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Occurrence and ecological implications of pyrophosphate in estuaries

Abstract—Loading of bioavailable phosphorus, traditionally measured as soluble reactive phosphorus (SRP), contributes to the eutrophication of aquatic ecosystems. However, polyphosphates are also bioavailable but escape detection by the standard method used for measuring SRP. ^{31}P nuclear magnetic resonance spectrometric analysis of sediment extracts and enzymatic assay of surface waters reveal heretofore unreported presence of pyrophosphate (Ppi) in coastal wetlands. We show that the accumulation of Ppi (the smallest chemical form of polyphosphate) in coastal wetlands is related to human impact and can occur in quantities that exceed that of SRP. We further demonstrate that Ppi is readily utilized by microbes in coastal wetland sediments in the presence of nitrogen and carbon and can serve as a reservoir of orthophosphate. Thus, Ppi accumulation in estuaries will subsidize the in situ biogeochemical phosphorus cycle. This has important ecological implications for trophic responses and estuarine productivity.

Phosphorus (P) plays a vital role in controlling biotic production in a wide range of ecosystems, ranging from freshwater lakes (Hecky and Kilham 1988) to open oceans (Clark

Table 1. Relative concentrations of SRP, sulfide, and Ppi and the degree of high-impact urban (HIU) areas at sampling sites. SRP and sulfide are concentrations in marsh pore water (geometric mean of monthly measurements for 2 yr integrated over 1 m depth). HIU is high-intensity urban area within a 5-km radius around the sampling site. Ppi is pyrophosphate abundance in NaOH + EDTA extract from surface sediments expressed as (% of NMR-visible and as mg kg^{-1} dry sediment for selected sites). Sediment total P (TP), total P in NaOH + EDTA extract, and extraction efficiency (% of sediment TP) are reported. Key: FM, BM, and SM are intertidal freshwater, brackish, and salt marsh, respectively. FSM and FOR are salt marsh fertilized with $(NH_4)_2HPO_4$ and maritime forest, respectively. SM1 and SM2 are impacted and unimpacted salt marsh, respectively. ND, no data.

Estuary	Site	SRP (μM)	S^{2-} (μM)	HIU ($\times 10^6$ m^2)	Ppi (% of total NMR visible P)	TP sediment (mg kg^{-1})	TP extract (mg kg^{-1})	Extract efficiency (%)	Ppi (mg kg^{-1})
Cooper River	FM	3	5	0.29	4.2	1752	840	48	35.3
	BW	11	15	3.9	12.5	921	396	43	49.5
	SW	35	1,034	14.4	56.9	572	282	49	160.5
North Inlet	SM	4.4	127	0.09	0	64.5	39	61	0
	FOR	ND	ND	0.09	0	ND	ND	—	—
	FSM	35	151	0.09	0	ND	ND	—	—
Edisto River	SM1	46	166	1.16	5.5	933	383	41	21
	SM2	69	451	0.21	0	626	300.5	48	0
Plum Island	BM	1.7	2.3	1.64	3.9	ND	ND	—	—
	SM	7.1	508.9	0.66	0	ND	ND	—	—

et al. 1998). Phosphorus occurs naturally in various organic and inorganic forms. Because estimation of these P pools is based on analytical procedures that target specific classes of P compounds, significant analytical overlap complicates the interpretation of results. For instance, the soluble reactive phosphorus (SRP) pool and the true ''orthophosphate'' pool are both considered as bioavailable P. However, research from lakes has shown that analytical procedures employed to measure SRP overestimate the concentration of orthophosphate because the acidic conditions of the reaction also hydrolyze some organic P compounds (e.g., Tarapchak et al. 1982). Nevertheless, novel techniques have been developed that circumvent these problems (Karl and Tien 1992; Thomson-Bulldis and Karl 1998). Although, studies of eutrophication have traditionally focused on SRP, other forms of P, such as organic P compounds and polyphosphates, are also important. However, some of these forms cannot be detected by the standard method used for measuring SRP (Strickland and Parsons 1972). For this reason, considerable effort is being expended to estimate the relative proportion of various P pools and their ecological significance in a wide range of ecosystems. In fact in certain samples, it is often observed that the sum of the concentration of P in various analytically defined P pools is less than the total P in the sample. This discrepancy was attributed to a missing P pool, the occurrence of which suggests (although indirectly) the presence of inorganic polyphosphates in the sample (Thomson-Bulldis and Karl 1998). Central to these investigations is the quest to understand the sources and ecological relevance of these operationally defined P pools. Although it has been shown that certain organic P pools are enzymatically remineralized and that such remineralization is indeed ecologically significant (e.g., Clark et al. 1998), bioavailability studies and characterization of missing and other nonreactive P pools is far from complete. We show here that pyrophosphate ($P_2O_7^{(\text{variably charged } 1- \text{ to } 4-)}$), the smallest chemical form of inorganic polyphosphate, can also contribute to the total bioavailable P pool in coastal wetlands and that its accumulation in coastal wetlands is related to the degree of anthropogenic impact.

