

# Phosphorus Transformations during Decomposition of Wetland Macrophytes

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The microbially mediated transformation of detrital P entering wetlands has important implications for the cycling and long-term sequestration of P in wetland soils. We investigated changes in P forms in sawgrass (*Cladium jamaicense* Crantz) and cattail (*Typha domingensis* Pers.) leaf litter during 15 months of decomposition at two sites of markedly different nutrient status within a hard-water subtropical wetland (Water Conservation Area 2A, Florida). Leaf litter decomposition at the nutrient enriched site resulted in net sequestration of P from the environment in forms characteristic of microbial cells (i.e., phosphodiester and pyrophosphate). In contrast, low P concentrations at the unenriched site resulted in little or no net sequestration of P, with changes in P forms limited to the loss of compounds present in the initial leaf litter. We conclude that under nutrient-rich conditions, P sequestration occurs through the accumulation of microbially derived compounds and the presumed concentration of endogenous macrophyte P. Under nutrient-poor conditions, standing P pools within wetland soils appear to be independent of the heterotrophic decomposition of macrophyte leaf litter. These conclusions have important implications for our ability to predict the nature, stability, and rates of P sequestration in wetlands in response to changes in nutrient loading.

## Introduction

Decomposition of macrophytes influences the biocycling, retention, and downstream release of nutrients in wetland systems. The often cited model of wetland macrophyte decomposition set out by Webster and Benfield (1) identifies three distinct yet overlapping phases of decomposition: (i) an initial rapid leaching of water-soluble components, (ii) microbial colonization and decomposition, and (iii) mechanical and invertebrate mediated fragmentation of material. Of these, the second stage - microbial colonization and decomposition - involves the most dynamic alteration of nutrient forms. Whether microbes mineralize or sequester inorganic nutrients during decomposition of senesced bio-

mass has implications for both the internal nutrient dynamics of wetlands (2) and overall nutrient sequestration, an important function of wetlands in the landscape (3).

Phenological characteristics of the litter appear to influence initial decomposition processes, after which decomposition rates are increasingly governed by gross nutrient ratios (4) and the nutrient status of the environment (5, 6). Anthropogenic perturbation of nutrient availability in aquatic systems can cause shifts in trophic status (7), changes in the composition of plant communities (8, 9), and alteration of microbial eco-physiological processes (e.g. refs 10 and 11). Observed alterations in catabolic processes appear to follow predicted changes in resource reallocation (12) with an increase in bioavailable P leading to a reduction in investment in P acquisition, e.g. a reduced release of extracellular phosphatase enzymes by microbes (13, 14).

Although numerous studies have investigated factors that influence changes in tissue total P during wetland macrophyte decomposition (e.g. refs 15–17), transformations of the functional forms of P during decomposition are less well studied. Since the chemical nature of P forms impacts both abiotic stabilization in the environment (18) and biological turnover (19), it is vital to understand how environmental conditions impact the composition of P forms present during the decay continuum. For terrestrial systems, studies of temporal changes in P functional groups in decomposing plant tissue indicates both the accumulation of microbial phosphodiester (20) and the synthesis of polyphosphates by fungi (21). Other studies have sought to partition biogenic P in soils into various microbial and plant sources (22), and transposed position within a soil profile for time, with the aim of tracking general transformations within the organic matter of forest soils (23). In wetlands, attempts have been made to characterize leachate from macrophyte leaves (24), but changes in P forms in the autochthonously derived organic matter of wetland systems remain poorly understood.

The objective of this study was to use solution <sup>31</sup>P nuclear magnetic resonance (NMR) spectroscopy to characterize the functional forms of P throughout a macrophyte decay continuum. In addition, we aimed to determine how litter quality and site characteristics regulate changes in both total P and its functional composition. It was hypothesized that in a P-enriched setting, the accumulation of microbial derived P and reduced catabolic breakdown of macrophyte P would result in net sequestration of certain P groups, whereas in an oligotrophic setting there would be close coupling of biogenic P production and its subsequent hydrolysis, limiting the accumulation of microbially derived P forms.

