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Dynamics of phosphorus speciation and the \textit{phoD} phosphatase gene community in the rhizosphere and bulk soil along an estuarine freshwater-oligohaline gradient

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**Abstract:**

Estuarine tidal marshes play a key role in phosphorus (P) retention and cycling; however, they are suffering
from small but significant increases in tidal saltwater intrusion. The likely impacts of these low-level saltwater intrusions on P availability and microbial activity are unclear. Here, we investigated soil P speciation, alkaline phosphatase (ALP) activity, and the phoD phosphatase gene community along a freshwater-oligohaline gradient in the Min River estuary, southeast China. The results indicated that with the transition from freshwater to oligohaline water, the levels of soil-water salinity, pH and sulfate (SO$_4^{2-}$) content were greater, and ALP activity was lower, which were associated with higher concentrations of organic P, available P, aluminum-bound P, calcium-bound P, and occluded P and lower levels of iron-bound P. There was a strong shift in the phoD phosphatase community composition in response to the freshwater-oligohaline gradient. Our findings showed that with the transition from freshwater to an oligohaline environment, in addition to the associated increases in salinity and soil pH and decreases in general microbial and biological activity and soil organic carbon, there is a shift in soil P toward more recalcitrant and immediately available fractions with less labile forms.

**Keywords:** Phosphorous; phoD phosphatase gene; Saltwater intrusion; Rhizosphere; Estuarine tidal marsh
1. Introduction

Tidal estuarine marshes, of which freshwater (salinity <0.5 ppt) and oligohaline (salinity = 0.5–5.0 ppt) marshes may represent a significant area (Odum 1988; Weston et al. 2014), are globally distributed ecosystems that play vital roles in ecological processes and nutrient cycling (Kirwan and Megonigal 2013; Tong et al. 2017). The complex biogeochemical cycles characteristic of these systems reflect various external stressors, such as human activity, hydrodynamics, and varying salinity, leading to spatial heterogeneity and uncertainty in the distribution of elements (Hu et al. 2018a). For example, estuarine studies of primary production have shown shifts in phosphorus (P) limitation to nitrogen limitation due to the transition between freshwater and seawater environments (Gireeshkumar et al. 2013; Hartzell et al. 2017). While nutrient cycling in coastal salt marsh systems has been well studied, little is known about nutrient dynamics in a low-salinity gradient from freshwater to oligohaline marshes, in which there are various microbial biogeochemical processes and plant communities (Weston et al. 2014). This knowledge gap currently limits the understanding of the wetland geochemical processes that drive nutrient cycling and the associated environmental responses.

Phosphorus is an essential element for living organisms and plays an important role in the regulation of primary productivity and ecosystem function in wetlands (Lin and Guo 2016). The immobilization and release processes of P in estuarine and coastal sediments are used in the quantification of the global P cycle due to the considerable levels of P sequestration and the potential associated contributions to water eutrophication (Gireeshkumar et al. 2013; Hartzell et al. 2010). Environmental conditions may drive the chemical speciation of P that subsequently determines its environmental fate, cycling, and bioavailability in estuarine sediments (Lin and Guo 2016). However, responses among P species to changes along a freshwater-saltwater gradient are known to differ. For example, in the transition from freshwater to saltwater, Gireeshkumar et al. (2013) reported decreased proportions of Fe-bound P (Fe-P) but increased proportions of calcium-bound P (Ca-P) and
total sulfur (TS) due to changes in sediment texture and redox conditions. In contrast, Paludan and Morris (1999) showed that aluminum-bound P (Al-P) was an important inorganic P (IP) pool, regardless of salinity, likely as a consequence of changes in ionic strength and aluminum availability. Previous studies of responses of P speciation to freshwater-saltwater gradients have tended to focus on the effects of wide ranges in salinity (Bai et al. 2017; Caraco et al. 1990; Gireeshkumar et al. 2013), while P responses to relatively narrow ranges in salinity, such as from freshwater to oligohaline, are poorly understood.