We related the relative abundance and distribution of pyrophosphate (Ppi) in marsh sediments from 10 locations in four different estuaries within South Carolina and Massachusetts, U.S.A., to the degree of urban impact around these sites (Table 1). These estuaries differ in their nutrient status and the degree of anthropogenic impact. Subsequently, we explored the implications of the presence of Ppi in coastal wetland sediments in the context of bioavailable P pools.

Study sites—For the purpose of this study, we categorized our sampling locations in the following categories based on the urban impact estimates around each site (*see below*). Out of a total of 11 sampling locations in four different estuaries in South Carolina and Massachusetts, five were classified as impacted, three as minimally impacted reference sites, one as maritime forest, and two as fertilized sites located in a minimally impacted estuary (Table 1). The impacted sites included three intertidal marshes located along the estuarine salinity gradient on the urbanized Cooper River estuary in South Carolina, a salt marsh (SM1) on the Edisto River (ACE Basin) in South Carolina, and a brackish marsh (BM) in the Plum Island estuary, Massachusetts. Three other salt marshes located in the minimally impacted North Inlet estuary (in South Carolina), the Edisto River (ACE Basin) (SM2), and the Plum Island estuary served as reference sites. Furthermore, two fertilized plots, adjacent to the reference site in the North Inlet estuary, were sampled to test the effect of duration of fertilization and the type of fertilizer used on the occurrence of Ppi. One of the fertilized plots has been fertilized with a commercial P fertilizer, Triple Superphosphate, for 2 yr with an annual dosage of 15 mol P m^{-2}. The second experimental plot was fertilized for 15 yr with reagent-grade ammonium phosphate to achieve a final annual dosage of 30 mol N m^{-2} and 15 mol P m^{-2}. Additionally,

one maritime forest site in the North Inlet estuary was also sampled.

Estimation of urban impact—The location of each sampling site in South Carolina and Massachusetts was determined using global positioning system (GPS), and a buffer radius of 5-km was generated around each site using geographic information system (GIS). The buffer around each sampling site was overlaid with the respective land use and land cover data to determine the type (e.g., urban, open water) and quantity of land use and land cover within it. For the South Carolina study sites on the Cooper River estuary, the North Inlet estuary, and the Edisto River (ACE Basin), the source data for the land use and land cover data layer were SPOT 20-m satellite images (classified according to the Anderson level I/II scheme) acquired during leaf-off conditions in 1989 and 1990 and rectified using a statewide database of GPS points as ground control points. For the Massachusetts study sites on the Plum Island estuary, the statewide 1 : 25,000 land use data layer was used to determine the land use characteristics around each site, developed originally from 1 : 25,000 aerial photography acquired in 1971. Updates to the original land use data layers were completed using 1 : 40,000 9″ by 9″ color infrared photographs acquired during 1984 and 1985. Further updates to the coastal areas of Massachusetts, including our study sites, took place in 1991 when the classification system was also revised to include additional classes (http://www.magnet.state.ma.us/mgis/lu-doc.htm). We expressed urban impact as the high-impact urban (HIU) area within the 5-km radius around our sampling sites, estimated using ArcView® and the land use classification as defined under the National Land Cover Data (NLCD). The HIU category includes the two NLCD class definitions that target high-intensity residential areas (including apartment complexes and row houses but excluding single-family housing units which typically dominate suburban development) and urban areas under commercial/industrial/transportation use.

