} concentration (Figure E.1). Sequential P extraction showed that P in surficial and deep sediments had low mobility, with the relative abundance of H_2O and $NaHCO_3$ extractable P fractions at ~22% and the total abundance of NaOH, HCl, and residual P fractions at ~78% of total P (Figure E.2a). Total P contents were 1.07 and 0.93 g g^{-1} (or 33.4 and 29.1 µmol g^{-1}) in surficial and deep sediments, respectively (Figure E.2b). The P fractions in these two sediment samples showed limited differences in P contents and speciation, with the dominant presence of orthophosphate with minor amounts of organic phosphates and polyphosphate (Figure E.2a-c). Powder XRD patterns of freeze-dried sediment samples indicated that the main crystalline phases were kalinite, illite, montmorillonite, and quartz (Figure E.4a), though the presence of other poorly crystalline or amorphous phases could not be ruled out due to the intrinsic limitations of XRD measurement.

6.4.2 Polyphosphate hydrolysis in surficial sediment (0.5–2 cm) incubation in the presence of minerals or enzymes

During the sediment incubation, we observed a gradual increase in orthophosphate during the early stage, which reached a steady state at the middle stage, and slightly decreased toward the late stage of sediment incubation (Figure 6.1). The continuous release of orthophosphate is attributed to the hydrolysis of polyphosphate catalyzed by minerals or phosphatases, which share a similar degradation mechanism via one-by-one cleavage of the terminal phosphate groups in

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polyphosphate molecules, as detailed in our recent studies (Huang et al., 2018b; Wan et al., 2019a, b). The release of orthophosphate into the solution was due to the competitive adsorption on mineral surfaces by short-chained polyphosphate produced during mineral-catalyzed hydrolysis or its replacement by functional ligands in enzyme protein structure (Huang et al., 2018b; Wan et al., 2019a; Wan et al., 2020).

Additionally, regardless of sediment sterilization method, the trends and extents of orthophosphate release were similar among the five different treatments (Figures 6.1a-e), suggesting that microbial activity had no significant effect on polyphosphate hydrolysis under the experimental condition and duration. In the five treatments, the hydrolysis extent followed the order of alkaline phosphatase > acid phosphatase ≥ birnessite ≥ hematite > boehmite in the initial stage of surficial sediment incubation (Figures 6.1a-e). In control experiment (Control Set in Table E.1), the sterilized surficial sediment could also hydrolyze polyphosphate at a low rate, probably caused by the mineral phases (such as clays) in raw sediments (Figures 6.1f and E.4a).

To directly compare polyphosphate hydrolysis by different minerals and enzymes, efforts were taken to quantify the hydrolysis rates. Polyphosphate degradation by minerals or enzymes is a multi-step process, involving one-by-one cleavage of the terminal phosphate groups (Huang et al., 2018b; Wan et al., 2019b), the resulting gradual shortening of polyphosphate chain length, and the subsequent complexation/precipitation of orthophosphate with Mg^{2+} or Ca^{2+} in artificial seawater. Since polyphosphate hydrolysis by minerals and enzymes shares similar reaction mechanism, we used a first-order kinetic model to fit the decrease of polyphosphate (in total P concentration) within the initial stage, similar to our previous approach (Huang et al., 2018b; Wan et al., 2019b). We refer to this change as the apparent hydrolysis rate. The fitting results for apparent hydrolysis rates were generally good with R^2 values of 0.71–0.99 (Figure E.3).

Figure 6.1 Dynamics of orthophosphate production from polyphosphate hydrolysis by birnessite (birn), hematite (hem), boehmite (boeh), acid phosphatase (acid pase), and alkaline phosphatase (alkaline pase) during the incubation with surficial sediment with (a) no pre-treatment of the sediment, or sterilization using (b) autoclave, (c) high temperature (HT), (d)

UV-light, and (e) NaN₃. (f) shows the control experiment using sediments treated with HT+NaN₃ sterilization and no amendment of minerals or phosphatases.

The hydrolysis rates of polyphosphate by each mineral or phosphatase were similar among the five sterilization treatments, consistent with the trends observed for polyphosphate hydrolysis extents (Figure E.3). For example, polyphosphate hydrolysis rate by birnessite was around 0.03 d⁻¹ and that for alkaline phosphatase was around 0.28 d^{-1} for the five treatments. Thus, we averaged the experimental data from all five pre-treatments for polyphosphate hydrolysis in each mineral or phosphatase system in surficial sediment incubation (Figure 6.2). The average rates of polyphosphate hydrolysis were 0.03 ± 0.004 d^{-1} by birnessite, 0.024 ± 0.004 d^{-1} by hematite, 0.011 ± 0.002 d^{-1} by boehmite, 0.053 ± 0.009 d^{-1} by acid phosphatase, 0.261 ± 0.027 d^{-1} by alkaline phosphatase, and 0.009 ± 0.001 d^{-1} by raw sediments (Control Experiment). This result indicated that natural minerals such as birnessite and hematite might likely contribute to polyphosphate mineralization in marine sediments at levels comparable to enzymatic processes depending on the specific mineral phases and contents.

Figure 6.2 Fitted apparent rate constants of polyphosphate hydrolysis in surficial sediment incubation with the amendment of birnessite, hematite, boehmite, acid phosphatase, and

alkaline phosphatase. Control experiment using surficial sediment treated with HT+NaN₃ sterilization and no addition of minerals or phosphatases.

SEM-EDX was applied to investigate the distribution of different elements in 150-day incubation products (Figure 6.3). SEM-EDX maps in Figure 6.3a showed the abundant occurrence of magnesium-aluminum-silicate clays in the incubation product of alkaline phosphatase-surficial sediment system with no pre-treatment. EDX spectra of selected spots (Ca hotpots in Ca Kα1 map) showed different Ca and P contents/distribution. The weight ratios of Ca to P were 32.4 (11.36/0.35 in wt%) for spot 1 and 2.46 (1.85/1.33 in wt%) for spot 2. The high contents of Ca and P indicated the possible presence of residual calcium salts (or stable calcium minerals) at spot 1 and Ca-phosphate solids at spot 2. Meanwhile, SEM-EDX maps of 150-day incubation product of alkaline phosphatase-surficial sediment system with autoclaved sterilization showed the abundant presence of magnesium-aluminum-silicate clays and high contents of Ca and P near Ca and P hotpots (Figure 6.3b). The weight ratios of Ca to P were 1.65 (1.52/0.92 in wt%) for spot 3 and 1.3 (1.57/1.21 in wt%) for spot 4, indicating the potential presence of Ca-phosphate solids (the Ca:P ratio of ACP solids is 1.8) and adsorbed P species at these two spots. The SEM-EDX results suggested the potential formation of bulk Ca-phosphate solids after polyphosphate hydrolysis in surficial sediment incubation.

Figure 6.3 SEM images, elemental maps (upper right small panels), and EDX spectra (lower panels) of surficial sediment samples after 150-day incubation in the presence of 0.2 unit L⁻¹ alkaline phosphatase in artificial seawater. (a) No pre-treatment of the sediment. (b) surficial sediment was autoclave sterilized before incubation.

We then applied P K-edge XANES to analyze solid P speciation of incubation products (Figure 6.4). In both no treatment and autoclave sterilization, P XANES spectra of 150-day incubation products did not show two well-separated peaks in the post-edge region, but presented a shoulder peak at ~2164 eV, implying that crystalline Ca-phosphate minerals did not contribute to a significant fraction of solid P species. Meanwhile, we observed a shoulder peak after the main peak at 2155.8 eV (Figures 6.4a-b). These two characteristics suggested that newly formed Ca-phosphate solids were amorphous, similar to the findings in our recent studies for enzyme- and mineral-catalyzed polyphosphate hydrolysis in the presence of Ca^{2+} (Huang et al., 2018b; Wan et al., 2019b). The same peak features were found in the P XANES spectra of the reaction products under UV-light, high temperature, and NaN_3 sterilization (Figures 6.4c-d and E.5). As incubation time increased from 9 days to 150 days, the relative intensity of the shoulder peak at 2164 eV slightly increased, suggesting that ACP solids might have formed rapidly during the early stage and its content slowly increased with increasing reaction time (Figures 6.4c-d). We then conducted laboratory and synchrotron XRD measurements to further identify the phase and crystallinity of solid P species (e.g., amorphous or crystalline Ca-phosphate minerals) (Figures 6.5 and E.4b-c). Laboratory XRD patterns of the incubation products did not show diffraction peaks of crystalline Ca-phosphate minerals (Figures E.4b-c), likely due to the low crystallinity and/or low abundance of newly formed Ca-phosphate solids as compared to the background signals from highly crystalline clays and quartz, consistent with SEM-EDX observations (Figure 6.3). Synchrotron XRD analyses of the sediment incubation products with no treatment or autoclave sterilization also did not observe the presence of crystalline Ca-phosphate minerals (Figure 6.5). Combined with P XANES results, XRD analyses also supported the formation of ACP solids upon polyphosphate hydrolysis by two enzymes and three minerals in surficial sediment incubations.

Figure 6.4 P K-edge XANES spectra of the 150-day reaction products from surficial sediment incubations amended with birnessite, hematite, boehmite, acid and alkaline phosphatases (Pase) with no pre-treatment (a) or autoclave sterilization (b); and by hematite (c) and alkaline phosphatase (d) with high temperature (HT), UV-light, or NaN₃ sterilizations.

Figure 6.5 Synchrotron XRD patterns of 150-day surficial sediment incubations for polyphosphate hydrolysis by birnessite (a), hematite (b), boehmite (c), acid phosphatase (d), alkaline phosphatase (e), and raw sediment (f) (HT+NaN₃ sterilization) with no treatment or autoclave sterilization.

6.4.3 Polyphosphate hydrolysis in no Mg^{2+} artificial seawater during surficial sediment (0.5–2 cm) incubation

Even after a long-term incubation, we could not observe the transformation from ACP solids into crystalline Ca-phosphate minerals, such as hydroxyapatite which was observed in our Fe oxide system (Chapter 4). We hypothesize that this is attributed to the high Mg^{2+} concentration (54.6 mM) in ASW, which may stabilize ACP and inhibit ACP transformation into crystalline Ca-phosphate minerals even with long-term aging (Hilger et al., 2020). We thus conducted sediment incubation Experimental Set II, which used ASW with no Mg^{2+} and with no sediment pre-treatment (Figure 6.6a) or with high temperature sterilization (Figure 6.6b).

Figure 6.6 Dynamics of orthophosphate production from polyphosphate hydrolysis by birnessite, hematite, acid, and alkaline phosphatases in surficial sediment incubations in no Mg^{2+} artificial seawater with no treatment (a) or high temperature (HT) sterilization (b).

The results of batch experiments showed the rapid release of orthophosphate into solution at first 9 days. However, orthophosphate concentrations only reached approximate 100 µM at the early stage and then decreased to about 60 µM at the late stage of surficial sediment incubations (Figure 6.6). No obvious difference in the trends and extents of orthophosphate production was observed in each mineral or phosphatase system between no treatment and high temperature sterilization, consistent with our *Section 6.3.2* observation.

Figure 6.7 SEM images, elemental maps (upper right small panels), and EDX (lower panels) spectra of polyphosphate 150-day hydrolysis products in surficial sediment incubation (no Mg²⁺ artificial seawater) in the presence of 0.2 unit L⁻¹ alkaline phosphatase with no treatment (a) or high temperature (HT) sterilization (b).

First-order kinetic fitting results indicated that the apparent hydrolysis rates in initial time range were ~0.031 d^{-1} for alkaline phosphatase and ~0.013 d^{-1} for birnessite, hematite, and acid phosphatase (date not shown). The absolute values of orthophosphate release extents and polyphosphate apparent hydrolysis rates were much lower than those in ASW with the presence of 54.6 mM Mg^{2+}. The lower release of aqueous orthophosphate was likely attributed to the formation of Ca-phosphate mineral(s) in these sediment incubations using no Mg^{2+} artificial seawater, which contains 10.5 mM Ca^{2+} but no 54.6 mM Mg^{2+}. The calculated speciation distribution of orthophosphate in seawater (20 $^\circ$C, 34.8 ppt salinity, and pH 8) showed that aqueous $MgHPO_4^\circ$ could account for 41.4% of total P species (Ruttenberg, 2014) and the formation of this complex might decrease the activity of orthophosphate chelating with Ca^{2+} to form Ca-phosphate solids.

SEM-EDX results clearly showed the distribution of different elements in 150-day sediment incubation products of alkaline phosphatase-catalyzed polyphosphate hydrolysis in no Mg^{2+} artificial seawater (Figure 6.7). For the incubation product without sterilization treatment, SEM-EDX map showed the occurrences of not only abundant magnesium-aluminum-silicate clays but also Ca hotpots in the middle of tested SEM region (Figure 6.7a). EDX spectra of three selected spots suggested that the weight ratios of Ca to P were 2.25 (6.93/3.08 in wt%) for spot 1, 2.75 (1.87/0.68 in wt%) for spot 2, and 2.08 (2.0/0.96 in wt%) for spot 3. The Ca hotpots 1 and 3 displayed Ca:P weight ratios similar to that of hydroxyapatite (2.08). This result suggested the potential formation of hydroxyapatite in surficial sediment incubation in no Mg^{2+} artificial seawater. SEM-EDX map of alkaline phosphatase-catalyzed polyphosphate hydrolysis with high temperature sterilization showed a large amount of magnesium-aluminum-silicate clays and abundant Ca and P (Figure 6.7b). The weight ratios of Ca to P were 1.87 (1.87/0.95 in wt%) for

spot 4, 98.8 (19.75/0.2 in wt%) for spot 5, and 2.04 (1.16/0.57 in wt%) for spot 6, indicative of potential hydroxyapatite formation in spots 4 and 6. Similar elemental distribution and Ca:P ratios were also observed in 150-day incubation product of acid phosphatase-catalyzed polyphosphate hydrolysis in no Mg^{2+} artificial seawater under high temperature sterilization (Figure E.6). Further mineralogical characterizations of solid products will be performed using P K-edge XANES and synchrotron XRD analysis (data collection delayed by COVID-19) to provide direct evidences for the potential formation of crystalline Ca-phosphate minerals (e.g., hydroxyapatite).