## Materials and Methods

**Site Description.** Water Conservation Area 2A (WCA-2A) is a diked and hydraulically controlled 424 km<sup>2</sup> portion of the northern Everglades, characterized as a freshwater peat system underlain by limestone bedrock. Historically, productivity in the northern Everglades has been limited by P availability, but anthropogenic loading from upstream agricultural practices has resulted in a distinct nutritional and concomitant vegetation gradient in WCA-2A (e.g. refs 25 and 26). There is a distinct transition from native Everglades marsh dominated by sawgrass (*Cladium jamaicense* Crantz), to dominance by cattail (*Typha domingensis* Pers.) in areas impacted by nutrient-rich inflow water (9). The nutrient-enriched areas have increased rates of heterotrophic decomposition (5, 11) and a reduced extracellular phosphatase activity (10, 14).

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**TABLE 1. Site Characteristics for Enriched and Unenriched Study Sites within WCA-2A<sup>a</sup>**

	WCA-2A location	
	enriched site	unenriched site
latitude (N)	26° 21.230'	26° 16.382'
longitude (W)	80° 20.967'	80° 21.502'
distance from inflow structure (km)	1.93	10.05
average water depth (cm; max, min) <sup>b</sup>	12.8 (11.9, 14.0)	11.9 (11.0, 13.6)
overlying water <sup>c</sup>		
total P ( $\mu\text{g P L}^{-1}$ )	52.4 $\pm$ 6.7	9.6 $\pm$ 0.9
ortho P ( $\mu\text{g P L}^{-1}$ )	27.8 $\pm$ 4.4	1.7 $\pm$ 1.3
detritus		
total P ( $\mu\text{g g}^{-1}$ )	1334 $\pm$ 904	206 $\pm$ 53
total C ( $\text{mg g}^{-1}$ )	414 $\pm$ 27	387 $\pm$ 16
total N ( $\text{mg g}^{-1}$ )	26 $\pm$ 12	14 $\pm$ 3
soil (0– 1.5 cm)		
total P ( $\mu\text{g g}^{-1}$ )	1312 $\pm$ 40	468 $\pm$ 40
total C ( $\text{mg g}^{-1}$ )	421 $\pm$ 90	406 $\pm$ 21
total N ( $\text{mg g}^{-1}$ )	26 $\pm$ 3	26 $\pm$ 2

<sup>a</sup> Detritus and soil samples average ( $n = 4$ )  $\pm$  one standard deviation. Overlying water characteristics based upon published values. <sup>b</sup> Stage data from South Florida Water management (23/01/03 through 04/21/04). Sampling stations WCA2E1 and WCA2U1. <sup>c</sup> Site water characteristics, South Florida Water management (6/21/94 through 9/27/94). Sampling stations WCA2E1 and WCA2E5. Available through DBHYDRO (<http://my.sfwmd.gov/dbhydroplsql/>).

Two sites along a well documented P gradient were selected for this study: one enriched site 1.93 km from the northern inflow structure S10-C and a second within an area considered unenriched by P loading (Table 1). Site detritus and surface soil (core diameter 15 cm  $\times$  1.5 cm deep) were sampled ( $n = 4$ ) from the study locations prior to experimental setup and analyzed for total C, N, P, and P composition by solution <sup>31</sup>P NMR spectroscopy.

**Litterbag Study.** Senescent leaf material of both *Typha* and *Cladium* were collected in bulk from locations proximate to both the enriched and unenriched sites. Material included only standing dead intact lamina of unknown age but presumed < 2 y. Bulk material was rinsed of adhering particulates, cut into 10 cm sections, and dried at 60 °C to a constant weight. Litter was analyzed for total C, N, P, and P forms by solution <sup>31</sup>P NMR spectroscopy. Litter quality was determined by a modified proximate forage analysis (27) utilizing the semiautomated Ankom A200 (Ankon Technology, Fairport, NY).