^{31}P nuclear magnetic resonance (NMR) analysis of sediments—Duplicate surface sediment (0–10 cm) cores were pooled and extracted overnight at room temperature (using a soil : solution ratio of approximately 10 g dry sediment : 100 ml 0.5 M NaOH + 0.1 M ethylenediaminetetraacetic acid [EDTA]) (Cade-Menun and Preston 1996). Extraction efficiencies were calculated in a parallel experiment. Sediment total P was measured using x-ray fluorescence and wet combustion techniques. Total P in NaOH + EDTA extract were measured by acid persulfate digestion. Extraction efficiencies are reported in Table 1. Comparable extraction efficiencies suggest that the relative differences in distribution and abundance of P species at and among sites were not due to extraction artifacts. For ^{31}P NMR analysis, sediment extracts were centrifuged at 3,200 rpm for 10 min, and 3 ml of the supernatant was used for NMR analysis after adding 0.3 ml D_2O. The pH of sediment extracts was between 11.5 ± 0.4 pH units. All spectra (202.46 MHz) were collected on a Varian Inova 500 spectrometer. Data were collected with a 200 ppm window centered at 0 ppm. A 45° pulse width with 2.1 s interpulse delay was used to collect 24,320 complex data points, which were processed with 10 Hz line broadening. Signal averaging varied with each sample as shown (Fig. 1). Chemical shifts are relative to 85% phosphoric acid. External standards were run as a mixture of 1 mM solution of inorganic orthophosphate (KH_2PO_4), representative phosphomonoesters (orthophospho-L-serine and DL-glycerophosphate), and pyrophosphate (tetrasodium Ppi, $Na_4P_2O_7$), in a matrix of 0.5 M NaOH + 0.1 M EDTA. Peak assignments based on external standards (Fig. 1A) and literature (e.g., Cade-Menun and Preston 1996) are as follows: orthophosphate at 5.2 ppm, phosphomonoesters (between 3.4 and 4.8 ppm), and a diester peak at around 0 ppm and a Ppi peak at around −5.6 ppm. Higher order polyphosphates (e.g., sodium tripolyphosphate, adenosine triphosphate [ATP], and ammonium tetrapolyphosphate) typically have multiple peaks at chemical shifts < -6 ppm (Robitaille et al. 1991). To estimate the Ppi concentration in NaOH + EDTA extract of wetland sediments, we relied on the total P in the extract and the percent contribution of Ppi to this total P as calculated from the ^{31}P NMR spectrum for corresponding sites. These values were converted to Ppi (mg kg^{-1} dry sediment) for select sites based on corresponding sediment total P and the extraction efficiency.

Ppi in surface and pore water samples—Ppi in surface and pore water was estimated using an enzymatic assay (Sigma product P-7275) after optimizing it for natural samples. This method was originally developed to monitor the production of Ppi in biochemical studies (e.g., O'Brien 1976), and consists of a cascade of enzymatic reactions, of which the first enzyme is Ppi dependent. In this assay, 2 mol of the reduced form of nicotinamide adenine dinucleotide (NADH) is oxidized to NAD for every mole of Ppi consumed. This reaction is monitored spectrophotometrically at 340 nm. Because organic acids also absorb strongly at this wavelength, potential problems can arise when attempting to use this technique to directly quantify the concentration of Ppi in NaOH + EDTA extract of wetland sediments. This is because large amounts of organic matter is also extracted (as highly colored material) from the sediments in the NaOH + EDTA extraction scheme, which could potentially interfere with the absorbance at the wavelength of interest. Prior to the use of the enzymatic assay (to measure Ppi in surface and pore water samples), we performed a laboratory test to evaluate the effect of changes in ionic strength of the water sample on the performance of the kit. We found that the kit performed very well in a simulated salinity gradient created using NaCl. Additionally, a comparison of actual and estimated Ppi concentration in a series of standards (5 μM to 200 μM) showed that this enzymatic kit predicts the Ppi concentration in water samples very well (slope = 1.09, r^2 = 0.96, n = 10). However, for Ppi concentrations that are below 50 μM, it is helpful to maximize the ratio of sample volume : reagent mixture volume while keeping the total volume of the reaction mixture constant. To the best of our knowledge, this is the first use of this method to detect Ppi in natural water samples.

Pore water nutrients—SRP (Strickland and Parsons 1972) and sulfides (Otte and Morris 1994) in marsh pore water