6.4.4 Influence of minerals on polyphosphate hydrolysis in deep sediment (10–12 cm) incubation

Experimental Set III focused on mineral-catalyzed polyphosphate hydrolysis in deep sediment (10–12 cm) in comparison with surficial sediment (0.5–2 cm) incubation, considering the variation in mineralogy and geochemical conditions at two different depths (Figures E.1 and E.4a). Bulk experiment results showed that the concentration of orthophosphate gradually increased to approximate 300 µM at the first 30 days, followed by a steady state and eventually decreased at the late stage of incubation (Figures 6.8a-b), consistent with the trends for orthophosphate release observed in surficial sediment incubations (Figure 6.1). Additionally, no significant difference in polyphosphate hydrolysis extents was observed in deep sediment incubations among no treatment and high temperature sterilization. Within the first 18 days, the hydrolysis extents followed the order of birnessite > hematite > boehmite ≈ raw sediments. We chose the first 18 days as the initial reaction range to fit polyphosphate hydrolysis rates using first-order kinetic model, and the fitting results were shown in Figure E.7. The change of polyphosphate concentration (as total P) in logarithm space showed obvious difference in polyphosphate hydrolysis extents by birnessite, hematite, and boehmite (Figures E.7a-c). The fitted apparent

hydrolysis rates were ~ 0.045 d^{-1} for birnessite, ~0.03 d^{-1} for hematite, ~0.016 d^{-1} for boehmite, and ~0.016 d^{-1} for raw sediments (Figure E.7d). These values of deep sediment incubations were relative higher than those for the surficial sediment incubation, which was likely caused by the mineralogical difference at two sediment depths (Figure E.4a). For example, the deep sediment contained less quartz than the surficial one (Figure E.4a).

Figure 6.8 Dynamics of orthophosphate production during polyphosphate hydrolysis by birnessite, hematite, and boehmite minerals in deep sediment incubation under no treatment (a) or high temperature (HT) sterilization (b). (c – e) are P K-edge XANES spectra of 70-day reaction products from polyphosphate hydrolysis by birnessite (c), hematite (e), and boehmite (d) in sediment incubation, respectively.

Additionally, due to the presence of high Mg^{2+} concentration in artificial seawater, P K-edge XANES spectra of 70-day incubation products did not show the well-separated peaks at 2164 eV, indicating that the formed Ca-phosphate solids in deep sediment upon polyphosphate hydrolysis were also amorphous phase (Figure 6.8c-d). P XANES spectra of 150-day incubation products will be collected in our next beamtime scheduled in December 2020.

6.5 Discussion

In the Control Experimental Set, the sterilized raw sediments were able to hydrolyze polyphosphate, leading to the continuous release of orthophosphate into solution (Figures 6.1f and 6.5). This finding suggested that minerals (e.g., clays) of raw sediments played an important role in catalyzing polyphosphate hydrolysis. The addition of birnessite, hematite, or boehmite further enhanced polyphosphate hydrolysis at different extents (Figure 6.1), as evidenced by the increase in apparent hydrolysis rates with mineral amendments (Figure 6.2). The presence of acid and alkaline phosphatases can rapidly hydrolyze polyphosphate, but the hydrolysis rates were much lower than our previously report values due to the lower phosphatase concentration used in this study (0.2 *vs.* 500 unit L^{-1}) (Huang et al., 2018b). Chapter 5 showed that the estimated contents of Fe oxides, Mn oxides, Al oxides, acid phosphatase, and alkaline phosphatase in the sediments were 0.0111–0.494 g g^{-1}, 0.02–0.2632 g g^{-1}, 0.0091–0.029 g g^{-1}, 0.00004–1.17 unit g^{-1}, and 0.00005–12.02 unit g^{-1}, respectively. Manganese oxides are abundant in some specific environmental samples such as marine Fe-Mn crusts and nodules, reaching up to 0.7002 g g^{-1} (Guan et al., 2019). In this study, the calculated contents were 0.133 g g^{-1} for hematite, birnessite, and boehmite, and 0.077 unit g^{-1} for acid and alkaline phosphatases in sediment incubations, and these contents were still within the range of their corresponding environmental contents. The surficial sediment (#1,

0.5–2 cm) incubation showed that polyphosphate hydrolysis rates were 0.03 d^{-1} for birnessite, 0.024 d^{-1} for hematite, 0.011 d^{-1} for boehmite, 0.053 d^{-1} for acid phosphatase, 0.261 d^{-1} for alkaline phosphatase, respectively. these values imply that mineral-catalyzed polyphosphate hydrolysis was comparable to enzyme-mediated ones. Thus environmental minerals might play a critical role in polyphosphate mineralization in marine sediments, even though alkaline phosphatase had a relatively higher rate for polyphosphate hydrolysis. Iron and Mn oxides showed the higher reactivity in hydrolyzing polyphosphate than Al oxides, consistent with Chapter 2–4 results.

Polyphosphate hydrolysis by minerals and phosphatases shared a similar mechanism via one-by-one cleavage of terminal phosphate groups, which first form metal-phosphate complexes with metal atoms on mineral surface or enzyme metal co-factors (Huang et al., 2018b; Wan et al., 2019b; Yong et al., 2014). Coordinated hydroxyl groups on mineral surface or enzyme structure then attack these complexes to break down P–O–P bonds, leading to polyphosphate hydrolysis and orthophosphate release. Due to the competitive surface complexation by the shortening polyphosphate, the produced orthophosphate is released into the solution and complexes with aqueous Ca^{2+} to form ACP solids instead of surface Ca-phosphate precipitates. A previous study suggested that direct homogenous precipitation of aqueous Ca^{2+} and orthophosphate led to the formation of ACP solids, which can finally transform into hydroxyapatite upon long-term aging (Li et al., 2012b). Indeed, mineral- and enzyme-catalyzed polyphosphate hydrolysis led to the rapid formation of ACP solids in 9-day incubation products (Figure 6.4). After 20-day incubation, the gradual decrease of orthophosphate production indicated that most polyphosphate had been degraded and transformed into ACP solids, resulting in the further decrease of orthophosphate concentrations in solution. However, after 150-day incubation, we did not observe the transformation of ACP solids into crystalline Ca-phosphate minerals. In contrary, the aging of

ACP solids into hydroxyapatite was observed in our Fe oxide-catalyzed polyphosphate hydrolysis in the presence of Ca^{2+} (Chapter 4). The presence of highly concentrated Mg^{2+} may have stabilized the formed ACP solids via poisoning of crystal growth propagation for hydroxyapatite and eventually inhibits ACP transformation into hydroxyapatite (Hilger et al., 2020). When Mg^{2+} was removed from incubation suspensions, lower orthophosphate concentrations were detected in solution (Figure 6.6) and the formation of crystalline Ca-phosphate minerals (e.g., hydroxyapatite) might be potentially detected in solid products, as our Chapter 4 and SEM-EDX results suggested (Figure 6.7). The role of Mg^{2+} in crystalline Ca-phosphate (trans)formation warrants further investigation at the molecular level and is beyond the scope of the current study.

Additionally, polyphosphate mineralization by three environmental minerals was studied via mesocosm sediment incubation from two different depths (0.5–2 *vs.* 10–12 cm) to test the effect of initial sediment and geochemical conditions (Figures 6.1 and 6.8). Although these two sediments had low/no oxygen in the porewater, our incubation experiments were conduct in ambient atmosphere. The reasons for conducting these experiments in oxic conditions are: (1) this study mainly focuses on the comparison of mineral- *vs.* enzyme-catalyzed polyphosphate hydrolysis and marine sediment is chosen to be a representative environment; (2) sediment sterilization (e.g., autoclaved, high temperature, and UV-light) is more convenient under atmospheric conditions; (3) anoxic environments might complicate our experiments due to the potential reduction of Fe and Mn oxides by pre-existing organic matters and/or microbes. The promotive hydrolysis of polyphosphate by three minerals in both sediments showed a similar trend in hydrolysis extents and rates, with the order of birnessite > hematite > boehmite. However, the fitted apparent rates of polyphosphate hydrolysis in deep sediments were higher than those in surficial sediments (Figures 6.2 and E.7d), likely caused by different background solid

compositions. Although the concentrations of Mn^{2+} in the porewater of surficial sediments were high in the range of 3.5–554 µM (Figure E.1), aqueous Mn^{2+} concentration in sediment incubation was hugely diluted into the possible range of 0.09-14.3 µM, which might not significantly affect mineral- and enzyme-catalyzed polyphosphate hydrolysis (Huang et al., 2018b; Wan et al., 2019b). Due to the large dilution in sediment incubations, the effect of slurry metal cations on polyphosphate hydrolysis might be limited. Synchrotron XRD analysis of 150-day incubation products from two Control Sets showed similar mineralogical compositions, but the higher diffraction intensity of surficial sediments incubation at 19° indicated that the surficial sediment (0.5–2 cm) contained more quartz than the deep sediment (10–12 cm) (Figure E.8). The higher quartz contents in surficial sediment may be a possible reason for the relatively lower rate of polyphosphate hydrolysis. Thus, mineral-catalyzed polyphosphate hydrolysis is highly dependent on the mineral phase and abundance in the sediments.

Due to the presence of highly concentrated Mg^{2+} in seawater, sedimentary P burial as apatite may be inhibited due to the stabilization of ACP solids as a metastable phase. However, a large amount of detrital apatite deposits into bottom sediments in aquatic environments (Figure 1.1) (Defforey and Paytan, 2018; Ruttenberg, 2014). Detrital apatite could serve as seeds facilitating heterogeneous nucleation of authigenic apatite. Polyphosphate can be viewed as a highly concentrated potential P source to create a supersaturated micro-environments for crystalline Ca-phosphate heterogeneous nucleation on detrital apatite surface. For example, X-ray fluorescence (XRF) micrograph and fluorescence spectra observed the co-presence of solid polyphosphate granules and crystalline apatite in phosphorus-rich regions of Effingham Inlet sediments (Diaz et al., 2008). A recent study showed the formation of low crystallinity hydroxyapatite via the direct surface precipitation of orthophosphate on calcite surface (Wan et al.,

2016). Thus, the mechanisms leading to marine P sequestration as crystalline apatite should be further studied under environmental conditions to provide detailed explanations for marine P burial in the sediments. This study compared mineral- *vs.* enzyme-catalyzed polyphosphate hydrolysis via mesocosm sediment incubation for the first time. Mineralogical (abiotic) control could be a important factor governing polyphosphate mineralization and transformation in marine sediments when compared with biological control (enzymatic hydrolysis). The current study can greatly advance our current understanding of global P cycle/marine P burial under the influences of both mineralogical and biological factors and help explain the potential formation of crystalline Ca-phosphate minerals in marine environments.

Supplementary Information for Chapter 6 can be found at **APPENDIX E. MINERAL AND ENZYME FACILITATED POLYPHOSPHATE TRANSFORMATION AND PHOSPHORUS SEQUESTRATION IN MARINE SEDIMENT**.

CHAPTER 7. CONCLUSIONS AND PERSPECTIVES

The largest pathway for marine phosphorus (P) burial is via *in situ* (i.e., authigenic) formation of stable calcium phosphate minerals (e.g., apatite) in oceanic sediments. However, under most marine conditions, apatite formation is kinetically inhibited. A possible mechanism explaining apatite marine burial flux was proposed to be the precipitation of apatite minerals from exogenous polyP intermediates as fine-grained particles. PolyP is a group of P-containing molecules that are produced by a wide range of microorganisms and human activities and can strongly associate with Ca^{2+}. Exploring the geochemical behaviors of polyP in the environment and its critical roles in P cycling is thus significant from both geological and environmental perspectives. This dissertation systematically investigates the biotic and abiotic polyP mineralization and transformation processes catalyzed by enzymes (acid and alkaline phosphatases) and minerals (Fe, Al, and Mn oxides) in complex environmental settings.

Four common Mn oxides can rapidly hydrolyze polyP via one-by-one cleavage of terminal P−O−P bonds (Chapter 2), and the hydrolysis rates follow the order of α-MnO_2 > δ-MnO_2 > birnessite > β-MnO_2. The hydrolysis rates for longer chained polyP are relatively higher than those of shorter chained ones. The presence of common metal cations (Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}) also showed promotion effect on polyP hydrolysis by δ-MnO_2. Formation of cation-polyP ternary surface complexes is likely the dominant mechanism for the cation promotion effect (Figures 7.1a-d). Solid calcium polyP granules can also be hydrolyzed by Mn oxides and transform into amorphous calcium phosphate (ACP) solids, and the content of ACP in solid products increases as pH increases (Figure 7.1e).