Soil microbes are key drivers of P transformation and dominate the composition of P forms (Fraser et al. 2015; Stout et al. 2014). Alkaline phosphatase (ALP) describes a large group of enzymes that generally originate from soil microbes and recycles organic P (OP) to orthophosphate via enzymatic hydrolysis (Ragot et al. 2015). Three ALP-encoding gene families, comprising phoD, phoA, and phoX, have been identified (Acuña et al. 2016). Among these genes, the distribution of the phoD phosphatase gene is widespread, and this gene is considered the key ALP gene in marine sediment. The abundance of the phoD phosphatase gene has been used as a measure of ALP bacterial diversity and distribution in a range of ecosystems (Fraser et al. 2015; Lagos et al. 2016). The soil ALP activity and phoD phosphatase genes are affected by biotic and abiotic factors. For example, Huang and Morris (2003) showed that ALP activity in tidal freshwater wetlands was positively correlated with aboveground plant biomass and negatively associated with soluble reactive P concentration. Acuña et al. (2016) found that ALP gene abundance in rhizosphere soils was positively correlated with ALP activity but negatively correlated with P availability. Increased levels of salinity have been associated with shifts in ALP activity, albeit with variable responses. Morrissey et al. (2014) reported a positive association between increased salinity and ALP activity as a consequence of increases in the bioavailability of organic substrates and changes in microbial community structure. However, Jackson and Vallaire (2009) observed that an increase in salinity to 3.5 ppt decreased the phosphatase activity by almost 20%. Therefore, systematic studies of P speciation, ALP activity, and phoD phosphatase genes would allow the evaluation of P dynamics.
in tidal estuarine wetlands and the prediction of eutrophication risk due to P mobilization.

The Min River estuarine tidal marsh is the largest in southeastern China and is characterized by a transition from freshwater to oligohaline water (Tong et al. 2017), providing an ideal model environment to study responses of soil P availability and phoD gene community to variation along a freshwater-oligohaline gradient. Previous studies in this estuary have found greater levels of porewater sulfate (SO$_4^{2-}$), chloride concentrations (Hu et al. 2019), and plant biomass, along with a larger pool of iron oxides and lower levels of sulfide (Luo et al. 2019) in oligohaline marshes than in freshwater. However, variation in soil P dynamics and phoD phosphatase genes between freshwater and oligohaline marshes remains unclear. Thus, the objectives of this study were to (1) evaluate the P dynamics and differences in P speciation and the associated drivers in estuarine marshes and (2) quantify the responses of ALP activity and phoD community composition to freshwater and oligohaline transition and the associated interactions with P availability. We hypothesized that soil P availability is greater at oligohaline sites due to shifts in P-related physicochemical properties (first hypothesis) and that the transition from freshwater to oligohaline reshapes the bacterial phoD gene community and modulates ALP activity due to the associated changes in nutrient levels (second hypothesis).

2. Materials and methods

2.1. Study sites

The study area at the Min River estuary (Fig. 1) is in a region with a humid, subtropical monsoon climate, where the average annual temperature and precipitation are 19.85 °C and 1905 mm, respectively (Tong et al. 2017). The tides are semidiurnal over a 24-h cycle, and the soil surface is completely exposed at low tide (Tong et al. 2014). Further details of the Min River estuary are described in our previous studies (Luo et al. 2019; Tong et al. 2017). We selected three tidal marsh study sites (Fig. 1) that included a freshwater site in the Tajiaozhou wetland (A; 25°56′59.9″N, 119°21′07.8″E) with an average salinity of 0.08 ±0.02 ppt and two
oligohaline sites at the mouth of the Min River estuary in the Bianfuzhou (B; 26°03′12.0″N, 119°33′25.1″E) and Shanyutan (C; 26°0′50.7″N, 119°40′28.4″E) wetlands that are affected by tidal saltwater intrusion and have average salinities of 1.27 ±0.09 and 3.31 ±0.14 ppt, respectively (Luo et al. 2019). At each marsh, we selected an area dominated by the native sedge grass *Cyperus malaccensis*.

### 2.2. Soil and water sampling

In each marsh, two plots (each with 1 m × 1 m dimension) were established in 2018, one vegetated and the other unvegetated. Three soil samples were randomly collected from each plot. For rhizospheres, entire plants with roots were sampled from the vegetated plots, and rhizosphere soil was collected by scraping the soil that was attached to the roots. For bulk soils, surface soil (0-20-cm deep) was collected from the unvegetated plots and immediately placed in sterile, vacuum-sealed polyethylene bags that were transported to the laboratory in a portable refrigerator containing ice within 12 h. The overlying water was simultaneously collected with the soil samples from each plot during low tide, when the soil surface was exposed, and filtered using a 0.45-µm cellulose membrane filter (Millipore Sigma, MA, USA).

At the laboratory, soil samples were sieved through a 2-mm mesh within an anaerobic glove box and then divided into three subsamples. One subsample was immediately stored at -80 °C prior to DNA extraction and bacterial *phoD* gene analysis, one subsample was stored at 4 °C for the measurement of ALP activity, and the remaining subsample was immediately freeze-dried and stored in a glass vacuum desiccator for the determination of soil physicochemical properties.