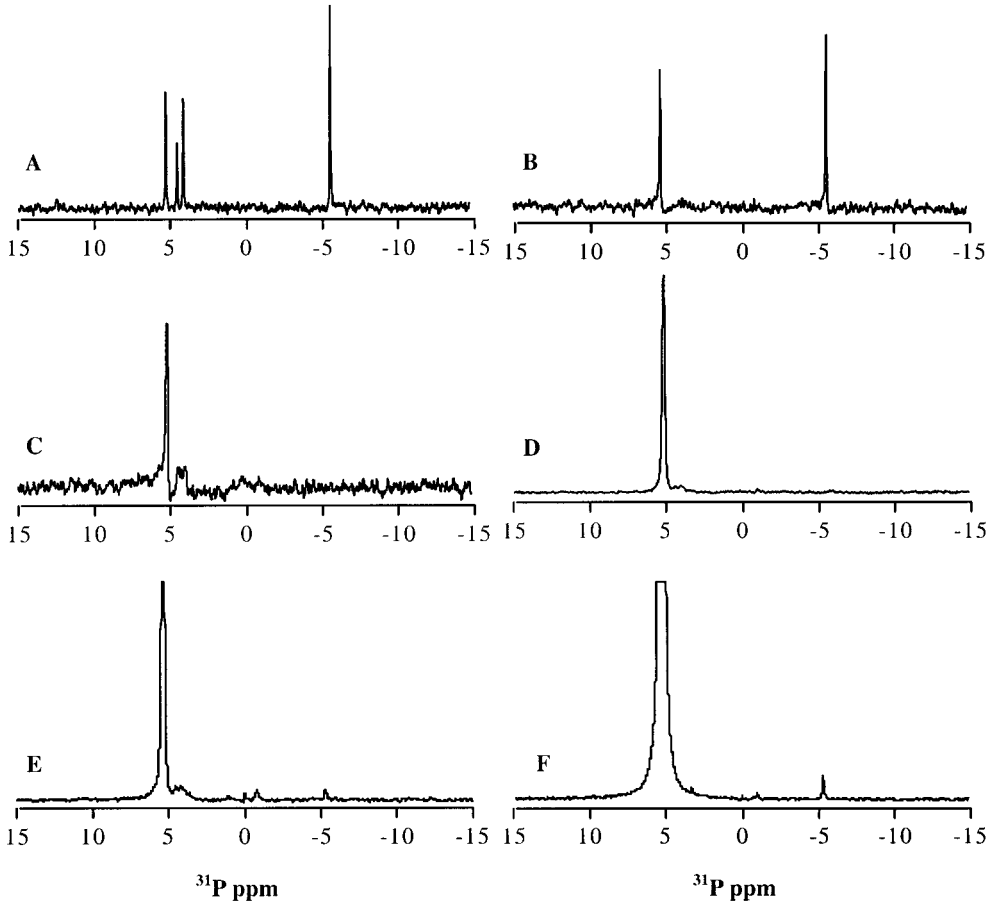

Fig. 1. Solution ^{31}P NMR spectra of sediment extracts from four representative salt marsh sites from two watersheds. A peak corresponding to Ppi is found at −5.6 ppm in the NMR scans. (A) External standards run in the NaOH + EDTA matrix. (B) Salt marsh located on the heavily industrialized Cooper River. Total number of scans = 112,000. (C) A salt marsh located in the pristine North Inlet estuary. (D) A fertilized plot in the pristine North Inlet estuary, fertilized with ammonium phosphate (a laboratory chemical that does not contain Ppi). (E) An experimentally fertilized plot in North Inlet estuary (fertilized with commercial Triple Superphosphate). (F) Saturated solution of commercial fertilizer Triple Superphosphate. Total number of scans = 102,000 for (C) and (F). Total number of scans = 40,000 for (C) and (D). Note the absence of a peak corresponding to Ppi (−5.6 ppm) in (C) and (D) and its presence in (A), (B), (E), and (F). Additional details on peak assignment are provided in the text titled *^{31}P NMR analysis of sediments.*

were measured using diffusion samplers equilibrated for 1 month (Sundareshwar and Morris 1999). Pore water at each site was sampled in triplicates at 10, 25, 50, 75, and 100 cm depths. Depth-integrated geometric means of at least 12 sampling dates at each site are reported here. These pore water constituents were measured to evaluate the occurrence of Ppi in coastal wetlands in relation to the biologically available SRP and redox conditions.

Bioavailability of Ppi—The bioavailability of Ppi was tested in two different experiments (Fig. 3A,B). In the first experiment (Fig. 3A), surface sediment from salt marshes on the heavily urbanized Cooper River estuary and the relatively pristine North Inlet estuary, South Carolina, were slurried and used as a source of microbial inocula. These were used to inoculate an aqueous medium supplied with Ppi (500 μM) as the sole source of added P and ammonium acetate (500 μM) as a source of nitrogen and carbon. SRP was measured (Strickland and Parsons 1972) in an aliquot after a 48-h incubation. Appropriate controls were carried out with or without the addition of either Ppi or the microbial inocula. In addition, we also included a killed control, where toluene was added as a poison. All treatments were in triplicate. The treatment codes are detailed in the figure legend. The second experiment (Fig. 3B) was a parallel experiment designed as above. However, here we used sediments from only the salt marsh on the Cooper River estuary. This experiment was run in duplicate, and the appropriate treatment codes are detailed in the figure legend. Duplicates of a treatment with added Ppi with sediment slurry inoculum were run in three batches sacrificed after 24, 48, and 72 h of incubation in the dark at room temperature. The two controls—Ppi addition without inoculation and microbial inoculation of the aqueous medium without the addition of Ppi—were sacrificed after 3