Figure 7.1 (a-d) Possible structure configuration and reaction schemes for polyphosphate hydrolysis catalyzed by Mn oxide surfaces in the presence or absence of Ca²⁺. (a) and (c) can be the potential coordination structures of polyphosphate on Fe and Al oxides. (e) is a schematic illustration for the overall abiotic processes of polyphosphate mineralization and transformation on oxide minerals into crystalline calcium-phosphate minerals. Note: Ca^{2+} is in octahedral coordination with O atoms, but for simplicity only the O atoms shared with Mn and P atoms are shown in panels a-d.

On the surface of Al oxide γ-Al$_2$O$_3$ and in the presence of Ca^{2+}, the rate of polyP hydrolysis decreases with increasing mineral particle size (Chapter 3). The main surface P species on γ-Al$_2$O$_3$ are 1) amorphous calcium phosphate precipitates; 2) phosphate groups in polyphosphate that formed direct bonds with the mineral surface as inner-sphere complexes; and 3) phosphate groups in polyphosphate that were not directly bonded to the mineral surfaces.

Figure 7.2 Summary on enzymatic and mineral-catalyzed polyphosphate transformation.

Fe oxides can also strongly hydrolyze polyphosphate in the presence of Ca^{2+}, following the hydrolysis rate of lepidocrocite > hematite > ferrihydrite > goethite (Chapter 4). A terminal-only pathway via one-by-one cleavage of terminal phosphate groups was the dominant hydrolysis mechanism. At alkaline pH conditions, ACP solids formed during polyphosphate hydrolysis in the presence of Ca^{2+}, and the fraction of ACP in total solid P increased as the pH value increased. The newly formed ACP solids eventually transformed to crystalline hydroxyapatite upon long-term aging (Figure 7.1e). However, the hydrolysis rate of polyphosphate and the percentage of ACP

formed was relatively low in artificial seawater, possibly due to the strong aggregation of Fe oxides at highly ionic strength and the subsequently reduced reactive surface area.

Besides polyphosphate, organic and short chained condense phosphates are both viewed as important P sources that can potentially mediate apatite formation. Chapter 5 systematically investigated enzymatic and mineral-catalyzed hydrolysis of six organic phosphates and three condense phosphates. Acid and alkaline phosphatases, two common phosphomonoesterases, can only hydrolyze organic phosphate monoesters and condense phosphates, but the hydrolysis rate was not significantly impacted by the presence of low concentrations of metal cations. In the presence of Ca^{2+}, hematite, boehmite, and birnessite can hydrolyze three condense phosphates (pyrophosphate, tripolyphosphate, and P_{45}) and four organic phosphates (glucose 6-phosphate, glycerol 3-phosphate, adenosine 5-monophosphate, and adenosine 5-triphosphate), but barely degraded inositol phosphate and diphosphopyridine nucleotide. The presence of other metal cations (Mg^{2+}, Zn^{2+}, and Mn^{2+}) promoted mineral- and enzyme-catalyzed hydrolysis at different levels as compared to Ca^{2+}. After normalization to the contents/reactivity of enzymes and minerals in soils and sediments, the hydrolysis rates of condensed phosphates on hematite and birnessite are comparable to those by acid and alkaline phosphatases. It is also worth noting that phosphatases and minerals showed different preferential hydrolysis for organic phosphate monoester and polyphosphate.

Chapter 6 reported the results from mesocosm incubation experiments using marine sediments amended with representative enzymes and minerals, in order to explore polyphosphate hydrolysis and its roles in the precipitation of calcium phosphate minerals in marine environments. The hydrolysis of polyphosphate in the amended sediments followed the order of alkaline phosphatase > acid phosphatase ≥ birnessite ≥ hematite > boehmite. Mineral-catalyzed

polyphosphate hydrolysis rate was also comparable to those catalyzed by enzymes. Upon polyphosphate hydrolysis, ACP first formed but its transformation into crystalline Ca-phosphate minerals was not observed, likely due to the presence of highly concentrated Mg^{2+}. When Mg^{2+} was removed from the reaction solution, equilibrium P concentrations were significantly lower due to ACP formation, which might transform into hydroxyapatite during the late stage of sediment incubation. Comparing the incubations of surficial and deep sediments, no obvious difference on solid P speciation (mainly as adsorbed P species and ACP) was observed in the reaction products. The rates of mineral-catalyzed hydrolysis in deep sediment were higher than those in surficial sediment, likely due to the mineralogical differences between these two sediments.

This dissertation underlines the important roles of natural minerals in controlling P cycling, both as a P sink (i.e., phosphate surface adsorption) and a source of orthophosphate by degrading complex P molecules such as polyphosphate (Figure 7.2). These studies provide new insights for understanding abiotic processes controlling polyphosphate transformation at the mineral-water interface, as well as kinetic and mechanistic explanations for the occurrence of crystalline calcium phosphate minerals in marine environments, and will greatly advance our current understanding of abiotic and biotic factors in controlling P cycling at sediment-water interfaces of natural environments.

Future studies are warranted to explore polyphosphate mineralization and transformation in different geological environments (e.g., freshwater *vs* seawater, presence of organic matter, varied-sediment incubation). Future research may also consider comparing the relative contributions of abiotic (e.g., mineral-catalyzed) *vs* biotic (e.g., enzymatic) processes in mediating polyphosphate hydrolysis using different normalization methods. These potential research directions can further help us understand the processes influencing the transformation, fate, and

bioavailability of complex phosphate-containing molecules in natural environments. For example, organic matters, widely existing in lake and marine sediments, may compete for surface adsorption sites on oxide minerals or directly form complexes with polyphosphate. These processes can reduce the adsorption density of polyphosphate on mineral surfaces and subsequently decrease the polyphosphate hydrolysis capacity of oxide minerals. Additionally, in Chapter 5, we used data from the initial reaction period to perform linear fitting to obtain hydrolysis rates of different complex phosphate molecules by minerals and enzymes. Future studies might consider calculate and normalize the hydrolysis rates of different phosphate species by enzymes and minerals using Michaelis–Menten kinetic equation. Finally, although this dissertation solved some critical questions on polyphosphate mineralization/transformation and the formation of crystalline calcium phosphate minerals under the conditions representative of marine sediment-water interfaces, a better understanding of polyphosphate behaviors in other natural environments warrants future investigations.

APPENDIX A. MANGANESE OXIDE CATALYZED HYDROLYSIS OF POLYPHOSPHATE

Text S1. Characterizations of P compounds and Mn oxides

Solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy was used to characterize the dissolved phosphate reagents (40 mM total P concentration) (Figure A.1). P_2 displayed a peak at -6.28 ppm, while P_3, P_6, P_{10}, and P_{45} showed signals between -6 and -24 ppm. As shown in Figure A.1, with increasing chain length, there was a decrease of the terminal P group signal (around -6 ppm) and an increase of the middle P group signal (around -22 ppm). Previous studies proposed the determination of polyphosphate chain length based on the ratio of the peak area between the phosphate end-groups and middle-groups for solution ^{31}P NMR spectrum (Choi et al., 2010; Fang et al., 2015; Huang et al., 2018b). P_2 only displayed the signal from end-group phosphate and P_{45} mainly showed the signal from middle-group phosphates, consistent with the absence of middle-group in P_2 and the small ratio between end-group to middle group (2:43) for P_{45}. Using this approach, analysis of the ^{31}P NMR spectra (Figure A.1d) indicated that the average chain length of the polyphosphate sodium salt (Na-polyP) is ~10. We thus approximate the molecular formula of Na-polyP to be $Na_{12}P_{10}O_{31}$ with 10 monomers ($-PO_3Na$). Similar approach was used to estimate the average chain length of other polyphosphate reagents, which were determined to be between 3 and 45.

Calcium polyphosphate (Ca-polyP) granules were synthesized by mixing 100 mL of 40 mM $CaCl_2$ solution and 100 mL P_{10} solution with a total P concentration of 40 mM, according to previous procedures for synthesizing other Ca phosphate minerals (Wan et al., 2016). The synthesized solids were repeatedly washed by deionized (DI) water, centrifuged, and freeze-dried. Solid state ^{31}P NMR analysis of the synthesized solids showed two chemical shifts at -9.09 and

24.13 ppm (Figure A.2), consistent with the reported values for Ca phosphate glass (Jäger et al., 2000), and different from NMR spectra of common Ca phosphate minerals (e.g., brushite, hydroxylapatite) (He et al., 2007a).

Four types of Mn oxides with different structure were used for this study, including α-MnO_2, β-MnO_2, δ-MnO_2, and birnessite. β-MnO_2 was purchased from Alfa Aesar. Other Mn oxides were synthesized according to previously reported methods for α-MnO_2 (Inman et al., 2001), δ-MnO_2 (Ginder-Vogel et al., 2009), and birnessite (McKenzie, 1971; Zhu et al., 2012). All solids were repeatedly rinsed with DI water and freeze-dried. Mn oxide structure was characterized by X-ray diffraction (XRD) using a Panalytical Empyrean diffractometer (Cu Kα radiation) or a Bruker D8 Advanced X-ray diffractometer (Mo radiation). All oxides were confirmed to be phase pure (Figure A.3). Specific surface area of Mn oxides was determined by Brunauer–Emmett–Teller (BET) gas adsorption analysis using an Autosorb-1-MP surface pore analyzer (Quantachrome Corp.). The surface areas of α-MnO_2, β-MnO_2, δ-MnO_2, and birnessite were determined to be 146.43 ± 0.32, 2.03 ± 0.07, 125.72 ± 0.6, and 27.4 ± 0.6 m^2 g^{-1}, respectively.

Text S2. Synthesis of P XAS reference standard: α-MnO$_2$ adsorbed orthophosphate

Before orthophosphate adsorption, a 2.0 g L^{-1} α-MnO_2 suspension was dispersed in 0.1 M NaCl and adjusted to a final pH of 6.0 ± 0.05 using HCl and NaOH. Samples containing 10.0 mL of stock suspension were then added to 10.0 mL of a series of P-contained solutions (100, 200, 400, 600, 800, 1000 µM) at pH 6.0 in 0.1 M KCl. The suspensions were equilibrated by shaking at 200 rpm for 24 h, during which the pH of each batch sample was manually adjusted to pH 6.0 $\pm$ 0.05 at 1, 6, 12, and 24 h, respectively. After 24 h of equilibration, the suspensions were centrifuged at 16,000 *g* for 10 min, and the supernatants were filtered through a 0.22-µm Millipore membrane to analyze orthophosphate concentration and adsorption density. The centrifuged wet

pastes with highest adsorption density was freeze-dried for P XANES analysis as a reference standard (referred to as α-MnO$_2$ adsorbed orthophosphate).

Text S3. Mn K-edge XAS analysis

Mn K-edge XAS analysis was conducted for Mn oxides to investigate their average oxidation state. Mineral powders were finely ground and packed in Teflon sample holders covered with Kapton tape. XAS data was collected at Beamline 5-BM-D at the Advanced Photon Source (APS, Lemont, IL) using a vortex detector and Si(111) monochromators (with 40% detuning to avoid higher order harmonics). Energy calibration used Mn foil. Two scans were collected and averaged. Analysis of the Mn XANES spectra for each sample showed no photo-reduction under the X-ray beam. XAS data analysis was performed using Ifeffit (Ravel and Newville, 2005). Linear combination fitting (LCF) of the Mn XANES region was conducted to determine the relative percentage of Mn(II), Mn(III), and Mn(IV) species and the average oxidation state (AOS) following the Combo method (Manceau et al., 2012) which was used in previous studies.(Wang et al., 2018) Specifically, best quantitative results are obtained when the unknown spectrum is fit to a weighted sum of all 17 reference spectra in the database with the fractions of species constrained to be non-negative (Combo method) (Manceau et al., 2012). For the fitting of our samples, five Mn reference standards were finally chosen to represent the best quantitative result. They contained (1) REF4_2: Ramsdellite, Mn(IV)O$_2$ for Mn(IV); (2) REF4_3: $Ca_2Mn_3^{IV}O_8$ for Mn(IV); (3) REF4_4: potassium birnessite (KBi), $K_{0.296}(Mn_{0.926}^{IV}\square_{0.074})O_2 \cdot 0.4H_2O$ for Mn(IV); (4) REF3_4: $Mn_2^{III}O_3$ for Mn(III); and (5)REF2_8: MnSO$_4$·xH$_2$O for Mn(II).

Table A.1 First-order kinetics fitting parameters for data shown in Figure 2.2.

Experimental condition			Reaction time (h)	k (h⁻¹)	R²
PolyP chain length	Mineral	Ca²⁺ concentration			
P_{10}	α-MnO$_2$	–	120	0.155	0.828
P_{10}	α -MnO$_2$	500 µM	120	0.441	0.917
P_{10}	β-MnO$_2$	–	120	0.001	0.932
P_{10}	β-MnO$_2$	500 µM	120	0.002	0.983
P_{10}	δ-MnO$_2$	–	120	0.011	0.935
P_{10}	δ-MnO$_2$	500 µM	120	0.109	0.972
P_{10}	Birnessite	–	120	0.001	0.969
P_{10}	Birnessite	500 µM	120	0.007	0.970
P_2	α-MnO$_2$	–	48	0.043	0.943
P_2	α-MnO$_2$	500 µM	48	0.069	0.994
P_3	α-MnO$_2$	–	48	0.223	0.874
P_3	α-MnO$_2$	500 µM	48	0.205	0.987
P_6	α-MnO$_2$	–	48	0.263	0.940
P_6	α-MnO$_2$	500 µM	48	0.488	0.983
P_{45}	α-MnO$_2$	–	48	0.678	0.930
P_{45}	α-MnO$_2$	500 µM	48	1.258	0.995

Table A.2 Linear combination fitting results of P K-edge XANES data on the solid products after Ca-polyphosphate granule reaction with α-MnO₂ at pH 6–9 for 5 d.