### 2.3. Analysis of soil and water physicochemical properties

Soil pH was measured *in situ* using an IQ150 meter (IQ Scientific Instruments, Carlsbad, USA), and electrical conductivity (EC) was monitored using an EC meter (FieldScout 2265FS, Spectrum Technologies, Aurora, USA). Soil total organic carbon (TOC) and TS concentrations were determined using a Vario MAX element...
analyzer (Elementar, Frankfurt, Germany), and carbonate was removed using 10% HCl before TOC determination. Soil particle size distribution was determined using a Malvern Mastersizer-2000 laser particle size analyzer (Malvern Instruments, Malvern, UK). The overlying water SO$_4^{2-}$ concentration was measured using ion chromatography (Dionex, Sunnyvale, USA), salinity was measured directly using a Salt 6+ salinity meter (Oakton Instruments, IL, USA), and total P concentration (TP$_w$) was determined using a continuous flow analyzer (Auto Analyzer 3, Bran+Luebbe, Germany) following digestion with K$_2$SO$_4$.

### 2.4. Soil P speciation

Phosphorus speciation was characterized as total P (TP), inorganic P (IP), or organic P (OP) and was analyzed according to the protocol method presented in Ruban et al. (1999) and Ruban et al. (2001). Further, the IP fraction was classified as aluminum-bound P (Al-P), Fe-bound P (Fe-P), calcium-bound P (Ca-P), and occluded P (O-P) and was determined following a sequential extraction procedure based on differential solubility in different chemical extractants (Table S1), as described by Chang and Jackson (1957) and modified by Hartikainen (1979). This method has previously been used for analysis of freshwater and saltwater sediments (Laakso et al. 2016; Rahutomo et al. 2019; Ray et al. 2018; Zhang et al. 2015). Available P (AP) was measured following a 0.5 M NaHCO$_3$ extraction (Olsen and Sommers 1982). The P concentrations of extracts were analyzed using the molybdenum blue spectrophotometry method (Paludan and Morris 1999).

### 2.5. Soil ALP activity assay

We estimated the ALP activity from the production of p-nitrophenol (pNP) from p-nitrophenyl phosphate (p-NPP), as described by Tabatabai and Bremner (1969), where 1 g of soil (dry weight equivalent) was incubated using para-nitrophenyl phosphate (Macklin Biotechnology, Shanghai, China) as the substrate in a modified universal buffer at pH 11 (Tabatabai and Bremner 1969). Following incubation at 37 °C for 1 h, the reactions were terminated using 1 M NaOH and centrifuged at 4000 rpm for 15 min. The colorimetric determination of
pNP formation in the supernatant was analyzed using a spectrophotometer at 410 nm (Thermo Fisher Scientific, MA, USA), and the ALP activity was expressed as micrograms of pNP released by 1 g of soil (dry weight equivalent) per hour (µmol g⁻¹ h⁻¹).

2.6. Bacterial phoD gene analysis

Total genomic DNA was extracted from 0.5 g of fresh soil using Fast DNA SPIN extraction kits (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer’s instructions and was stored at -20 °C prior to analysis. The quantity and quality of extracted DNAs were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively.

The primer set ALPS-F730 (5’-CAGTGGGACGACCACGAGGT-3’) and ALPS-1101 (5’-GAGGCCGATCGGCATGTCG-3’) was used to amplify the bacterial phoD gene (Sakurai et al. 2008), where PCRs (30 µL) comprised 15 µL of Phusion High-Fidelity PCR Master Mix (New England Biolabs), 0.2 µM of forward and reverse primers, and approximately 10 ng of template DNA. Amplification was performed in a thermal cycler with an initial denaturation at 98 °C for 2 min, followed by 25 cycles of denaturation at 98 °C for 15 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s, with a final extension for 5 min at 72 °C (Huang et al. 2017). Purified amplicons were quantified on a microplate reader (BioTek, Vermont, USA) using a Quant-iT PicoGreen dsDNA assay kit (Invitrogen, P7589) and were subsequently pooled in equal amounts. Paired-end (2×300 bp) sequencing was performed using an Illumina MiSeq platform with a MiSeq rReagent kit (v3) at Shanghai Personal Biotechnology (Personal Biotechnology Co., Ltd., Shanghai, China).

Although the ALPS primer have an amplification bias toward Alphaproteobacteria (Ragot et al. 2015; Tan et al. 2013), this set of primers has been conducted by most of the previous studies (Acuña et al. 2016; Chen et al. 2019a; b; Fraser et al. 2015; Fraser et al. 2017; Hu et al. 2018b; Huang et al. 2019; Luo et al. 2017; Matsuoka et al. 2019; Sun et al. 2019; Tan et al. 2013; Valdespino-Castillo et al. 2014; Wan et al. 2019; Wei
et al. 2019), and making it possible to compare phoD-harboring bacterial community among studies.