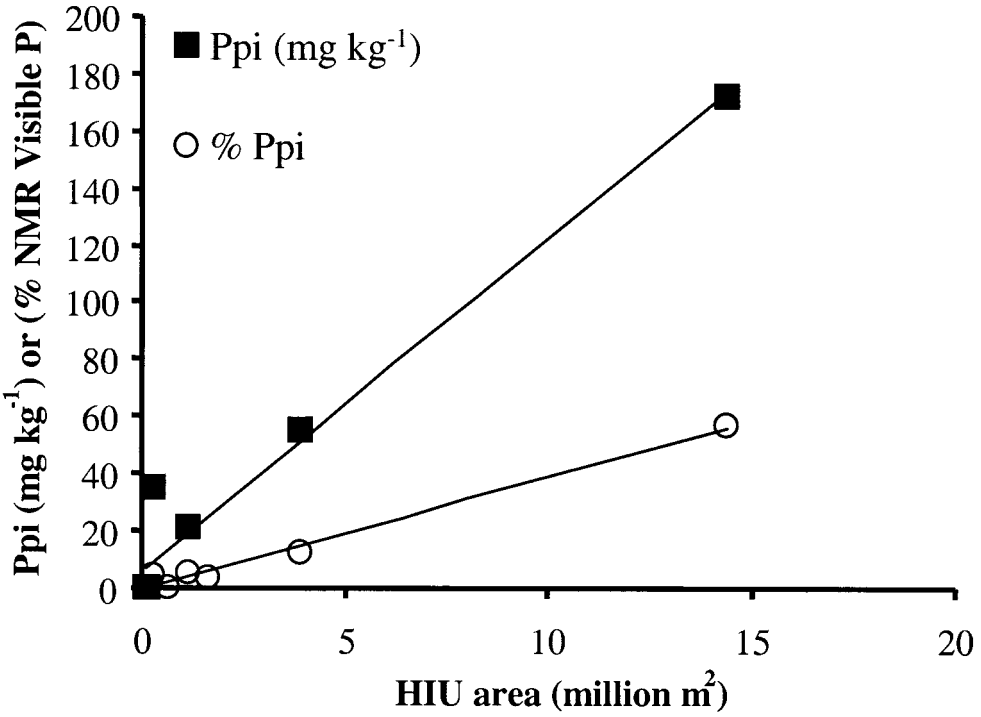

Fig. 2. Relationship between Ppi occurrence and high-impact urban (HIU) area. Two relationships are shown, where (1) Ppi is expressed as mg kg^{-1} sediment dry wt; $Y = 11.65X + 5.95$; $r^2 = 0.963$, $P < 0.0001$, $n = 8$, excluding the two Plum Island estuary (PIE) sites, and (2) Ppi is expressed as % NMR-visible P; $Y = 3.94X - 0.58$; $r^2 = 0.989$, $P < 0.0001$, $n = 10$.

d of incubation. The net production of SRP and consumption of added Ppi were monitored in aliquots from each replicated treatment during the course of the experiment.

Ppi occurrence and urban impact—We detected the presence of Ppi in sediments from the coastal wetlands that were classified as impacted (Table 1). For instance, sediments from all three locations along the salinity gradient on the urbanized Cooper River showed the presence of Ppi. Similarly, we detected Ppi in sediments from the impacted salt marsh (SM1) in the Edisto River (ACE Basin), which is influenced by a local marina and a nearby housing development. The impacted brackish marsh in the Plum Island estuary that occupies a more developed, suburban landscape (when compared to the reference site in the Plum Island estuary) also showed the presence of Ppi in marsh sediments. The contribution of Ppi to the total NMR-visible P at these sites varied and was related to the degree of urban impact estimates for the corresponding sites. For instance, the greatest concentrations in sediment, pore water, and surface waters among the sites examined were in the salt marsh near the city of Charleston, South Carolina (Fig. 1B). This site (SM) is at the mouth of the highly urbanized Cooper River estuary. Ppi in sediments at this site accounted for approximately 57% of the total extractable P that was NMR-visible. We also measured a concentration of 33.8 ± 7.3 μM of Ppi in surface waters, where the corresponding concentration of SRP was only about 2 μM. Although in minimally impacted or oligotrophic ecosystems the contribution of SRP to the total P pool is higher than that of any other P pool, ecosystems that are affected by anthropogenic activities may exhibit a divergence from this pattern. Such a shift in relative contribution of various P pools to the total P obviously will depend on the forms in which P loading occurs. For instance, in contrast to the high concentration of Ppi found in Cooper River salt marsh sediments (160.5 mg kg^{-1}), sediments from a salt marsh receiving inconsequential terrigenous inputs from a small, undeveloped watershed did not contain detectable levels of Ppi (Fig. 1C). Similarly, sediments from the reference site in the ACE Basin (SM2, in South Carolina) did not show the presence of Ppi, even though this site supported similar pore water concentrations of phosphate and sulfides to the impacted site (SM1) in the ACE Basin (Table 1). The third reference site (SM), located in the Plum Island estuary, Massachusetts, also did not show the presence of Ppi (Table 1). Furthermore, in contrast to previous reports (Gressel et al. 1996; Preston and Trofymow 1998), we did not detect Ppi from a forest site (FOR) located in the undeveloped watershed of the North Inlet estuary. Collectively, our data show a trend of increasing Ppi concentration with increasing degree of urbanization (Fig. 2).