Litterbags (15 cm  $\times$  15 cm) were constructed from gray polyethylene mesh (1 mm) allowing entry of micro fauna and local microbial assemblages. Approximately 15 g dry weight of litter was sealed into individual litter bags and placed (January 2003) on the surface of both the enriched and unenriched sites, secured in place with polyethylene stakes. Three replicate bags were recovered from each site after 16, 33, 75, 204, and 454 days. Litterbags were returned on ice to the laboratory, where they were gently washed with deionized water to remove surface debris, frozen, and lyophilized. Litter was then removed from the bags for mass loss determination and ground to pass a 2 mm mesh using a Wiley mini mill (Thomas Scientific, Swedesboro, NJ). Samples of coarsely ground material were then analyzed for total P and P composition and after further grinding, total C and N. All samples were stored in sealed containers in the dark at ambient lab conditions until analysis.

**Analysis of Biogeochemical Properties.** Initial site detritus, surface soils, and recovered litter were analyzed for total N and C simultaneously using a Costech Model 4010 Elemental Analyzer (Costech Analytical Industries, Inc., Valencia, CA). Loss on ignition (LOI) and total P were determined by a modified ashing method (28) using 550 °C for 4 h, dissolution of the ash in 1 M H<sub>2</sub>SO<sub>4</sub>, and subsequent

P determination by discrete autoanalyzer (AQ2+, SEAL Analytical, Fareham, UK) and standard molybdate colorimetry (USEPA, 1993).

**Phosphorus Composition.** Initial site detritus, surface soil, and selected leaf litter samples recovered from the decomposition study were analyzed by solution <sup>31</sup>P NMR spectroscopy. Given practical and financial constraints of analyzing multiple samples with <sup>31</sup>P NMR spectroscopy, only *Cladium* and *Typha* sourced from the enriched site were analyzed, as these were expected to provide clear P signals during NMR spectroscopy. Samples were analyzed from time steps that were considered able to provide insight into mechanistic processes (i.e., initial and final material and after maximum total P leaching). Additional samples from the enriched site were analyzed to provide information on P accumulation rates.

**Phosphorus Extraction in NaOH-EDTA.** A standard alkaline extraction (29) was applied to detritus, soil, and litter samples using combined field replicates and a 1:30 solid to solution (0.25 M NaOH plus 50 mM EDTA) ratio. Samples were shaken at ambient room temperature for 3 h and then centrifuged (Sorvall RC6 centrifuge, SLA 1500 Rotor; Thermo Fisher Scientific, Waltham, MA, USA) at 6500 rpm for 10 min. After centrifugation a subsample (20 mL) of the supernatant was removed to a scintillation vial and combined with 1 mL of 50 mg P L<sup>-1</sup> methylenediphosphonic acid (MDP) as an internal standard. Mixed samples were immediately frozen (-80 °C) and lyophilized prior to NMR spectroscopy. A second subsample was analyzed for total P (NaOH<sub>TP</sub>) by a modified double-acid digest using H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> and a discrete molybdate colorimetric detection method.

Residual P (total P - NaOH<sub>TP</sub>) is by definition unidentified, and its chemical stability is presumed to indicate its recalcitrance in the environment. For mineral soils it has been assigned as recalcitrant organic (29) and alkali-stable (acid-soluble) inorganic P (30). Although there is little information on its chemical nature in wetland soils, given relatively large concentrations of acid-extractable P recovered from detritus (31) and surface soils (32) in WCA-2A it seems likely that at least a proportion of the residual P was inorganic Ca-phosphates.

**Solution <sup>31</sup>P Spectra Acquisition.** Spectra were acquired using a Bruker Avance 500 Console with a Magnex 11.75 T/54 mm magnet using a 10 mm BBO Probe. Lyophilized samples (~300 mg) were resuspended in 0.3 mL of D<sub>2</sub>O and 2.7 mL of a solution containing 1 M NaOH and 0.1 M EDTA, vortexed, and then transferred to a 10 mm tube. Spectra acquisition was carried out at a stabilized 25 °C with a calibrated (~30°) pulse length, a zgig pulse program, and a 2 s T1 delay. Results presented here are of ~30,000 scans accumulated as three sequential experiments, with FIDs summed post acquisition by Bruker proprietary software.