Reaction pH	Relative percentage of fitted reference compounds (%)			R-factor
	Ca-polyP	Amorphous Ca phosphate	α-MnO₂ adsorbed orthophosphate	
6.0	12.0 ± 4.5	59.4 ± 5.2	28.6 ± 7.4	0.0198
7.5	12.0 ± 4.6	76.7 ± 5.3	11.3 ± 7.6	0.0279
9.0	-	77.4 ± 3.6	22.6 ± 5.6	0.0103

Figure A.1 ^{31}P solution NMR spectra of polyphosphate stock solutions prepared from corresponding Na-salts at total P concentration of 40 mM each: (a) P_2, (b) P_3, (c) P_6, (d) P_{10}, and (e) P_{45}.

Figure A.2 ³¹P solid-state NMR spectra of the synthetic Ca-polyphosphate granules. Spin side bands are marked with asterisks.

Figure A.3 X-ray diffraction (XRD) data of α-MnO₂ (a), β-MnO₂ (b), δ-MnO₂ (c), and birnessite (d).

Figure A.4 Change of total phosphorus concentration during mineral catalyzed hydrolysis of P$_{10}$ by α-MnO$_2$ (a), β-MnO$_2$ (b), δ-MnO$_2$ (c), and birnessite (d) at pH 6.0 with or without 500 μM Ca^{2+}. The total phosphate concentration for polyphosphate added was approximate 500 μM.

Figure A.5 Concentration of Ca^{2+} in solution after polyphosphate reaction with Mn oxides for 5 min or 5 d at pH 6.0. All experiments used polyphosphate with total phosphate concentration of 500 μM and Mn oxide loading of 0.4 g L^{-1}. (a) P$_{10}$ reaction with different Mn oxides; (b) α-MnO$_2$ reaction with polyphosphates with varied chain length (P$_2$ to P$_{45}$); and (c) α-MnO$_2$ reaction with Ca-polyP granules.

Figure A.6 Total phosphorus concentration as a function of time during mineral catalyzed hydrolysis of various polyphosphates by α-MnO₂ at pH 6.0 with or without 500 μM Ca²⁺: (a) P₂, (b) P₃, (c) P₆, and (d) P₄₅. The total phosphate concentration for polyphosphate added was approximate 500 μM.

Figure A.7 ^{31}P solution NMR spectra of the liquid supernatant after P_{10} reaction with α-MnO_2 (without Ca^{2+}) at pH 6.0 for 10 min (refer to Figure 2.4).

Figure A.8. Time-resolved ^{31}P solution NMR spectra of the liquid supernatant after P_3 reaction with α-MnO_2 at pH 6.0. The concentrations of α-MnO_2, P_3, and Ca^{2+} were 0.04 g L^{-1}, 1 mM, and 3 mM, respectively.

Figure A.9 Effect of Ca²⁺ concentration and different metal cations on mineral catalyzed hydrolysis of polyphosphate P₁₀ by δ-MnO₂ at pH 6.0. All experiments were conducted with P₁₀ (total phosphate concentration 500 μM), δ-MnO₂ concentration of 0.1 g L⁻¹, and background electrolyte of 0.1 M NaCl. (a) Total P concentration in the supernatant after reaction in the presence of 0–500 μM Ca²⁺ for 2–48 h, and (b) corresponding Ca²⁺ concentration in the supernatant at 48 h. (c) Total P concentration in the supernatant after reaction in presence of 500 μM metal cations for 2–48 h, and (d) corresponding metal ion concentrations in the supernatant at 48 h (with a starting concentration of 500 μM each).

Figure A.10 Phosphate adsorption isotherm on δ-MnO₂ (reaction condition: α-MnO₂ = 1.0 g L⁻¹, orthophosphate concentration = 50–500 μM, NaCl = 0.1 M, pH = 6.0). Dashed lines are the fitting results using Langmuir or Freudnlich models. Error bars represent the results from duplicates.

Figure A.11 ³¹P solution NMR spectra of the supernatant after Ca-polyP granule reaction with α-MnO₂ at pH 6.0 (a), pH 7.5 (b), and pH 9.0 (c) for 1 h or 5 d.

Figure A.12 Mn XANES spectra (a) (black lines) and linear combination fitting (LCF) results (red dash lines) of α-MnO₂ phases using reference from the previous study.(Manceau et al., 2012) Reference compounds used for LCF are: REF4_2: Ramsdellite, Mn(IV)O₂; REF4_3: $Ca_2Mn_3^{IV}O_8$; REF4_4: potassium birnessite (KBi), $K_{0.296}(Mn_{0.926}^{IV}\square_{0.074})O_2 \cdot 0.4H_2O$; REF3_4: $Mn_2^{III}O_3$; REF2_8: MnSO₄·xH₂O. Relative percentage (b) of Mn(II), (III), and (IV) in α-MnO₂ phases, determined by LCF results in Figure A.12a.

APPENDIX B. POLYPHOSPHATE ADSORPTION AND HYDROLYSIS ON ALUMINUM OXIDES

Text S1. Characterization of γ-Al₂O₃ samples

γ-Al$_2$O$_3$ samples of three different particle sizes (5, 35, and 70 nm) were characterized by powder X-ray diffraction (XRD), transmission electron microscopy (TEM), and N$_2$ gas adsorption-desorption BET (measured at 77 K). TEM images confirmed that the average particle diameters were around 5, 35 and 70 nm, respectively (Figure B.1). The particles of the 5 and 35 nm γ-Al$_2$O$_3$ samples were needle shaped, while the particles of the 70 nm sample appear to be rhombohedral. Their powder XRD patterns indicated increasing crystallinity with increasing particle size, and the 35 nm sample contained some small amount of α-Al$_2$O$_3$ (Figure B.2). BET specific surface areas (SSA) of the 5, 35, and 70 nm samples were 325, 167, and 126 m^2 g^{-1}, respectively, consistent with the increasing particle size. More detailed characterization information can be found in the previous study (Yan et al., 2015). Compared to environmental Al oxide minerals (e.g., corundum, boehmite, gibbsite and bayerite), γ-Al$_2$O$_3$ has larger surface area and higher reactivity for P adsorption (Li et al., 2013a; Yan et al., 2015). The point of zero charge (PZC) values of amorphous Al(OH)$_3$, boehmite, corundum, and gibbsite were reported to be 9.3, 9.2, 8.9 and 8.6, respectively (Chang et al., 2006; Li et al., 2013a; Yan et al., 2014b). The PZC values of 5, 35, and 70 nm γ-Al$_2$O$_3$ were reported to be 9.3, 9.1 and 8.5, respectively (Yan et al., 2013), and are similar to those of the environmentally relevant Al oxide minerals. Previous studies have also shown that hydration at pH > 5 can slowly transform γ-Al$_2$O$_3$ to gibbsite and/or bayerite (Roelofs and Vogelsberger, 2006). The short hydration time employed in this study (overnight) might lead to minor transformation of the surface of γ-Al$_2$O$_3$ particles, but was not detected by XRD in our preliminary studies.

Text S2. Characterization of the Na-polyphosphate salt

The average chain length of the polyphosphate samples were calculated based on the ratio of the peak area between the end groups and middle groups in polyphosphate solution ^{31}P nuclear magnetic resonance (NMR) spectrum (Fang et al., 2015). Quantitative analyses of the ^{31}P NMR spectra of the Na-polyphosphate (Na-polyP) salt (Figure A.1d) indicated an average chain length of 10 using this NMR method. Thus, the molecular formula of this sample can be defined as $Na_{12}P_{10}O_{31}$. More details can be found in Appendix A. *Text S1.*

Text S3. NMR data collection and analysis

After 9-day reaction, the reaction suspensions were centrifuged to separate the solid and supernatant. The supernatant samples were directly used for solution ^{31}P NMR spectroscopy analysis. The wet pastes were freeze-dried for analysis of solid state ^{31}P NMR spectroscopy.

Solution ^{31}P NMR spectra were collected on a Bruker AMX 400 MHz spectrometer operated at 162 MHz and 297 K. A 90° pulse width, 6.5k data points (TD) over an acquisition time of 0.51 s, and relaxation delay of 2 s were applied. Chemical shift was calibrated using 85% H_3PO_4 as the external standard. At least 1500 scans were collected for each spectrum (> 2 h).

Solid-state ^{31}P NMR spectra were acquired using single pulse/magic angle spinning (SP/MAS) and proton decoupling on a Bruker Avance 400 spectrometer operated at a ^{31}P frequency of 161.9 MHz. Solid samples (~20 mg) were packed into the inserts of a 4 mm diameter zirconia rotor with Kel-F Caps (Wilmad, NJ) and spun at 10 kHz. Direct polarization (DP) data collection parameters were 2048 data points (TD) over an acquisition time (AQ) of 24.6 ms, a recycle delay (RD) of 180 s, and 256 scans. Variable RD experiments were conducted, and 180 s were verified to be sufficient to prevent signal saturation during data acquisition. The DP-MAS ^{31}P

NMR spectra were acquired with a ^{31}P 90° pulse of 5.0 μs and an attenuation level (PL1) of 12.1 dB. Chemical shifts were externally referenced to $NH_4H_2PO_4$ at 0.72 ppm. ^{31}P spin counting experiments were based on previous reports where $NH_4H_2PO_4$ was used as an external intensity standard and its ^{31}P DP NMR spectrum was acquired in one scan after equilibration for 1000 s (16.7 min). ^{31}P{^{1}H} Cross-polarization (CP)/MAS spectra were also collected for those samples. The CP contact time was set at 1 ms and 256 scans were collected for samples. CP kinetics curves were measured at a spinning rate of 10 kHz with continuous wave (CW) irradiation at the n = −1 sideband match condition, varying the contact time from 0.3 to 7 ms. Proton decoupling was employed during acquisition of all ^{31}P{^{1}H} CP/MAS spectra.

The SP/MAS ^{31}P NMR spectra were deconvoluted into component resonance lines using Origin 2016. The resonance line shape chosen was 100% Gaussian. All spectra were deconvoluted into three resonance lines with fixed peak positions based on our assignments for surface P species on γ-Al_2O_3 in *Section 3.3.3*, including 1) calcium phosphate precipitates, 2) phosphate groups in polypohosphate that are complexed on the mineral surfaces as inner-sphere species (to polyP-P_{bonded}), and 3) phosphate group in polyphosphate that are not associated with the mineral surface (to polyP-$P_{unbonded}$). We did not observe the chemical shift for orthophosphate (produced from polyphosphate hydrolysis) adsorption on γ-Al_2O_3, possibly due to the strong competitive adsorption between orthophosphate and residual polyphosphate or low surface coverage of orthophosphate on γ-Al_2O_3 surface (if any). Therefore, we did not include adsorbed orthophosphate species during peak deconvolution of surface P speciation.

Two-dimensional (2D) ^{31}P{^{1}H} heteronuclear correlation (HetCor) spectra were collected to obtain ^{1}H spectra indirectly. The HetCor experiments employed a spinning rate of 12 kHz and a CP contact time of 3 ms, using a linear ramp of the ^{31}P B1 field and 42 kHz ^{1}H field.

We collected 150 hypercomplex points in t1 with a 10 μs increment, corresponding to a 100 kHz spectral window in F1. For spectra containing narrow ^{1}H peaks, standard linear prediction methods were used to complete the signal decay in F1 to avoid truncation artifacts. For each spectrum, 48 scans were collected for each point at a 1 s relaxation delay. The spectra were acquired in [1]H-coupled mode, with no homonuclear ^{1}H decoupling pulses applied during t1. The ^{1}H NMR chemical shifts (δ_H) are referenced with respect to tetramethylsilane (TMS) using adamantane as a secondary reference set to $\delta_H = 2.0$ ppm.

Solid-state SP/MAS ^{27}Al NMR spectra of γ-Al$_2$O$_3$ loaded with polyphosphate and standard samples (aluminum polyphosphate) were collected on a Bruker Avance 400 spectrometer operated at a [27]Al frequency of 104.2 MHz, with samples contained in 4 mm (o.d.) ZrO$_2$ rotors at a spinning rate of 10 kHz. The [27]Al chemical shifts (δ_{Al}) are reported relative to an external 1 M Al(NO$_3$)$_3$ solution set to $\delta^{Al} = 0$ ppm. The pulse delay was optimized at 5 s, and approximately 128 (Experiment Set I) or 1024 (Experiment Set II) scans were collected for spectra to obtain an acceptable signal-to-noise ratio.