Of course, the type of urban impact is probably a stronger determinant of Ppi loading than is the total urban area around a site. For instance, total urban area (expressed as a percentage of total land cover) around the Cooper River brackish marsh is 1.02%, but the HIU area here is 3.9 million m^2, whereas the total urban area (expressed as a percentage of total land cover) around the Plum Island estuary brackish marsh is 13.17%, but the HIU area here is only 1.64 million m^2. This reflects the relative differences in the type of urban

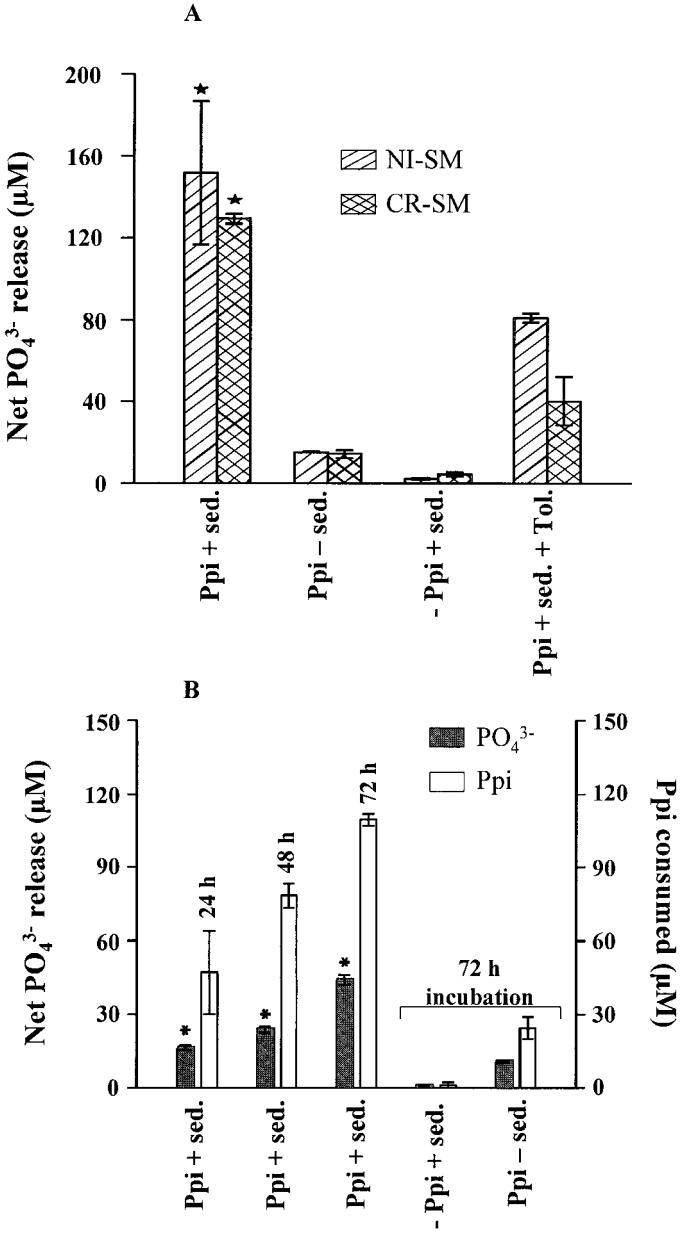

Fig. 3. (A) Utilization of Ppi by microbial inocula from North Inlet (NI-SM) and Cooper River (CR-SM) salt marsh sediments in an aqueous media supplied with Ppi as the sole source of P and ammonium acetate as a source of nitrogen and carbon. Shown is the mean (±1 SE, $n = 3$ per treatment) production of orthophosphate by treatment following a 48-h dark incubation at room temperature, where + and − indicate the presence or absence, respectively. Toluene (Tol.) was added in the killed controls to inhibit microbial activity. Significant differences (Tukey at $\alpha = 0.05$) in orthophosphate release between Ppi + sed. and Ppi − sed. treatments are shown as *. (B) Ppi breakdown in aqueous medium inoculated with Cooper River–SM sediment slurry. Shown are the production of orthophosphate and the hydrolysis of Ppi following 1, 2, and 3 d of incubation. The controls are shown as −Ppi + sed. and Ppi − sed. Note that the net release of SRP and the hydrolysis of Ppi from these controls are over a 72-h period. A significant increase ($\alpha = 0.05$) in orthophosphate concentration during 1,2, and 3 d of incubation over Ppi − sed. is shown as *. Error bars represent ±1 SD, $n = 2$.

impact around these sites. The urban area around the brackish marsh site on the Cooper River is dominated by industries, whereas the type of urban area around the brackish marsh site on the Plum Island estuary is mainly suburban development. Ppi and other polyphosphates have wide industrial and domestic applications (e.g., Cordon et al. 1997), and correspondingly, the Cooper River brackish marsh site supports relatively higher concentration of Ppi than the Plum Island estuary brackish marsh site (Table 1). Thus, the HIU area within a 5-km radius around sampling sites is a good indirect index of Ppi loading (Fig. 2).