Spectra interpretation was conducted using NMR Utility Transform Software (Acorn NMR Inc., Livermore, CA). After applying 15 Hz line broadening, spectra were referenced and integrated against the internal standard, MDP, set as 17.46 ppm (compared to externally held 85% phosphoric acid). Integration over set spectral windows were chosen to correspond with known P bonding environments (33). The region between 8 and 3 ppm was further elucidated on spectra processed using 3 Hz line broadening by a deconvolution subroutine applied to identify and quantify orthophosphate (6.21  $\pm$  0.02 ppm) separately from phosphomonoesters.

**Data Analysis.** All statistical tests were performed in SPSS for windows version 17.0.0 statistical software (SPSS Inc. 2008). Mass remaining, P concentration, and mass of P were analyzed by a four-way univariate ANOVA using site of decomposition (site), species of litter (species), source of litter (source), and time as independent variables. Given the homogeneous nature of initial material, time = 0 was

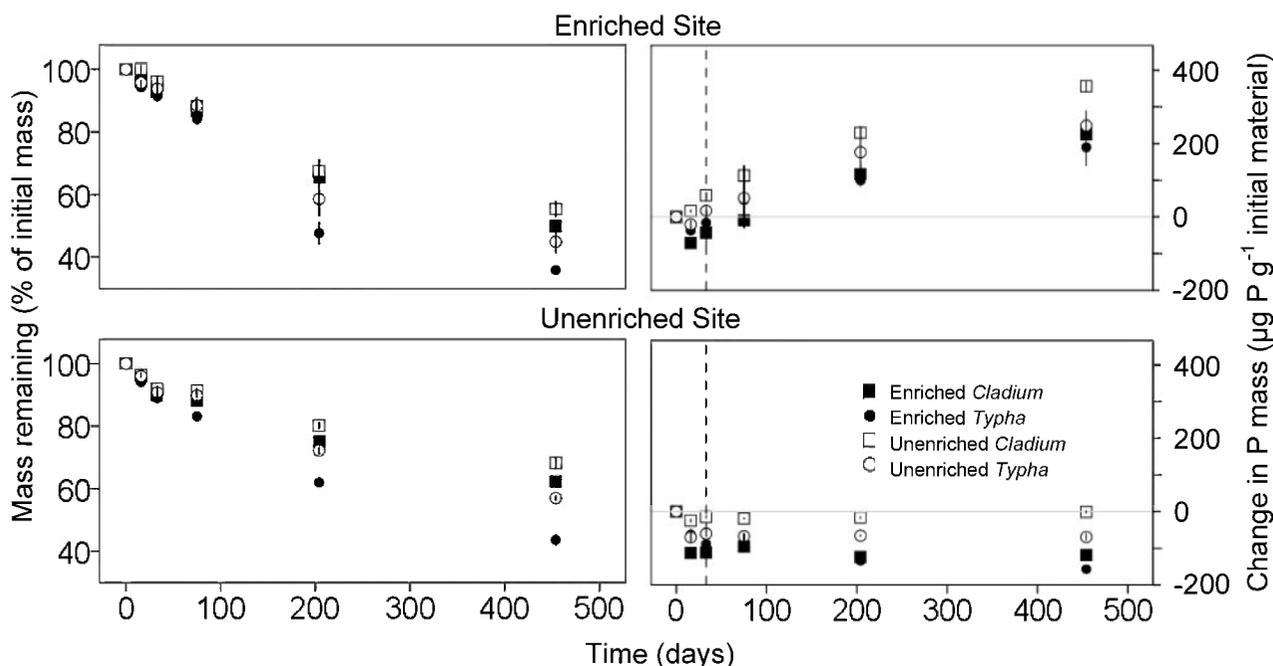


FIGURE 1. Time series of mass remaining (left panel) and change in total mass of P (right panel) in four litter types placed at two distinct sites within WCA-2A. Symbols represent averages ( $n = 3$ ) with error bars showing one standard error.