Text S4. CP/MAS spectra kinetics

The signal intensity and area vary with CP contact time (s) according to the classical biexponential equation.

$$I(\tau) = I_0[1 - \frac{T_{PH}}{T_{1\rho,H}}]^{-1}[\exp\left(-\frac{\tau}{T_{1\rho,H}}\right) - \exp\left(-\frac{\tau}{T_{PH}}\right)] \qquad \text{Eq. (1)}$$

Where T_{PH} is the characteristic time for [1]H → [31]P magnetization transfer and $T_{1\rho,H}$ is the time constant for decay of the ^{1}H magnetization in the rotating frame (Kolodziejski and Klinowski, 2002). Typical CP kinetic curves were shown in Figure B.10, along with least-square fits to Eq. (1), which illustrated the effects of CP contact time on the peak areas. The peak areas of varied P

species in these three samples built up polarization quickly, reached a maximum area around 1 ms contact time, and then declined sharply with further increase in contact time (Figure B.10).

Text S5. P K-edge XANES data collection and analysis

Freeze-dried samples after reaction were analyzed by P K-edge XANES at Beamline 14-3 at the Stanford Synchrotron Radiation Lightsource (SSRL), Menlo Park, CA. Sample powders were brushed evenly onto P-free Kapton tape and mounted to a sample holder maintained under helium atmosphere. XANES data were collected in fluorescence mode using a PIPS detector. Energy calibration used $AlPO_4$ (edge position at 2152.8 eV). XANES spectra were collected at 2100–2485 eV.

Additionally, a suite of P reference compounds were prepared for XANES analysis: (1) polyphosphate sodium salt (Na-polyP) (Sigma Aldrich) and synthetic Ca-polyP granules (synthesized via 1:1 mixture of 10 mM Ca^{2+} and polyphosphate at total P concentration of 10 mM at pH 7.0. The same mothed was used to synthesize Al-polyP), representing solid polyphosphate phases; (2) orthophosphate sorbed on γ-Al_2O, prepared at pH 6.0 following previous method (Yan et al., 2015) and was centrifuged after 6-h adsorption reaction time, representing Al oxide associated orthoP; (3) polyphosphate sorbed on γ-Al_2O_3, prepared at pH 6.0 using the same way for orthophosphate sorbed samples, representing Al oxide associated polyP; (4) amorphous calcium phosphate (ACP), octacalcium phosphate (octaCa), and hydroxyapatite (Huang and Tang, 2015a), representing calcium phosphate precipitates. XANES spectra of all reference compounds were collected in the same manner as for unknown samples.

Data analysis used the software Ifeffit (Ravel and Newville, 2005). All spectra were carefully examined for energy calibration, merged, and normalized. Linear combination fitting

(LCF) was conducted on the XANES spectra at energy range of 15 to 50 eV relative to the edge energy. The goodness of fit was evaluated using the residual factor (R-factor), and fit with smallest R-factor was decided as the best fit.

Table B.1 Experimental design and reaction conditions.

Experiment Set	Experiment #	PolyP concentration (as total P)	γ-Al$_2$O$_3$ particle size	γ-Al$_2$O$_3$ concentration	pH	Metal cation and concentration
Set I	I-1	2 mM	5, 35, 70 nm	0.4 g/L	6.0, 8.0	–
	I-2	2 mM	5, 35, 70 nm	0.4 g/L	6.0, 8.0	Ca^{2+} (1 mM)
Set II	II-1	1 mM	5 nm	0.1 g/L	6.0	Ca^{2+}, Mg^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+} (0.5 mM)
	II-2	1 mM	5 nm	0.1 g/L	8.0	Ca^{2+}, Mg^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+} (0.5 mM)

Table B.2 Relative percentage of orthophosphate and polyphosphate in solution calculated based on the NMR data in Figure 3.2 and solution data in Figure 3.1 and B.4.

γ-Al$_2$O$_3$ particle size (nm)	Ca^{2+} (mM)	pH	NMR analysis		Wet chemistry analysis	
			Orthophosphate (%)	Polyphosphate (%)	Orthophosphate (%)	Polyphosphate (%)
5	–	6	9.2	90.8	6.4	93.6
35	–	6	8.29	91.71	5.44	94.56
70	–	6	9.18	90.82	5.17	94.83
5	1	6	29.94	70.06	19.33	80.67
35	1	6	28.4	71.6	16.72	83.28
70	1	6	26.08	73.92	15.19	84.81
5	–	8	5.56	94.44	6.54	93.46
35	–	8	6.25	93.75	4.93	95.07
70	–	8	6.93	93.07	4.69	95.31
5	1	8	55.54	44.46	47.49	52.51
35	1	8	47.87	52.13	35.37	64.63
70	1	8	40.87	59.13	26.78	73.22

Figure B.1 Transmission electron microscope (TEM) images of 5 nm (a), 35 nm (b), and 70 nm γ-Al₂O₃. Initial TEM images can be found in supplementary materials of the previous study (Yan et al., 2015).

Figure B.2 X-ray diffraction (XRD) patterns of γ-Al₂O₃ with average 5, 35, and 70 nm particle sizes. Asterisks indicate the presence of small amount of α-Al₂O₃ in the 35 nm sample. Initial XRD patterns can be found in supplementary materials of (Yan et al., 2015).

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Figure B.3 Dynamic of dissolved total P concentration during hydrolysis of polyphosphate hydrolysis by 5 nm (a, d), 35 nm (b, e), and 70 nm (c, f) γ-Al₂O₃ (Experiment Set I). Reaction condition: polyphosphate (2 mM as total P), γ-Al₂O₃ 0.4 g/L, pH 6.0 and 8.0, with/without 1 mM Ca²⁺.

Figure B.4 Hydrolysis kinetics of polyphosphate hydrolysis with (a) 5 nm, (b) 35 nm, and (c) 70 nm γ-Al₂O₃ (Experiment Set I). Reaction condition: polyphosphate (2 mM as total P), γ-Al₂O₃ 0.4 g/L, pH 6.0 and 8.0, with/without 1 mM Ca²⁺. Solid lines are the first-order kinetic model fitting results.

Figure B.5 Final concentration of Ca^{2+} in solution after polyphosphate reaction with 5, 35, and 70 nm γ-Al₂O₃ for 5 min or 9 d (Experiment Set I). Reaction condition: polyphosphate (2 mM as total P), γ-Al₂O₃ 0.4 g/L, pH 6.0 and 8.0, with/without 1 mM Ca^{2+}.

Figure B.6 Schematic illustration for possible coordination structure for polyphosphate at the surface of γ-Al₂O₃.

Figure B.7 ^{31}P and ^{27}Al solid-state NMR spectra of synthetic Al-polyphosphate and Ca-polyphosphate compounds. Asterisks denote spinning side bands.

Figure B.8 ^{27}Al solid-state NMR spectra of γ-Al$_2$O$_3$ with 5 nm (a), 35 nm (b) and 70 nm (c) particle size after reaction with polyphosphate (Experiment Set I). Reaction condition: polyphosphate (2 mM as total P), γ-Al$_2$O$_3$ 0.4 g/L, pH 6.0 and 8.0, with/without 1 mM Ca^{2+}, 9 d reaction time.

Figure B.9 Deconvolution of single-pulse ^{31}P MAS NMR spectra of the reaction products from polyphosphate with different sized γ-Al₂O₃ (Experiment Set I). Reaction condition: polyphosphate (2 mM as total P), γ-Al₂O₃ 0.4 g/L, pH 8.0, 1 mM Ca^{2+}, 9 d. Blue: calcium phosphate precipitates; Green: phosphate groups in polyphosphate that are complexed on

mineral surface as inner-sphere species (to polyP-P$_{bonded}$); Pink: phosphate groups in polyphosphate that are not directly bonded with mineral surface (to polyP-P$_{unbonded}$).

Figure B.10 ^{31}P{^{1}H} **CP/MAS kinetics of the two areas (from –2 to –16.5 and from –16.5 to –30 ppm chemical shifts) for 5 nm γ-Al$_2$O$_3$ reacting with polyphosphate (2 mM as total P) with Ca^{2+} at pH 6.0 (a), without Ca^{2+} at pH 8.0 (b), and of the three areas (from 3.9 to –2.0; from –2 to –16.5; from –16.5 to –30) for 5 nm γ-Al$_2$O$_3$ reacting with polyphosphate with Ca^{2+} at pH 8.0 (c). (d) is the zoom of (c) for peak area in the range of 3.9 to –2 ppm. Experiments were conducted at a spinning rate of 12 kHz varying contact time from 0.1 to 7 ms.**

Figure B.11 Effects of different metal cations (0.5 mM) on polyphosphate (1 mM as total P) hydrolysis (Experiment Set II) by 5 nm γ-Al$_2$O$_3$ (0.1 g/L) at pH 6.0 (a, b) and 8.0 (c, d). (a) and (c) is the orthophosphate release; (b) and (d) is the total P concentration in the supernatant.

Figure B.12 Metal cation concentration in the supernatant after polyphosphate hydrolysis (Experiment Set II) by 5 nm γ-Al₂O₃ for 9 d at pH 6.0 or 8.0. Initial concentration of metal ions is 0.5 mM.

Figure B.13 ^{31}P solid-state NMR spectra of γ-Al₂O₃ reacting with polyphosphate (Experiment Set II) in the presence of 0.5 mM Cu^{2+} (a) and Mn^{2+} (b) at pH 6.0 or 8.0.

Figure B.14 (a–b) Normalized P K-edge XANES spectra of the reaction products from polyphosphate hydrolysis by γ-Al₂O₃ (Experiment Set II) with 0.5 mM metal ions at pH 6.0 or 8.0. (c) Normalized P K-edge XANES spectra of phosphate reference compounds used for linear combination fitting of reaction products.

Sample labels: Al₂O₃_polyP (Al₂O₃-adsorbed polyphosphate); Al₂O₃_orthoP (Al₂O₃-adsorbed orthophosphate); Ca/Mg/Cu/Zn/Mn_polyP, 6 or 8 refer to polyP hydrolysis experiments conducted in the presence of metal cations (Ca²⁺, Mg²⁺, Cu²⁺, Zn²⁺, or Mn²⁺) at pH 6.0 or 8.0; sodium polyphosphate salt (Na-polyP), synthesized calcium polyphosphate granules (Ca-polyP), amorphous calcium phosphate (ACP), octacalcium phosphate (octa Ca-P).

Figure B.15 ^{27}Al solid-state NMR spectra of the solid products after polyphosphate reaction with 5 nm γ-Al$_2$O$_3$ in the presence of 0.5 mM metal ions (Ca^{2+}, a; Mg^{2+}, b; Cu^{2+}, c; Zn^{2+}, d; Mn^{2+}, e) (Experiment Set II) at pH 6.0 or 8.0 after 9 d. (f) is the zoom image of Figure B.15d in chemical shift range of 40–0 ppm.

APPENDIX C. IRON OXIDE-CATALYZED HYDROLYSIS OF POLYPHOSPHATE AND THE PRECIPITATION OF CALCIUM PHOSHATE MINERLS

Text S1. Synthesis and characterization of Fe oxides

Ferrihydrite, goethite, hematite, and lepidocrocite were synthesized based on reported methods (Cornell and Schwertmann, 2004; Lanzl et al., 2012) and briefly described below.

For ferrihydrite synthesis, 0.3 M NaOH was slowly added into 250 mL of 0.1 M $FeCl_3$ solution under magnetic stirring to bring the pH to 7–8 (Cornell and Schwertmann, 2004).

For the synthesis of hematite, 100 mL of 1 M $FeCl_3$ solution was added dropwise to 1 L of boiling deionized water during vigorous magnetic stirring (Lanzl et al., 2012).

For the synthesis of goethite, 0.3 M NaOH was slowly added into 250 mL of 0.1 M $FeCl_3$ solution to reach a final pH of 7–8 under continuous magnetic stirring. The precipitated suspension was transferred to a closed polypropylene bottle and placed in an oven at 70 ºC for 60 hours (Cornell and Schwertmann, 2004).

For the synthesis of lepidocrocite, a 0.06 M $FeCl_2$ solution was titrated to pH 7.0 with 0.2 M NaOH, and the precipitates were oxidized with air at a rate of 200 mL min^{-1} (Cornell and Schwertmann, 2004). pH value of the suspension was maintained at 7.0 by the addition of 0.2 M NaOH and the reaction was carried out at room temperature with magnetic stirring and was completed within 3 h.

After the synthesis, all Fe-mineral suspensions were centrifuged, washed with deionized water until the electrical conductivity was less than 2 µS cm^{-1}, freeze-dried, and finely ground. The phase purity was confirmed by powder X-ray diffraction (XRD) (Figure C.1). Specific surface

area of each Fe mineral was determined by Brunauer–Emmett–Teller (BET) gas adsorption analysis using an Autosorb-1-MP surface pore analyzer (Quantachrome Corp.). The BET surface areas of ferrihydrite, hematite, and goethite are 236.11 ± 1.49, 117.96 ± 0.43, and 38.44 ± 0.39 m^2 g^{-1}, respectively. We did not measure the surface area of lepidocrocite and their surface areas in the literatures were reported to be 77 ± 1 m^2 g^{-1} using the same synthesis and measurement method (Alexandratos et al., 2017; Davantès et al., 2016).

Text S2. Characterization of Na-polyphosphate salt and preparation of P reference compounds

The average chain length of the polyphosphate sample was calculated based on the ratio of the peak area between the end P groups and middle P groups in polyphosphate solution ^{31}P nuclear magnetic resonance (NMR) spectrum (Fang et al., 2015). Quantitative analyses of solution ^{31}P NMR spectra of the Na-polyphosphate (Na-polyP) salt (Figure A.1d) suggested an average chain length of 10 using this method. We thus define the molecular formula of this polyphosphate sample as $Na_{12}P_{10}O_{31}$. More details can be found in Appendix A. *Text S1*.