The sources of Ppi in these coastal wetlands are exogenous. In addition to the link between Ppi occurrence and urban impact demonstrated above, the pattern of occurrence of Ppi in the experimentally fertilized plots located in the North Inlet estuary also supports this hypothesis. The experimental plot that has been fertilized annually with 30 mol N m^{-2} and 15 mol P m^{-2} for 15 yr with reagent-grade $(NH_4)_2HPO_4$ did not accumulate detectable levels of Ppi (Fig. 1D). In contrast, sediments from the second fertilized plot within the North Inlet estuary that was fertilized annually (15 mol m^{-2} of P) with a commercial P fertilizer (Triple Superphosphate) for 2 yr showed the presence of Ppi (Fig. 1E). In this case however, ^{31}P NMR analysis of this commercial P fertilizer confirmed that it contains Ppi (Fig. 1F). Thus, we detected Ppi in only those plots that were fertilized with commercial fertilizer containing Ppi.

This is the first report of the existence and sources of Ppi, the smallest chemical form of polyphosphate, in marine environments. Although it has been found in forest soils (Gressel et al. 1996; Preston and Trofymow 1998) and lakes (Hupfer and Gachter 1995; Carman et al. 2000), its origin was unclear or was suggested to be biogenic in nature. By contrast, in estuaries, we show a clear link between Ppi accumulation and human impact (Fig. 2).

Ppi and other polyphosphates have wide industrial applications (Monsanto 1996). Ppi has also been used as a P fertilizer in agriculture (Louge 1961), and from 1955–1960 in the United States alone, annual use of Ppi and sodium tripolyphosphate was at least 5.44 and 45.35×10^6 kg of P equivalent, respectively (Louge 1961). More recently, organo-P fertilizers, such as chitosan-polyphosphate complex, have also been synthesized (Frossard et al. 1994). Urban wastewater also contains Ppi and polyphosphates (Florentz et al. 1983). In addition, the more unstable long-chain polyphosphates may breakdown to the relatively more stable Ppi form, with an abiological half-life as great as 230 yr (Monsanto 1996). Consequently, the occurrence of Ppi in marsh sediments can be related to human impacts.

There are also biogenic Ppi sources, but they alone cannot explain its distribution among our sample sites. For instance, bacteria may accumulate polyphosphates in the presence of excess orthophosphate (e.g., Kromkamp 1987), but Ppi and pore water SRP concentrations were not correlated in our study sites ($P = 0.74$, $n = 9$) (Table 1). In addition, a fertilized plot (FSM) in the pristine North Inlet estuary that was fertilized with $(NH_4)_2HPO_4$ did not accumulate Ppi to detectable levels, even though the pore water SRP concentration at this site was similar to that at the SM site on the urbanized Cooper River estuary (Table 1). This indicates that

the presence of Ppi in Charleston Harbor is not a consequence of excessive inorganic orthophosphate loading per se. However, Ppi is involved in many cellular biochemical reactions (e.g., sulfate reduction) and has been proposed as an evolutionary precursor of ATP (Lipmann 1984). In biotic reactions, Ppi is hydrolyzed by specific enzymes such as pyrophosphatase, and some sulfate reduction reactions are ''pulled'' to completion by the hydrolysis of the generated Ppi (Ware and Postgate 1970). Furthermore, the presence or absence of Ppi and other polyphosphates have been speculated to be controlled by differences in redox conditions (Carman et al. 2000). In their study, they found Ppi in oxic sediments from the lakes tested but not from the Baltic Sea sediment samples. However, in our study the pore water sulfide concentrations for the reference sites (where we did not detect Ppi) ranged from 127 to 508.9 μM (Table 1), whereas the pore water sulfide concentrations in our impacted sites (where we detected Ppi to varying degree) ranged from 2.3 to 1,034 μM (Table 1). In our study, in contrast to the findings of Carman et al (2000), Ppi in sediments accounted for about 57% of the total NMR visible P at the SM site on the Cooper River estuary, which also supported the highest pore water sulfide concentration ($\sim$1 mmol L^{-1}). Interestingly, the SM site also had the highest degree of urban impact among our sites (Table 1). Furthermore, the two salt marsh sites in the ACE basin supported similar pore water sulfide and SRP concentrations, but we detected Ppi only in sediments from the impacted location. Thus, we detected Ppi in marsh sediments from sites that were classified as impacted and not from our reference sites (irrespective of the prevalent redox conditions and bioavailable SRP concentrations). Furthermore, the relationship between sulfide versus percent Ppi ($r^2 = 0.56$, $P = 0.02$, $n = 9$) is weaker than the relationship between HIU and percent Ppi ($r^2 = 0.989$, $P < 0.0001$, $n = 10$). This further suggests that the sources of Ppi are exogenous. The degree of accumulation of Ppi in coastal wetland sediments appears to reflect a legacy of historical trends in land use and urbanization within coastal watersheds. Collectively, our data suggest that the loading of Ppi in coastal wetland sediments is anthropogenic in origin, although its utilization is biological.