2.7. Pyrosequence data processing

Raw sequence reads with exact matches to barcodes were assigned to respective samples and identified as valid sequences. After chimera detection, the remaining high-quality sequences were classed into operational taxonomic units (OTUs) using a sequence similarity threshold of 97%. To minimize differences in sequencing depth across samples, an averaged, rounded rarefied OTU table was generated by averaging 100 evenly resampled OTU subsets at <90% of the minimum sequencing depth for further analysis (QIIME, v1.8.0).

Abundance at the phylum, class, order, family, genus, and species levels was compared among samples or groups using Metastats (White et al. 2009). Indices of OTU-level alpha diversity, such as the Chao1 richness estimator, abundance-based coverage estimator (ACE), Shannon’s diversity index and Simpson’s evenness index, were calculated using the OTU table in QIIME (Caporaso et al. 2010). We analyzed beta diversity to investigate the structural variation in microbial communities using UniFrac distance metrics based on the OTUs and visualized these results using principal coordinates analysis (PCoA) and pair-group method with arithmetic mean (UPGMA) hierarchical clustering (Ramette 2007).

2.8. Statistical analysis

When necessary, data (i.e., P concentrations and environment variables) were log-transformed to meet the ANOVA assumption of normality and homoscedasticity. Pearson's correlation coefficient was used to test the potential correlation between soil P concentration, phoD gene diversity, ALP activity, and environmental parameters, and the correlation matrix was visualized using the 'corrplot' package in R. Regression analysis was used to explore the relationship between AP concentration, ALP activity, and phoD gene diversity. Redundancy analysis (RDA) was performed to identify the main influencing factors of soil P dynamics using Canoco 4.5 (Microcomputer Power, Ithaca, USA). Overall distributions and variations in soil P fraction, environmental parameter, and phoD gene community among the study sites were summarized using a principal
components analysis (PCA) in Statistica 6.0 (StatSoft, Tulsa, USA).

3. Results

3.1. Soil and overlying water physicochemical properties

Physicochemical properties varied between the freshwater and oligohaline marshes (Table 1), where the levels of soil pH, EC, and TS were greater, but that of TOC was lower, at oligohaline sites. Overall, surface soil mainly comprised silt (55%), followed by sand (34%) and clay (11%). The proportions of silt and clay were greater, while that of sand was lower, at oligohaline sites. The salinity and SO$_4^{2-}$ concentration of overlying water at oligohaline site C were greater than at freshwater site A, and there was no difference in TP$_w$ concentration among the study sites. In general, there were no within-study site differences between the rhizosphere and bulk soil parameters; exceptions were for lower levels of pH, TS, and sand and higher levels of TOC and clay in the rhizosphere at oligohaline site B and respectively higher and lower levels of silt and sand in the rhizosphere at oligohaline site C (Table 1).

3.2. Soil P dynamics

There were some differences in soil P concentrations in the rhizosphere and bulk soils, where the rhizosphere TP concentration was lower at freshwater site A than at oligohaline site B. The bulk soil IP concentration was lower at the freshwater site than at oligohaline site B. The OP concentration was lower in rhizosphere and bulk soils at the freshwater site than at the two oligohaline sites (B and C). The AP concentration in rhizosphere soils was greater at oligohaline site C than at the freshwater site and oligohaline site B. The AP concentration in bulk soils was greatest at oligohaline site B and lowest at the freshwater site ($P < 0.05$; Figs. 2a-d). Overall, IP accounted for 66-89% of TP (Figs. 2a, b). Among the IP fractions, the concentrations of Fe-P in rhizosphere and bulk soils were greater at the freshwater site than at the two oligohaline sites, whereas the concentration
of Ca-P in the two soil profiles was lower (Figs. 2e, g). The Al-P concentration in the rhizosphere was lower at the freshwater site than at the two oligohaline sites and lower in the bulk soil at the freshwater site than at oligohaline site C (Fig. 2f). The O-P concentrations in the rhizosphere and bulk soils were lower at the freshwater site and oligohaline site B than at oligohaline site C (Fig. 2h). Al-P and O-P were the dominant forms of IP (38 and 30%, respectively), followed by Fe-P (21%) and Ca-P (11%) at all sites (Fig. S1). There were few within-site differences in the soil P concentrations between rhizosphere and bulk soils (Fig. 2). TP and Fe-P at the freshwater site were respectively lower and greater in the rhizosphere than in the bulk soils, while at oligohaline site B, the concentration of AP was lower in the rhizosphere than in bulk soils, but that of Fe-P was greater.