A suite of P reference compounds were prepared for P K-edge XANES measurement: (1) polyphosphate sodium salt (Na-polyP) (Sigma Aldrich) and synthetic Ca-polyphosphate granules [Ca-polyP, details in our recent study (Wan et al., 2019b)], representing solid polyphosphates. (2) orthophosphate-adsorbed samples on four Fe oxides at pH 6.0 using the reported method (centrifuged after 6-hour adsorption reaction) (Wang et al., 2013a), representing Fe oxide associated orthophosphate; (3) polyphosphate-adsorbed samples on four Fe oxides at pH 6.0 using the same condition for orthophosphate-adsorbed ones, representing Fe oxide associated polyphosphate; (4) amorphous calcium phosphate (ACP), octacalcium phosphate (octaCa), and hydroxyapatite (details in our previous study(Huang and Tang, 2015a)), representing calcium phosphate precipitates. P XANES spectra of all reference compounds were collected in the same

manner as for unknown samples.

Text S3. XRD, FTIR, TEM, NMR, and XAS analyses

X-ray diffraction (XRD). Powder XRD measurements were conducted on a Panalytical Empyrean diffractometer (Cu Kα radiation, λ = 1.5418 Å) at the IEN/IMAT Materials Characterization Facility at Georgia Institute of Technology. Powdered samples were placed on zero-background holders (MTI corp.) for the XRD measurement. Scan parameters were 0.04° step size and 9 second/step at 5–80° 2θ. Synchrotron XRD data were collected for the freeze-dried solids using X-rays of 58.6491 keV (λ = 0.2114 Å) at beamline 11-ID-B at the Advanced Photon Source (APS), with the sample-to-detector distances of 100 cm, respectively.

Fourier transformed infrared (FTIR) spectroscopy. FTIR analysis of the reaction products was conducted on a Thermo Scientific iS50 FT-IR Spectrometer via touch point one-touch sampling operation at the IEN/IMAT Materials Characterization Facility at Georgia Institute of Technology. Spectra were collected in the spectral range extending from 4000 to 500 cm^{-1} for an average of 64 scans at an instrument resolution of 4 cm^{-1}. FTIR spectra were performed 0-1 normalization based on the highest and lowest peak intensity in each spectrum, according to our previous studies (Wan et al., 2016; Wan et al., 2017a).

Transmission Electron Microscope (TEM). Normal TEM and high angle annular dark-field (HAADF) images were collected on FEI Tecnai F30 TEM (equipped with thermally assisted field emission (TFE) gun) operated at 300 kV at the IEN/IMAT Materials Characterization Facility at Georgia Institute of Technology. Small quantities of powdered reactants were dispersed in ethanol solution and ultrasonicated for 5 minutes. The samples were prepared by loading 10-μL aliquots of powdered reactants on carbon-coated copper grids. Chemical distribution maps were obtained

for elemental calcium (Ca) and iron (Fe) from the electron energy loss spectroscopy (EELS) spectral images using a power-law background model. The elemental abundance at selected points in HAADF image was record by an energy dispersion X-ray spectroscopy (EDS).

NMR analysis. At the end of reaction, the suspensions were centrifuged to separate the solid and supernatant. The supernatant samples were further filtered by 0.22-μm Millipore membrane and used for solution ^{31}P NMR spectroscopy analysis. Solution ^{31}P NMR spectra were collected on a Bruker AMX 400 MHz spectrometer operated at 162 MHz and 297 K. A 90° pulse width, 6.5k data points (TD) over an acquisition time of 0.51 s, and relaxation delay of 15 s were applied. Chemical shift was calibrated using 85% H_3PO_4 as the external standard. At least 2048 scans were collected for each spectrum (> 2 h).

XAS analysis. P K-edge XANES analysis was conducted at Beamline 14-3 at the Stanford Synchrotron Radiation Lightsource (SSRL), Menlo Park, CA. Sample powders were brushed evenly onto P-free Kapton tape and mounted to a sample holder maintained under helium atmosphere. P XANES spectra were collected in fluorescence mode using a PIPS detector and at the energy range of 2100–2485 eV. Energy calibration used $AlPO_4$ (edge position at 2152.8 eV). The list of P reference compounds and the preparation procedures can be found in Appendix B. *Text S2*. XANES data analysis used the software Ifeffit (Ravel and Newville, 2005). All spectra were carefully examined for energy calibration, merged, and normalized. LCF was conducted on the XANES spectra at energy range of –15 to 50 eV relative to the edge energy. The goodness of fit was evaluated using the residual factor (R-factor) and fit with smallest R-factor was decided as the best fit.

Table C.1 First-order kinetics fitting results of polyphosphate apparent hydrolysis rates on Fe oxides under various solution conditions (Figure C.2−3).

	Experimental condition			Reaction time (h)	k (10^{-3} h^{-1})	R^2
PolyP	Mineral	pH	Ca^{2+}			
P$_{10}$	Ferrihydrite	6.0	–	216	0.232	0.985
P$_{10}$	Ferrihydrite	6.0	1000 μM	216	0.818	0.992
P$_{10}$	Hematite	6.0	–	216	0.264	0.938
P$_{10}$	Hematite	6.0	1000 μM	216	1.88	0.998
P$_{10}$	Goethite	6.0	–	216	0.287	0.989
P$_{10}$	Goethite	6.0	1000 μM	216	0.984	0.977
P$_{10}$	Lepidocrocite	6.0	–	216	0.345	0.906
P$_{10}$	Lepidocrocite	6.0	1000 μM	216	1.62	0.990
P$_{10}$	Ferrihydrite	7.5	–	216	0.210	0.959
P$_{10}$	Ferrihydrite	7.5	1000 μM	216	1.43	0.924
P$_{10}$	Hematite	7.5	–	216	0.226	0.948
P$_{10}$	Hematite	7.5	1000 μM	216	2.0	0.915
P$_{10}$	Goethite	7.5	–	216	0.246	0.943
P$_{10}$	Goethite	7.5	1000 μM	216	0.620	0.970
P$_{10}$	Lepidocrocite	7.5	–	216	0.665	0.992
P$_{10}$	Lepidocrocite	7.5	1000 μM	216	2.98	0.905
P$_{10}$	Ferrihydrite	9.0	–	216	0.202	0.966
P$_{10}$	Ferrihydrite	9.0	1000 μM	216	0.851	0.892
P$_{10}$	Hematite	9.0	–	216	0.271	0.875
P$_{10}$	Hematite	9.0	1000 μM	216	1.42	0.853
P$_{10}$	Goethite	9.0	–	216	0.07	0.858
P$_{10}$	Goethite	9.0	1000 μM	216	0.259	0.842
P$_{10}$	Lepidocrocite	9.0	–	216	1.14	0.991
P$_{10}$	Lepidocrocite	9.0	1000 μM	216	1.54	0.873

Table C.2 Results of linear combination fitting (LCF) analysis of P K-edge XANES spectra of the solid products after long term reaction of polyphosphate with hematite or lepidocrocite in the presence of Ca^{2+} at pH 9.0 (Figure 4.4a).

Label	Sample information	Relative percentage (%)						R-factor
		HAP	ACP	adsorbed orthoP	adsorbed polyP	Ca_polyP	Na_polyP	
Sample 1	Hematite, 1 mM Ca^{2+}, 2 mM polyP (as total P), 150 days	11.2 ± 1.8	37 ± 7.6	26.3 ± 1.0	18.0 ± 0.8	N/A	0.075 ± 5.9	0.000531
Sample 2	Hematite, 2 mM Ca^{2+}, 1 mM polyP (as total P), 70 days	83.9 ± 21.7	N/A	11.1 ± 5.0	5.0 ± 6.2	N/A	N/A	0.006357
Sample 3	Lepidocrocite, 1 mM Ca^{2+}, 2 mM polyP as total P, 150 days	30.3 ± 2.0	20.8 ± 10.8	46.6 ± 4.7	N/A	N/A	2.2 ± 7.3	0.000738

Table C.3 First-order kinetics fitting parameters of polyphosphate apparent hydrolysis rates on Fe oxides in artificial seawater (Figure 4.5b).

Experimental condition				Reaction time (h)	k (10^{-3} h^{-1})	R^2
PolyP	Mineral	pH	Ca^{2+}			
P_{10}	Ferrihydrite	8.0	10.5 mM	216	0.287 ± 0.0426	0.777
P_{10}	Hematite	8.0	10.5 mM	216	0.592 ± 0.0463	0.926
P_{10}	Goethite	8.0	10.5 mM	216	0.290 ± 0.0129	0.973
P_{10}	Lepidocrocite	8.0	10.5 mM	216	0.901 ± 0.0209	0.993

Figure C.1 XRD patterns of ferrihydrite (a), hematite (b), goethite (c), and lepidocrocite (d).

Figure C.2 Dynamics of orthophosphate production from polyphosphate hydrolysis on ferrihydrite (a, b, c), hematite (d, e, f), goethite (g, h, i), and lepidocrocite (J, k, l) at different pH values.

Figure C.3 Hydrolysis of polyphosphate on Fe oxides (a, ferrihydrite; b, hematite; c, goethite; d, lepidocrocite) under various solution conditions. First-order kinetic model fitting based on orthophosphate production are shown in color lines. TP: total phosphate concentration; orthoP: orthophosphate.

Figure C.4 Dynamics of total P concentration change in solution during polyphosphate hydrolysis on ferrihydrite (a, b, c), hematite (d, e, f), goethite (g, h, i), and lepidocrocite (j, k, l) at different pH as a function of time (from 10 min to 216 h).

Figure C.5 The concentration of Ca^{2+} in the solution after 9-day reaction of polyphosphate with ferrihydrite, goethite, and hematite at different pH values.

Figure C.6 Normalized P K-edge XANES spectra of different P-containing reference compounds used for LCF analysis. ACP, amorphous calcium polyphosphate; octa Ca-orthoP, octacalcium phosphate; Na-polyP, sodium polyphosphate salts; Ca-polyP, calcium polyphosphate precipitates; Ferr_polyP, ferrihydrite-adsorbed polyphosphate; Ferr_orthoP, ferrihydrite-adsorbed orthophosphate; Hem_polyP, hematite-adsorbed polyphosphate; Hem_orthoP, hematite-adsorbed orthophosphate; Goet_polyP, goethite-adsorbed polyphosphate; Goet_orthoP, goethite-adsorbed orthophosphate; Lep_polyP, lepidocrocite-adsorbed polyphosphate; Lep_orthoP, lepidocrocite-adsorbed orthophosphate.

Figure C.7 Results of LCF analysis of P XANES spectra of 9-day reaction products of polyphosphate hydrolysis on ferrihydrite (a, Ferr), hematite (b, Hem), goethite (c, Goet), and lepidocrocite (d, Lep) with/without 1 mM Ca²⁺ at various pH values. Raw and fitted data are in open circles and solid lines, respectively.

Figure C.8 (a) XRD patterns of the aged products of polyphosphate hydrolysis on hematite (Hem) and lepidocrocite (Lep) in the presence of Ca^{2+} (1 mM or 2 mM) at pH 9.0. (b) is the TEM image of 70-day aged product of 1 mM polyphosphate (as total P) on hematite in the presence of 2 mM Ca^{2+} and (c) is the HRTEM image of a selected region. (d) and (f) are the RBG EELS map of Ca and Fe, respectively. Ca:P (1:2) indicates 1 mM Ca^{2+} and 2 mM polyphosphate as total P; Ca:P(2:1) indicates 2 mM Ca^{2+} and 1 mM polyphosphate as total P.

Figure C.9 STEM-EDS image of the products of 1 mM polyphosphate (as total P) reaction with hematite in the presence of 2 mM Ca^{2+} for 70 days. Points 1 and 2 indicate some large particles of highly aggregated hematite particles with surface adsorbed Ca^{2+} and orthophosphate. The dark particle indicated by Point 3 has a high concentration of Ca and P, implying the co-existence of fine hydroxyapatite particles that randomly distributed in the products.

Figure C.10 Dynamics of total phosphate concentration change in the supernatants during polyphosphate hydrolysis on ferrihydrite, hematite, goethite, and lepidocrocite in artificial seawater (ASW) at pH 8 as a function of time (from 10 min to 216 h).

APPENDIX D. REVISITING THE ROLES OF MINERALS IN THE PHOSPHORUS CYCLE

Text S1. Materials and reagents

<u>*Nine phosphorus (P) compounds*</u> representing natural abundant phosphates were selected for the reaction, including 6 organic phosphates and 3 condensed phosphates. All compounds were purchased from Sigma-Aldrich. The 6 organic phosphates are β-glycerophosphate (GP; product # G9422), D-glucose 6-phosphate (G6P; product # G7250), adenosine 5′-monophosphate (AMP; product # A2252), adenosine 5′-triphosphate (ATP; product # A2383), β-nicotinamide adenine dinucleotide (NP; product # N6522), and myo-inositol hexakisphosphate (IHP; product # P8810). The 3 condensed phosphates are pyrophosphate (P_2; product # P8010), tripolyphosphate (P_3; product # 238503), and polyphosphate (P_{45}; product # S4379). Details of their source, structure, and stock solution concentration are in Table 5.1.