We have found that the biological hydrolysis of Ppi is a function of the nutrient status of sediment microbes. When we provided Ppi as the sole source of P in the presence of excess nitrogen and carbon, microbial inocula from urbanized Cooper River and pristine North Inlet salt marsh sediment slurries produced 120 and 160 μM SRP, respectively (Fig. 3A). Note that the killed controls do show some release of SRP. This is most likely due to residual enzyme activity (of pyrophosphatase) released from lysed bacterial cells because of the addition of toluene. However, the amount of SRP liberated is still significantly lower than the treatment that includes both Ppi and live microbes. This clearly shows that utilization of Ppi is an active process, which occurs in both impacted and pristine sites. Algae and bacteria have been shown to scavenge polyphosphate in the presence of excess nitrate (Solorzano and Strickland 1968).

Additionally, in a parallel experiment where we monitored the net release of SRP and the consumption of Ppi, we found that the amount of net SRP released was a function of Ppi hydrolysis (under identical conditions) (Fig. 3B). Based on the consumption rate of Ppi in this experiment, we calculated the rate of biotic hydrolysis of Ppi to be approximately 7% d^{-1}, a rate that is consistent with a previous report (Al-Kanani and MacKenzie 1990). Consistent with earlier studies where Ppi has been shown to support the growth of various groups of bacteria from salt and freshwater marshes (Liu et al. 1982), we found that in the above experiment the slope of Ppi hydrolysis and net production of SRP was less than 2 (the theoretical slope of Ppi hydrolysis and the gross yield of SRP), suggesting that a fraction of the Ppi and the regenerated SRP may indeed have been used by bacteria for growth. In laboratory experiments, Ppi amendments stimulated bacterial production in sediments from the reference salt marsh in the North Inlet estuary (data not shown), although the hydrolysis of Ppi was not monitored in this case. These data demonstrate that the loaded Ppi is actively incorporated into the P biogeochemical cycle.

The physicochemical properties of polyphosphates, which make them suitable for treatment of hard water, also imply that the loaded polyphosphates will accumulate in the coastal zone where concentrations of complexing cations are the highest. This is consistent with the increase in precipitation of Ppi that we observed with increasing salinity (data not shown) and suggests that the marsh surface of coastal wetlands acts as a sink for Ppi. Another important factor that can regulate the sink strength of the coastal wetlands for P is the P sorption capacity of wetland sediments. For instance, the P sorption capacity of Cooper River wetland sediments declines along the salinity gradient (Sundareshwar and Morris 1999), whereas the mean depth-integrated (up to 1 m) pore water SRP concentration increases (Table 1). Interestingly, the pore water Ppi concentrations also increase along the salinity gradient on the urbanized Cooper River. For instance, pore water Ppi concentrations (at 10 cm deep) increased from 55.4 $\pm$ 53 μM at the freshwater site to 74.7 $\pm$ 18.6 μM at the brackish marsh site, with the salt marsh site supporting the highest concentration of 157 $\pm$ 55 μM. Thus, Ppi represents a greater source of available P than SRP, particularly at the salt marsh site on the Cooper River. Because the hydrolysis of 1 mol of Ppi yields 2 mol of orthophosphate, Ppi trapped in the sediment matrix at this site can act as an ecologically significant reservoir for P, depending on the activity of pyrophosphatase (an enzyme that hydrolyzes Ppi).

The bioavailability of Ppi and its ability to support the growth of heterotrophic bacteria from different environments (Liu et al. 1982) suggests that even in the presence of high ambient SRP concentrations, accumulation of Ppi will subsidize the estuarine P cycle and contribute to the maintenance of high in situ SRP concentrations. Importantly, this accumulated Ppi is not detectable by the standard molybdate method of SRP determination. Given the influence of P on important trophic compartments in estuarine and other ecosystems (e.g., Thingstad et al. 1998; Ulanowicz and Baird 1999), it will be necessary to examine other previously undetected forms of bioavailable P, such as Ppi, to fully understand the effects of nutrient loadings. Our results suggest that the full extent of bioavailable P accumulation in estuaries is unknown because of the presence of Ppi.

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