3.3. Soil ALP activities

Soil ALP activity was 7-fold greater in the freshwater (0.7 μmol g⁻¹·h⁻¹) than in the oligohaline marshes (0.1 g⁻¹·h⁻¹), but there were no within-study site differences in ALP activity between the rhizosphere and bulk soils (Fig. 3).

3.4. Richness and alpha diversity of the phoD phosphatase gene

A total of 73,350 qualified sequences of the *phoD* phosphatase gene were recorded from the soil samples. The richness and alpha diversity of the *phoD* phosphatase gene were greater in the rhizosphere than in the bulk soils at site B, and overall, these values were greatest at site B and lowest at site A (Table 2). There was no clear pattern in *phoD* gene diversity with the transition from freshwater to oligohaline environments. The PCoA showed that the bacterial *phoD* gene communities at freshwater site A were more loosely clustered and distinct from those at oligohaline sites B and C (Fig. 4).

3.5. phoD phosphatase gene community structure

Overall, the most abundant bacteria classes containing the *phoD* gene were the *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria*, accounting for 30-80% of total sequences (Fig. 5a). The
dominant genera in all samples were Pleomorphomonas, Streptomyces, Cupriavidus, Bradyrhizobium, and Pseudomonas (relative abundance >1%) (Fig. 5b). Specifically, Streptomyces, Cupriavidus, and Bradyrhizobium were the dominant genera at the freshwater marsh (relative abundance: 43, 19, and 9%, respectively), and Pleomorphomonas, Streptomyces, and Bradyrhizobium were dominant at the oligohaline sites (relative abundance: 52, 22, and 5%, respectively).

The relative abundance of the phoD-harboring bacterial shifted significantly along a freshwater-oligohaline gradient, where the relative abundance of the Alphaproteobacteria class was greater at oligohaline sites, but that of the Betaproteobacteria was greater at freshwater sites. The relative abundance of the Pleomorphomonas genus was greater, whereas those of Streptomyces and Cupriavidus genus were lower, at oligohaline sites. Hierarchical cluster analysis showed that bacterial communities formed three groups that represented the three study sites (Fig. S2). However, there were no within-site differences between rhizosphere and bulk soil communities.

3.6. Relationships between soil P and phoD gene communities with soil and overlying water variables

The concentrations of soil Al-P, Ca-P, O-P, and AP were positively correlated with soil pH, EC, TS and silt content and negatively correlated with soil TOC and sand content. The soil Fe-P concentrations were negatively correlated with pH, EC, and TS and positively correlated with soil TOC. The soil OP concentrations were positively correlated with soil pH, EC and overlying water salinity (Fig. 6). The concentrations of soil Ca-P and AP were positively associated with soil clay content. Soil Al-P, Ca-P, O-P, and AP were also found to be positively related to overlying water salinity and SO$_4^{2-}$ concentration.

We found that the relative abundance of Pleomorphomonas was negatively associated with the contents of TOC and sand and positively associated with other environmental variables; that of Streptomyces showed the opposite trend (Fig. 6). The soil ALP activity was positively correlated with soil TOC and sand content and negatively correlated with soil pH and EC, contents of TS, clay, and silt, and overlying water salinity and SO$_4^{2-}$.

We examined the relationships between the AP concentration, phoD gene community, and ALP activity (Fig. S3) and found that soil AP was positively correlated with the phoD gene diversity but negatively
correlated with the ALP activity. The soil AP was positively related to the relative abundance of
Pleomorphomonas and negatively related to the abundance of Streptomyces. The soil ALP activity was
negatively related to the bacterial phoD gene diversity and the relative abundance of Pleomorphomonas.

The first two axes of the RDA of the influence of biochemical variables (environmental variables, phoD
gene community, and ALP activity) accounted for 78.6% of the variation in P dynamics (P < 0.01) that
clustered into three groups (groups I, II, and III) representing the three study sites (A, B, and C, respectively)
(Fig. 7). The soil P dynamics in Group I (freshwater) were primarily influenced by the soil contents of TOC,
ALP activity, and the relative abundance of Streptomyces, while in Groups II and II (oligohaline), they were
primarily driven by overlying water salinity and SO$_4^{2-}$, soil TS and pH, phoD gene diversity, and the relative
abundance of Pleomorphomonas.