<u>*Alkaline phosphatase*</u> (ALP; product # P5931) from *Escherichia coli* and <u>*acid phosphatase*</u> (ACP; product #P1146) from potato were purchased from Sigma-Aldrich. On the basis of the information from the manufacturer, one unit of alkaline phosphatase can hydrolyze 1.0 μmol of *p*-nitrophenyl phosphate (*p*-NPP) per min at pH 10.4 at 37 °C; one unit of acid phosphatase can hydrolyze 1.0 μmol of *p*-NPP per min at pH 4.8 at 37 °C;

<u>*Boehmite*</u> (PURAL TM 100) was purchased from Sasol Germany GmbH. <u>*Hematite*</u> was synthesized by adding 100 mL of 1 M $FeCl_3$ solution drop wise into 1 L of boiling deionized (DI) water under vigorous magnetic stirring *(1)*. <u>*Birnessite*</u> was synthesized by pumping (1 mL min^{-1}) 45 mL of 6 mol L^{-1} HCl solution into 300 mL of boiling 0.667 mol L^{-1} KMnO4 solution under vigorous magnetic stirring *(2)*. For both hematite and birnessite, the obtained mineral suspensions

were allowed to cool down and the solid precipitates were centrifuged, washed by DI water until the conductivity was less than 2 μS cm^{-1}, and freeze-dried. The phase purity of hematite, birnessite, and boehmite were confirmed by X-ray diffraction (XRD) using a Panalytical Empyrean diffractometer (Cu Kα radiation) or a Bruker D8 Advanced X-ray diffractometer (Mo radiation) and their powder XRD patterns were showed in our previous studies (Chapter 2 and 4) *(3−5)*. The specific surface area of hematite, birnessite, and boehmite are 117.96, 27.4, and 150 m2 g^{-1}, respectively, as determined by Brunauer–Emmett–Teller (BET) nitrogen gas adsorption analysis using an Autosorb-1-MP surface pore analyzer (Quantachrome Corp.).

Text S2. Normalization of hydrolysis rate

Efforts were taken to normalize the hydrolysis rates obtained in enzyme and mineral systems in order to facilitate direct comparison of the relative contributions of different pathways. Phosphatases concentration is typically expressed as activities in unit per liter (***unit L$^{−1}$***), as compared to minerals contents in gram per liter (***g L$^{−1}$***). By dividing the fitted rate values in μM hr^{-1} (for enzymes) or μM d^{-1} (for minerals) (Fig. 2 and Table S1) by the concentrations of phosphatase (200 unit L^{-1}) and minerals (0.4 g L^{-1}) used in the hydrolysis experiments, the units for hydrolysis rates can be converted into ***μmol d$^{−1}$ unit$^{−1}$*** for phosphatase and ***μmol d$^{−1}$ g$^{−1}$*** for minerals (Table D.2). This conversion allows an initial and simple comparison of the hydrolysis rates by one unit of enzymes and one gram of minerals. 1 unit of alkaline phosphatase shows higher activity toward hydrolyzing organic phosphate monoesters than 1 unit of acid phosphatase and 1 g of three oxide minerals, but lower activity toward hydrolyzing condensed phosphates than 1 unit of acid phosphatase, and 1 g of birnessite and hematite (Table D.2). 1 unit of acid phosphatase shows similar rates for the hydrolysis of organic and condensed phosphates as compared to 1 g of

birnessite and hematite. For some P compounds (e.g., ATP, P_3 and P_{45}), the hydrolysis rates by 1 g of birnessite or hematite are much higher than those by 1 unit of acid phosphatase (Table D.2).

Text S3. Evaluating the relative contribution of enzyme vs. mineral catalyzed hydrolysis in soils and sediments

In order to evaluate the relative contributions of the studied enzyme vs. mineral catalyzed hydrolysis processes in environmental settings, the contents and distribution of these enzymes and minerals need to be considered. We obtained literature data on the content distribution of the studied enzymes and minerals from 26 studies *(6−31)*. This allows further normalization of the hydrolysis rates in environmentally relevant conditions, as detailed in the following description.

Enzymes. Acid and alkaline phosphatase concentration in soils and sediments is typically expressed as enzymatic activity, which assayed by incubating the soils/sediments with p-NPP at 30 ℃ for a specific reaction time (10, 32). Acetate buffer (50 mM, pH 5) is typically used for acid phosphatase and TRIS buffer (100 mM, pH 8.7) for alkaline phosphatase. The release of *p*-nitrophenol (*p*-NP) during the incubation is spectrophotometrically measured at 410 nm. Soil/sediment phosphatase activity is generally expressed as µmol *p*-NP (sometimes µg or mg) hr^{-1} g^{-1} solid. Acid/alkaline phosphatase activity data were collected from 17 studies and their units were converted to unit protein per gram soil/sediment (***unit g^{-1}***; Table D.3). The calculation processes are described in the following.

Based on the information provided by manufacturer (Sigma Aldrich), one unit of alkaline phosphatase can hydrolyze 1.0 µmol of *p*-NPP per min at pH 10.4 at 37 °C (or 60 µmol *p*-NPP hr^{-1} $unit^{-1}$); one unit of acid phosphatase can hydrolyze 1.0 µmol of *p*-NPP per min at pH 4.8 at 37 °C (or 60 µmol *p*-NPP hr^{-1} $unit^{-1}$) *(33)*. Previous studies showed that alkaline phosphatase

activity at pH 8.7 is ~67% of its activity at pH 10.4 *(34)*, and acid phosphatase activity at pH 6.0 is ~90% of its activity at pH 4.8 *(35)*. Reactions conducted at 30 °C showed no significant difference in enzymatic activities as compared to 37 °C *(34, 35)*. We conducted enzymatic hydrolysis experiments at 37 °C. Thus, we assume that in our system acid relative phosphatase activity is 54.0 µmol *p*-NPP hr^{-1} unit^{-1} (90% manufacturer's given activity) at pH 6.0 and alkaline phosphatase is 40.2 µmol *p*-NPP hr^{-1} unit^{-1} (67% manufacturer's relative activity) at pH 8.0.

Then, acid/alkaline phosphatase activities (***µmol/µg p-NPP hr^{-1} g^{-1}*** dry weight solids) collected from literatures are divided by their relative activity (***µmol p-NPP hr^{-1} unit^{-1}***) at our tested pH values (pH 6 for acid phosphatase; pH 8 for alkaline phosphatase). Their potential environmental activities or contents at these two pHs are then obtained and represented using the given unit of ***unit g^{-1}***. Based on the reported acid/alkaline phosphatase activities in soils and sediment *(8−11, 13−19, 21, 23, 25, 27−27, 31)*, the specific data points of acid/alkaline phosphatase activities are estimated (Figure D.4a) and the estimated content ranges are showed in Table D.3.

Minerals. The contents of Fe/Al/Mn oxide minerals in soils/sediments were obtained from 9 references *(6−7, 12, 20, 22, 24, 26, 29−30)* and is readily converted to the unit of gram mineral per gram soil/sediment (***g g^{-1}***, see Figure D.4b for specific data points and Table D.3 for the range of contents). For each mineral, more than 70 data points were obtained, and up to 185 data points were collected for Fe oxide concentration in soils. Because high concentrations of Mn oxide (up to 0.7002 g g^{-1}) are only found in specific environmental samples such as Fe-Mn crusts and nodules *(36)*, we did not include data from these samples.

Figure D.1 Dynamics of orthophosphate production from enzyme- and mineral-catalyzed hydrolyses of organic and condensed phosphates by acid phosphatase (a-c) at pH 6, alkaline phosphatase (d-f) at pH 8, as well as birnessite (g–i), hematite (j–l), and boehmite (m–o) at pH 6 and 8. Error bars indicate standard deviation (SD) of replicate experiments.

Figure D.1 Fitting of the initial linear range of orthophosphate production from the hydrolysis of organic and condensed phosphate by acid (a–c) phosphatase, alkaline phosphatase (d–f), Mn oxides (g–i), Fe oxides (j–l), and Al oxides (m–o).

Figure D.3 Time-resolved ^{31}P solution NMR spectra showing the evolution of aqueous P speciation during the hydrolysis of glycerophosphate (GP) and polyphosphate (P$_{45}$) by birnessite (a), hematite (b), and boehmite (c) at pH 6.

Figure D.4 Estimated content distribution of (a) acid (ACP) and alkaline phosphatases (ALP), and (b) Fe/Al/Mn oxide minerals in soils and sediments *(6-31)*. **Related data ranges are summarized in Table S3.**

Figure D.5 Dynamics of orthophosphate production from the hydrolysis of ATP in the presence of varied metal cations by (a) acid phosphatase at pH 6, (b) alkaline phosphatase at pH 8, as well as (c and d) hematite, (e and f) birnessite, and (g and h) boehmite at pH 6 and 8. Enzymes: 200 unit L^{-1}; Minerals: 0.4 gL^{-1}; ATP: 1 mM as total P; Metal cations: 0.5 mM.

Table D.1 Hydrolysis rates obtained by fitting the initial linear range of orthophosphate production from enzyme- and mineral-catalyzed hydrolysis of nine P-containing compounds at pH 6 and 8 as shown in Figure D.2.

P compound	Rate ($\mu M\ hr^{-1}$)		Rate ($\mu M\ d^{-1}$)					
	Acid phosphatase	Alkaline phosphatase	Birnessite		Hematite		Boehmite	
	pH 6	pH 8	pH 6	pH 8	pH 6	pH 8	pH 6	pH 8
GP	75.02 ± 14.8 (R^2=0.90)	203.41 ± 70.37 (R^2=0.89)	2.24 ± 0.87 (R^2=0.45)	6.34 ± 2.1 (R^2=0.55)	0.51 ± 0.20 (R^2=0.53)	3.63 ± 0.74 (R^2=0.80)	0.43 ± 0.10 (R^2=0.67)	1.16 ± 0.19 (R^2=0.78)
G6P	100.58 ± 14.7 (R^2=0.94)	330.51 ± 9.99 (R^2=0.99)	2.77 ± 0.89 (R^2=0.45)	9.68 ± 1.67 (R^2=0.81)	0.22 ± 0.08 (R^2=0.57)	3.46 ± 0.88 (R^2=0.77)	0.91 ± 0.26 (R^2=0.56)	0.63 ± 0.19 (R^2=0.53)
NP	21.68 ± 0.87 (R^2=0.99)	2.2 ± 0.70 (R^2=0.62)	0.95 ± 0.43 (R^2=0.37)	1.77 ± 0.39 (R^2=0.71)	0.27 ± 0.09 (R^2=0.63)	3.82 ± 0.49 (R^2=0.91)	0.69 ± 0.13 (R^2=0.74)	0.20 ± 0.22 (R^2=0.08)
AMP	117.26 ± 6.12 (R^2=0.99)	435.82 ± 90.9 (R^2=0.96)	3.23 ± 0.23 (R^2=0.95)	5.84 ± 0.16 (R^2=0.99)	0.54 ± 0.42 (R^2=0.21)	8.25 ± 0.53 (R^2=0.97)	1.44 ± 0.35 (R^2=0.63)	0.66 ± 0.28 (R^2=0.35)
ATP	52.55 ± 6.47 (R^2=0.96)	305.26 ± 9.91 (R^2=0.99)	40.89 ± 1.34 (R^2=0.99)	16.30 ± 1.07 (R^2=0.97)	11.39 ± 0.95 (R^2=0.96)	34.25 ± 1.13 (R^2=0.99)	3.78 ± 0.23 (R^2=0.96)	2.46 ± 0.55 (R^2=0.66)
IHP	5.61 ± 0.84 (R^2=0.88)	11.12 ± 1.83 (R^2=0.88)	3.25 ± 0.38 (R^2=0.89)	0.87 ± 0.42 (R^2=0.30)	0.46 ± 0.89 (R^2=0.04)	2.03 ± 0.56 (R^2=0.69)	0.59 ± 0.07 (R^2=0.87)	0.26 ± 0.06 (R^2=0.62)
P$_2$	1281.12 ± 129.0 (R^2=0.99)	203.72 ± 15.2 (R^2=0.99)	12.43 ± 1.70 (R^2=0.84)	4.80 ± 0.29 (R^2=0.96)	8.71 ± 0.26 (R^2=0.99)	36.03 ± 2.21 (R^2=0.98)	3.60 ± 0.76 (R^2=0.62)	2.04 ± 0.21 (R^2=0.91)
P$_3$	280.13 ± 32.62 (R^2=0.99)	240.91 ± 32.36 (R^2=0.98)	37.07 ± 3.93 (R^2=0.90)	17.34 ± 0.72 (R^2=0.98)	9.15 ± 2.05 (R^2=0.77)	28.76 ± 2.22 (R^2=0.97)	4.00 ± 0.19 (R^2=0.97)	5.72 ± 0.04 (R^2=0.99)
P$_{45}$	539.71 ± 20.72 (R^2=0.99)	68.28 ± 14.95 (R^2=0.84)	65.63 ± 5.38 (R^2=0.94)	45.12 ± 4.37 (R^2=0.91)	19.17 ± 2.75 (R^2=0.99)	59.93 ± 3.26 (R^2=0.98)	4.07 ± 0.47 (R^2=0.88)	4.99 ± 0.2 (R^2=0.98)

Table D.2 Estimation of the normalized hydrolysis rates for organic and condensed phosphates by phosphatases and minerals at pH 6 and 8.