4. Discussion

4.1. Soil P speciation responses to a freshwater-oligohaline gradient

Previous studies have indicated that P dynamics and associated speciation varied from freshwater to saltwater
due to shifts in soil physicochemical and microbial processes (Gireeshkumar et al. 2013; Paludan and Morris
1999). As expected, we observed higher of soil-water salinity, pH and SO$_4^{2-}$ content, lower soil TOC, and
greater associated soil AP concentration in the transition from freshwater to oligohaline sites (Table 1; Fig. 2),
supporting our first hypothesis that soil P availability is greater at oligohaline sites due to shifts in the P-related
physicochemical properties. It is possible that the salinity brought by seawater influences P dynamics through
changes to adsorption and desorption reactions triggered by an increase in ions in the sediment that compete
with phosphate ions (PO$_4^{3-}$) for the sorption sites (Qu et al. 2018). This response was demonstrated by the
positive correlation between the AP concentration and overlying water salinity (Fig. 6). The abundance of
electron-accepting SO$_4^{2-}$ in the oligohaline marshes (Table 1) may enhance the release of P as a result of sulfate
reduction (SR), but it may also compete with $PO_4^{3-}$ for anion adsorption sites (Caraco et al. 1990), thereby regulating P availability. However, we did not find a difference in soil IP concentration among the sites, even though the OP levels were greater in the oligohaline sites than in freshwater (Figs. 2b, c), possibly as a result of a slower capacity for P-mineralization in oligohaline soils than in freshwater, as indicated by the lower ALP activity in the oligohaline soils (Fig. 3).

The concentrations of Al-P, Ca-P, and O-P in the IP fraction increased from freshwater to oligohaline sites (Figs. 2f-h), while Fe-P markedly decreased (Fig. 2e), representing a shift from Fe-P to Ca-P and Al-P due to the reduction of Fe along a freshwater-oligohaline gradient. These findings are consistent with the greater availability of $SO_4^{2-}$ in oligohaline sites (Table 1). This greater availability promotes the formation of sulfide and subsequent Fe(II) sulfides due to the high rates of SR, which increase the release of $PO_4^{3-}$ due to the reduction of Fe(III)-bound-P, thus reducing Fe-P storage in the soil (Dierberg et al. 2011; Luo et al. 2019). It is also likely that greater levels of alkalinity generated by SR in anoxic soils may inhibit P sorption onto iron oxides in soils (Caraco et al. 1989). The shifts in soil physicochemical properties from freshwater to oligohaline sites also affected the P fraction. We found that soil concentrations of Al-P, Ca-P, and O-P were positively associated with silt content in the soil and negatively associated with sand content (Fig. 6), indicating that soil texture is a driver of P dynamics in estuarine marshes. This finding may be explained by differences in the substrate surface area and reactivity, as the greater surface area of silt particles provides more binding sites for $PO_4^{3-}$ adsorption in the soil (Gireeshkumar et al. 2013). Our findings also indicate that the transition from freshwater to an oligohaline environment produces more strongly occluded P in soil so that the long-term P storage capacity in soil is enhanced with moderate increases in salinity.

We found that the IP fraction of soil P was primarily controlled by Al-P (38%), followed by O-P (30%), Fe-P (21%), and Ca-P (11%) (Fig. S1). The Min River estuary is located in the subtropics, where the weathering of parent rock is relatively strong under the warm, humid climate, resulting in high levels of organic matter
rich in Fe/Al oxide (Luo et al. 2014; Luo et al. 2019). The release of Fe and Al facilitates the enrichment and
migration of P as the adsorption carriers, thereby controlling the IP fraction. In this study, the average soil TP
concentration across study sites in the estuary (698 mg·kg⁻¹) did not vary with the transition from freshwater
to oligohaline sites (Fig. 2a). In addition, the TP concentrations were similar to those recorded in estuaries in
Portugal (Coelho et al. (2004) and elsewhere in China (Jin et al. (2013) but lower than those recorded from
estuaries in the US (Jordan et al. 2008) and Sri Lanka (Gireeshkumar et al. 2013) (Table S2). Within-estuary
consistency in soil TP content may be the result of a combination of changing dominance of the actions of
various forms of P and their trade-offs because we found that while some P-fractions were more abundant at
oligohaline sites (AP and O-P), other, mostly moderately labile forms, were less abundant. Our finding that
soil TP, and its associated speciation, did not differ between rhizosphere and bulk soils was inconsistent with
previous studies (Hinsinger 2001; Luo et al. 2017). This inconsistency may be due to the periodic flooding
and associated variations in salinity that drive complex hydrological and nutrient cycling in estuarine
environments (Hu et al. 2018a), which partially offset possible underlying rhizosphere effects on soil P
dynamics. Moreover, the resuspension of sediment driven by tide and river runoff may have stimulated P
immobilization to change the transformation of soil P fractions (Labry et al. 2016). Thus, shifts in soil P
fractions from freshwater to oligohaline sites may be a consequence of the direct effects of salinity and the
indirect effects of altered soil/water physicochemical properties due to saltwater intrusion.

4.2. Effects of salinity on ALP activity and the phoD gene community

Greater rates of ALP activity were observed in the freshwater than the oligohaline sites, regardless of soil type
(rhizosphere/bulk) (Fig. 3). These findings corroborate a study by Jackson and Vallaire (2009) but are in
contrast to those by Morrissey et al. (2014) and Labry et al. (2016). It is known that pH plays a crucial role in
the regulation of phosphatase activity, and ALP activity is predominant in alkaline environments (Stout et al.
2014). However, and somewhat surprisingly, we did not observe this phenomenon in our study because the
soil ALP activity was negatively correlated with pH (Fig. 6). This absence of a positive effect of pH on ALP activity may be due to the limited variation in pH value (6.4-7.4; Table 1) or the low concentrations of PO₄³⁻ that drive ALP activity in P-deficient environments (Fraser et al. 2015; Labry et al. 2016). In this study, soil AP concentrations were greater in the oligohaline sites than the freshwater site, decreasing the requirement for ALP synthesis to facilitate P uptake in microbes and plants, as indicated by the negative association between ALP activity and soil AP (Fig. S3d). It should be noted that the ALP activity of this study refers to potential phosphatase activity rather than actual soil phosphatase activity because the pH has been modified, which allows it to be compared to other studies (Chen et al. 2019a; b; Fraser et al. 2015; Fraser et al. 2017).

Although phoD genes have been investigated extensively under various environmental conditions (Fraser et al. 2015; Ragot et al. 2015), the influence of variations with the transition from freshwater to oligohaline environments remains unclear. Here, the alpha-diversity of bacterial phoD genes did not vary with the transition (Table 2), possibly due to the relatively narrow range of salinity (0.03–2.92 ppt) at the study sites, which may not have been sufficient to result in differences in diversity. In contrast, the soil bacterial phoD gene community compositions differed between the freshwater and oligohaline sites (Fig. 5). Overall, Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria were the most abundant bacterial classes in the study sites (>59% of total bacteria; Fig. 5), supporting other studies of various types of soil (Lagos et al. 2016; Luo et al. 2017; Tan et al. 2013) and indicating that the phoD-harboring bacteria community composition is stable across different environments. The greater relative abundance of Pleomorphomonas and the lower abundance of Streptomyces at oligohaline sites relative to freshwater (Fig. 5) may reflect their responses and adaptations to nutrient availability and salinity fluctuations (Spohn et al. 2015).

Our analysis of the community composition of phoD-harboring bacterial genera showed that the relative abundance levels of some genera, such as Pleomorphomonas, were much greater at oligohaline sites, while that of Streptomyces was lower (Fig. 5). In addition to the direct influences of soil osmotic potential and water
stress caused by salinity (Chambers et al. 2013), changes in soil pH, TOC, and texture with the transition from freshwater to oligohaline environments may have affected phoD gene community composition. For example, we found that soil TOC was positively associated with the relative abundance of Acidobacteria (Fig. 6), likely because substrates rich in C favor growth of some phoD-harboring species, leading to increases in the abundance of the phoD genes and ALP activity (Luo et al. 2017). It is important to note that soil texture, which is correlated with variations in bacterial community composition, may affect the adsorption of chemicals by distinct microbial communities due to differences in surface properties and microenvironments (Hemkemeyer et al. 2015). Overall, these results support our second hypothesis that the transition from freshwater to oligohaline environments shapes bacterial phoD gene communities and influences ALP activity.