P compound	Rate (μmol d^{-1} unit^{-1}) *		Rate (μmol d^{-1} g^{-1}) *					
	Acid phosphatase	Alkaline phosphatase	Birnessite		Hematite		Boehmite	
	pH 6.0	pH 8.0	pH 6.0	pH 8.0	pH 6.0	pH 8.0	pH 6.0	pH 8.0
GP	9 ± 1.78	24.41 ± 8.44	5.6 ± 2.18	15.85 ± 5.25	1.28 ± 0.5	9.08 ± 1.85	1.08 ± 0.25	2.9 ± 0.48
G6P	12.07 ± 1.78	39.66 ± 1.2	6.93 ± 2.225	24.2 ± 4.175	0.55 ± 0.2	8.65 ± 2.2	2.28 ± 0.65	1.58 ± 0.475
NP	2.6 ± 0.1	0.26 ± 0.08	2.38 ± 1.08	4.43 ± 0.98	0.68 ± 0.23	9.55 ± 1.23	1.73 ± 0.33	0.5 ± 0.55
AMP	14.07 ± 0.73	52.3 ± 10.91	8.08 ± 0.58	14.6 ± 0.4	1.35 ± 1.05	20.63 ± 1.33	3.6 ± 0.88	1.65 ± 0.7
ATP	6.31 ± 0.78	36.63 ± 1.19	102.23 ± 3.35	40.75 ± 2.68	28.48 ± 2.38	85.63 ± 2.83	9.45 ± 0.58	6.15 ± 1.38
IHP	0.67 ± 0.1	1.33 ± 0.22	8.13 ± 0.95	2.18 ± 1.05	1.15 ± 2.23	5.08 ± 1.4	1.48 ± 0.18	0.65 ± 0.15
P_2	153.73 ± 15.48	24.45 ± 1.82	31.08 ± 4.25	12 ± 0.73	21.78 ± 0.65	90.08 ± 5.53	9 ± 1.9	5.1 ± 0.53
P_3	33.61 ± 3.91	28.91 ± 3.88	92.68 ± 9.83	43.35 ± 1.8	22.88 ± 5.13	71.9 ± 5.55	10 ± 0.48	14.3 ± 0.1
P_{45}	64.77 ± 2.49	8.19 ± 1.79	164.08 ± 13.45	112.8 ± 10.93	47.93 ± 6.88	149.83 ± 8.15	10.18 ± 1.18	12.48 ± 0.5

Note: * Normalized hydrolysis rates are calculated by dividing the fitted rate values in μmol L^{-1} hr^{-1} (for enzymes) or μmol L^{-1} d^{-1} (for minerals) (Table S1) by the concentrations of phosphatase (200 unit L^{-1}) and minerals (0.4 g L^{-1}) in hydrolysis experiments.

Table D.3 Estimated contents of Fe/Al/Mn oxide minerals and acid/alkaline phosphatases based on their environmental activity and/or concentration in soils and sediments.

Mineral	Contents in sediments (g g^{-1})	References	Content in soils (g g^{-1})	References
Fe oxides	0.0111–0.494	(*7, 12, 22, 24, 26, 29*)	0.0013–0.7364	(*6, 20, 30*)
Al oxides	0.02–0.2632	(*7, 12, 22, 24, 26, 29*)	0.0028–0.2867	(*6, 20, 30*)
Mn oxides	0.0091–0.029	(*7, 12, 22, 24, 29*)	0.0001–0.292	(*6, 20, 30*)
Enzyme	**Activity in sediments (unit g^{-1})**	**References**	**Activity in soils (unit g^{-1})**	**References**
Acid phosphatase	0.00004–1.17	(*10, 11, 13, 14, 16, 27*)	0.0004–1.06	(*8, 9, 17, 18, 21, 25, 28*)
Alkaline phosphatase	0.00005–12.02	(*10, 11, 15, 16, 23, 27, 31*)	0.00018–2.27	(*8, 9, 18, 19, 25, 28*)

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APPENDIX E. MINERAL AND ENZYME FACILITATED POLYPHOSPHATE TRANSFORMATION AND PHOSPHORUS SEQUESTRATION IN MARINE SEDIMENT

Text S1. Materials and characterization

The average chain length of polyphosphate salts were calculated based on the ratio of the peak area between the end groups and middle groups in polyphosphate solution ^{31}P nuclear magnetic resonance (NMR) spectrum (Fang et al., 2015; Wan et al., 2019a). Quantitative analyses of the ^{31}P NMR spectrum of sodium polyphosphate solution (Figure 6.S1) indicated an average chain length of 10 using this method. Thus, the molecular formula of this sample is defined as $Na_{12}P_{10}O_{31}$. More details can be found in Appendix A. *Text S1*.

Boehmite (PURAL TM 100) was purchased from Sasol Germany GmbH. Hematite was synthesized by drop-wisely adding 100 mL of 1 M $FeCl_3$ solution into 1 L of boiling deionized (DI) water under vigorous magnetic stirring (Lanzl et al., 2012). Birnessite was synthesized by pumping (1 mL min^{-1}) 45 mL of 6 mol L^{-1} HCl solution into 300 mL of boiling 0.667 mol L^{-1} $KMnO_4$ solution under vigorous magnetic stirring (Zhao et al., 2018). After synthesis, hematite and birnessite suspensions were allowed to cool down to room temperature and the solid precipitates were centrifuged, washed by DI water until the electrical conductivity was less than 2 μS cm^{-1}, and then freeze-dried. The phase purity of synthetic hematite and birnessite were confirmed by X-ray diffraction (XRD) measured on a Panalytical Empyrean diffractometer (Cu Kα radiation) or a Bruker D8 Advanced X-ray diffractometer (Mo radiation), and the results were reported in our previous studies (Wan et al., 2019a; Wan et al., 2017b). Specific surface areas of hematite, birnessite, and boehmite were determined by Brunauer–Emmett–Teller (BET) gas adsorption analysis using an Autosorb-1-MP surface pore analyzer (Quantachrome Corp.). The

surface areas of hematite, birnessite, and boehmite were determined to be 117.96, 27.4 and 150 m^2 g^{-1}, respectively. More details can be found in Appendix D. *Text S1.*

Text S2. Sediment collection and P characterizations

Voltammetric depth profiles of dissolved O_2, Mn^{2+}, Fe^{2+} as well as organic complexes of Fe(III) [org-Fe(III)] and iron sulfides (FeS_{aq}) were obtained by mercury/gold amalgam (Hg/Au) microelectrodes in intact sediment cores. The depth profiles of these species were shown in Figure 6.S2. Details on the sampling site, sediment collection, and core profiles for sediment Core_Z was previously reported (Eitel, 2018).

Sequential chemical extraction for phosphorus (P) speciation was conducted on the sediment Core_Z sample #1 (surficial) and #2 (deep) following the Hedley's method (Hedley et al., 1982; Huang and Tang, 2016). Briefly, 250 mg of solid samples were added to a 50 ml polypropylene centrifuge tube and sequentially extracted by 20 ml extraction solutions, including DI water (readily soluble P), 0.5 M $NaHCO_3$ (exchangeable P), 0.1 M NaOH (Fe/Al mineral adsorbed P), and 1.0 M HCl (insoluble phosphates), each lasting 16 hours under end-to-end shaking. The residual fraction and total P were measured after aqua regia digestion at 100 ℃. Replicate sets of extraction experiments were conducted. The aqueous phases and solids were separated by centrifugation and filtration (0.45 μm Millipore membrane) at the end of each step. The P-concentration in the solutions was determined with the molybdenum blue method using a UV-vis spectrometer (Carey 60, Agilent) (Murphy and Riley, 1962).

Liquid P extraction was also performed for the sediment sample #1 (surficial) and #2 (deep) to assess the speciation of the extractable P portion via solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy, which followed our previously published mothed (Huang and Tang, 2015a;

Wan et al., 2019a). A portion of the freeze-dried solid samples were extracted using a solution containing 0.25 M NaOH and 0.05 M EDTA at a solid:liquid ratio of 0.2:4 g mL^{-1} and was shaken at 150 rpm for 16 hours at room temperature. At the end of extraction, liquid extracts and solid residuals were separated by centrifugation. All extractions were conducted in duplicates. This NaOH/EDTA extraction method has been widely used for P extraction from various environmental matrix and has been proof as a relatively effective P extraction method with high P extraction efficiency and with the most diverse P peaks by solution ^{31}P NMR analysis, suggesting more representativeness and less destruction than other extraction methods (Huang and Tang, 2015a).

Text S3. Reference compounds for P XANES linear combination fitting (LCF) analysis

A suite of P reference compounds were prepared for P K-edge XANES analysis, including: (1) polyphosphate sodium salt (Na-polyP; Sigma Aldrich) and synthetic Ca-polyphosphate granules (Ca-polyP), as detailed in our recent study (Wan et al., 2019b), representing solid polyphosphates. (2) orthophosphate-adsorbed samples on birnessite, hematite, and boehmite at pH 6.0 using the reported method (centrifuged after 6-hour reaction) (Wang et al., 2013a), representing mineral-associated orthophosphate; (3) polyphosphate-adsorbed samples on birnessite, hematite, and boehmite at pH 6.0 using the same condition for orthophosphate-adsorption, representing mineral-associated polyphosphate; (4) amorphous calcium phosphate (ACP), octacalcium phosphate (octaCa), and hydroxyapatite [details in our previous study (Huang and Tang, 2015a)], representing calcium phosphate precipitates. P XANES spectra of all reference compounds were collected in the same manner as for unknown samples.

Table E.1 Experimental designs and conditions of mesocosm sediment incubation.

Experimental Set	Sediment	Sediment solid concentration	Solution	Mineral or enzyme addition	Additive concentration	Polyphosphate concentration (as total P)	pH
Set I	#1 (surficial)	~2.6 g L^{-1}	Artificial seawater	Birnessite; Hematite; Boehmite.	0.4 g L^{-1}	500 µM	8.0
				Acid and alkaline phosphatases	0.2 unit L^{-1}		
Set II	#1 (surficial)	~2.6 g L^{-1}	no Mg^{2+} artificial seawater	Birnessite; Hematite.	0.4 g L^{-1}	500 µM	8.0
				Acid and alkaline phosphatases	0.2 unit L^{-1}		
Set III	#2 (deep)	~2.6 g L^{-1}	Artificial seawater	Birnessite; Hematite; Boehmite	0.4 g L^{-1}	500 µM	8.0
Control Set	#1 (surficial) and #2 (deep)	~2.6 g L^{-1}	Artificial seawater	N/A	N/A	500 µM	8.0

Figure E.1 Depth profiles of dissolved O₂, Mn²⁺, Fe²⁺, organic complexes of Fe(III) [org-Fe(III)], and iron sulfide clusters (FeS_aq), in the sediment Core_Z from the Saltmarsh Ecosystem Research Facility (SERF) collected on Skidaway Island, GA on June 26, 2014. The data were provided by Prof. Martial Taillefert affiliated at the School of Earth and Atmospheric Sciences, Georgia Institute of Technology, United States.

Figure E.2 Relative distribution (a) and absolute contents (b) of various P species in chemical sequential extracts of surficial (#1; 0.5-2 cm) and deep (#2; 10-12 cm) sediments. Solution ^{31}P NMR spectra (c) of EDTA-NaOH liquid extracts of these two sediments.

Figure E.3 First-order kinetic fitting for the initial range of polyphosphate (in total P) hydrolysis by birnessite, hematite, boehmite, acid and alkaline phosphatases in surficial sediment (0.5-2 cm) incubation under no treatment (a) and four different sterilization methods of autoclave (b), high temperature (HT) (c), UV-light (b), or NaN₃; and by raw sediment (f) with HT + NaN₃ sterilization.

Figure E.4 Laboratory XRD patterns of raw sediment solids, reaction products from polyphosphate hydrolysis by environmental minerals (b) and phosphatases (c) after 150-day surficial sediment (0.5–2 cm) incubation with autoclave sterilization.

Figure E.5 P K-edge XANES spectra of 150-day reaction products from polyphosphate hydrolysis by birnessite (a), hematite (b), and boehmite (c) in surficial sediment (0.5–2 cm) incubation with high temperature, UV-light, or NaN₃ sterilizations.

Figure E.6 SEM images, elemental maps (upper right small panels), and EDX spectra (lower panels) of sediment 150-day surficial sediment (0.5–2 cm) incubation in the presence of 0.2 unit L^{-1} acid phosphatase in no Mg^{2+} artificial seawater with high temperature sterilization. The weight ratios of Ca to P were 2.01 (2.05/1.02 in wt%) for site 1, 23.2 (9.08/0.39 in wt%) for site 2, and 2.19 (0.9/0.41 in wt%) for site 3, indicative of potential hydroxyapatite formation in sites 1 and 3.

Figure E.7 First-order kinetic fitting for the initial range of polyphosphate (in total P) hydrolysis by birnessite, hematite, and boehmite minerals in deep sediment (10–12 cm) incubation with no treatment (a) or high temperature sterilization (b); and by raw sediment (f) with HT+NaN₃ sterilization. The fitting hydrolysis rates were presented and compared in Figure E.7d.

Figure E.8 Synchrotron XRD patterns of 150-day incubation products from polyphosphate hydrolysis by raw solids of surficial (0.5–2 cm) and deep (10–12 cm) sediments with high temperature (HT) plus NaN₃ sterilization.

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