4.3. Linking ALP activity and phoD gene community structure with P availability

Soil ALP activity has been regarded as an indicator of changes in organic P mineralization and bacterial phoD gene abundance in soils (Acuña et al. 2016; Huang and Morris 2003). The negative relation between the soil ALP activity and AP concentration (Fig. S3d) provides additional evidence that ALP activity is only induced at low P levels due to the inhibited synthesis of phosphatases at high AP concentrations (Acuña et al. 2016; Fraser et al. 2015). The differences in relative abundance of the dominant phoD gene community may play a key role in P availability because soil AP concentration was positively associated with the relative abundance of Pleomorphomonas (Fig. S3b) but negatively associated with that of Streptomyces (Fig. S3c). Both of these dominant genera are important for P solubilization and mineralization through the production of P-hydrolyzing enzymes (Acuña et al. 2016; Ragot et al. 2015). In general, potential ALP activity may have been directly regulated by the phoD phosphatase gene community due to the greater production efficiency of extracellular alkaline phosphatases (Luo et al. 2017). The high taxonomic diversity of the phoD gene community renders it better able to tolerate changes in salinity and potentially affects ALP production (Fraser et al. 2015). Moreover, the responses of the phoD gene
community to the freshwater-oligohaline transition might be partially depend on P availability (Fraser et al. 2015), and high levels of P might inhibit phoD gene expression (Vershinina and Znamenskaya 2002). However, this pattern only was observed in the phoD gene diversity, which showed a negative correlation with the ALP activity. Measuring the gene and transcript levels with more universal phoD primers is thus warranted (Ragot et al. 2015) and will provide a full understanding of ALP production and phoD gene diversity along an estuarine freshwater-oligohaline gradient.

4.4. Implications and uncertainties

In summary, our data clearly showed the variations of soil P fractions, environmental parameters, and phoD gene communities among the study sites, where the levels of salinity and pH were greater, the soil texture was finer, and the contents of O-P/Al-P were greater at the oligohaline sites, which were associated with greater levels of soluble and available P. In contrast, freshwater sites were characterized by coarser textured soils, higher levels of TOC and Fe-P, lower levels of O-P and AP, and higher levels of ALP activity, despite the low pH levels. These results indicate higher microbial and general biological activity, lower P retention capacity and greater biological effort required for P uptake in freshwater conditions. Furthermore, the overall PCA analysis (Fig. 8) indicated that the most diverse bacterial community and the higher, more evenly distributed stocks of P among the rhizospheres and bulk soils occurred at the moderately saline site (site B). Thus, we conclude that with the transition from freshwater to oligohaline sites, the associated increases in soil-water salinity and SO$_4^{2-}$ and decreases in general microbial activity are key drivers of P fractions and availability, which indicates that greater potential P losses may reduce nutrient availability for estuarine plants and microbes under longer-term, climate change-mediated rises in sea levels.

Although these patterns have important implications for our understanding of wetland geochemical processes that drive P cycling, some uncertainties and future works must be carefully considered. First, hydrological conditions, such as periodic tidal processes and seawater intrusion across the estuarine tidal
marshes, might have significant effects on P speciation and the phoD gene community. Therefore, increasing the consideration of tidal effects can contribute to more accurate estimates of soil P dynamics and microbial community along a freshwater-oligohaline gradient. Second, our results support the conclusion of Tan et al. (2013) and Ragot et al. (2015) that the ALPS primers seem to have an amplification bias caused by the primers specificity, for example, numerous sequences were assigned to Alphaproteobacteria, which suggested this primer set could be biased toward Alphaproteobacteria rather than the real distribution of phoD. Therefore, although the present study provided some insights into the phoD bacterial communities along an estuarine freshwater-oligohaline gradient, the interpretation and comparison of our results must be conducted with some caution. Future research should consider newly designed primers based on metagenome databases (Ragot et al. 2015), which probably provide better coverage of the phoD diversity.

5. Conclusions

Our results suggest that the transition from freshwater to an oligohaline environment drives the increases in soil-water salinity, pH and SO$_4^{2-}$ content, a decrease in soil TOC, the associated increases in concentrations of AP, Al-P, Ca-P, and O-P, and a decrease in Fe-P concentrations. These findings support our first hypothesis that soil P availability is greater at oligohaline sites due to shifts in P-related physicochemical properties. These findings also highlight the role of salinity as a substantial factor in P availability, where increases in salinity and the associated changes in soil-water physicochemical properties (SO$_4^{2-}$, pH, and TOC) as a consequence of saltwater intrusion may exacerbate losses of P to water, eventually leading to losses in P-fertility for plants and microbes. The lower levels of soil ALP activity and the altered composition of phoD gene communities at oligohaline sites indicate that even small increases in the salinity levels and the associated shifts in environmental factors can be caused by saltwater intrusion and may regulate microbial activity and reshape the phoD gene community composition in estuarine tidal marshes, supporting our second hypothesis. Our
results showed that the moderately saline study site (site B) was characterized by the most diverse bacterial community and higher and more evenly distributed stocks of P among the rhizosphere and bulk soils. Our results increase our understanding of the main processes and mechanisms involved in the estuarine P dynamics and *phoD* phosphatase gene communities. However, detailed studies and analyses of spatiotemporal coupling and tidal action that control the available soil P pools are required.

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**References**


sediments along a hydrologic gradient in a subtropical estuarine wetland, China. Estuarine Coastal and Shelf Science 154: 30-38.