excluded from the statistical analysis. Resulting model residuals were analyzed via P–P plot and visual inspection for normality. If required, original data were normalized using a natural log transformation. Significant results are reported alongside their calculated partial  $\eta^2$  values. Given complications of interpreting multiple interaction factors, non-significant higher order terms are not presented here. Due to practical constraints, solution  $^{31}\text{P}$  NMR data were acquired for pooled samples and as such are without a measure of variance at individual time steps. All integrated results using MDP as an internal standard were within  $\pm 20\%$  of determined  $\text{NaOH}_{\text{TP}}$  concentrations. Calculated rates of accumulation of specific P forms are based upon simple linear regression after initial leaching and equilibration period.

## Results

**Initial Litter Material.** *Typha* litter material from both the unenriched and enriched study sites contained greater concentrations of total N (4.5 and 5.4  $\text{mg g}^{-1}$ ) and P (135 and 261  $\mu\text{g g}^{-1}$ ) and had a higher loss on ignition (indicating less biosilica) than *Cladium* (4.1 and 3.8  $\text{mg g}^{-1}$  total N and 46 and 171  $\mu\text{g g}^{-1}$  total P in unenriched and enriched sites, respectively) (Table S1). Although *Typha* litter had a noticeably larger proportion of neutral-detergent extractable C (Table S1), the proportion of lignin and cellulose was similar, with LCI (Lignin to Cellulose Index) between 0.170 and 0.197 for all litters sampled. Within species, there was a distinct difference between material collected at the enriched and unenriched site, with *Cladium* showing an approximate 4-fold increase in total P and *Typha* showing a 2-fold increase. Using both species, collected from enriched and unenriched settings allowed four litter types to be used within the subsequent decomposition study.

**Mass Loss.** Between 36 and 70% of original mass of litter remained after 15 months in the field (Figure 1). Rates of decomposition varied significantly (ANOVA  $p < 0.001$ ) between litter types, in terms of both macrophyte species and litter source, as well as between study sites (Table S2). In addition, decay rate constants based upon a simple exponential decay (Table S3) followed expected patterns, with increased rates of mass loss in response to increased

litter quality and exogenous nutrients (16). However, decomposition rate constants were relatively constrained, varying from  $0.00275 \text{ day}^{-1}$  for enriched *Typha* material at the enriched site to  $0.00093 \text{ day}^{-1}$  for unenriched *Cladium* at the unenriched site. Significant interaction terms between time and both litter species and source demonstrated the continued and differential influence of initial material characteristics on the long-term stability of organic matter (Table S2).

**Phosphorus Content.** Total P concentrations of decomposing leaf litter (Figure S1) changed significantly with time. At the enriched site all litter types except unenriched *Cladium* showed an initial drop in P, as expected from a rapid leaching event, but then subsequently increased to between 5 and 10 times the original P concentration. In contrast, at the unenriched site after an initial decrease in P, concentrations did rise but with the exception of unenriched *Cladium* never recovered to their initial levels (Figure S1). Univariate four-way ANOVA (Table S4) showed significant differences dependent not only upon the site of decomposition ( $p < 0.001$ ) but also on the nature of the initial litter, as shown by highly significant effect of both litter species ( $p < 0.001$ ) and source ( $p < 0.001$ ). In addition, significant interaction terms (litter quality with time) suggest different responses in P concentration of litter types over time.

Changes in P concentration during decomposition may result from two distinct processes: a change in litter mass that results in alteration of the endogenous P concentration or the loss or gain of P from the environment. This distinction was explored by plotting changes in the mass of P over time, standardized for the initial amount of litter used in each litter bag (Figure 1). A conservative breakpoint at 33 days (indicated by a dashed line in Figure 1) marked the switch from initial abiotic processes and site equilibration to long-term microbial action. From 33 days to the end of the study there was a distinction in changes in mass of P between sites. Linear regression demonstrated that the mass of P in all litter types at the enriched site showed a significant ( $p < 0.001$ ) increase of between 0.447 and 0.685  $\mu\text{g P g}^{-1}$  initial material  $\text{day}^{-1}$  between day 33 and the end of the study (Table

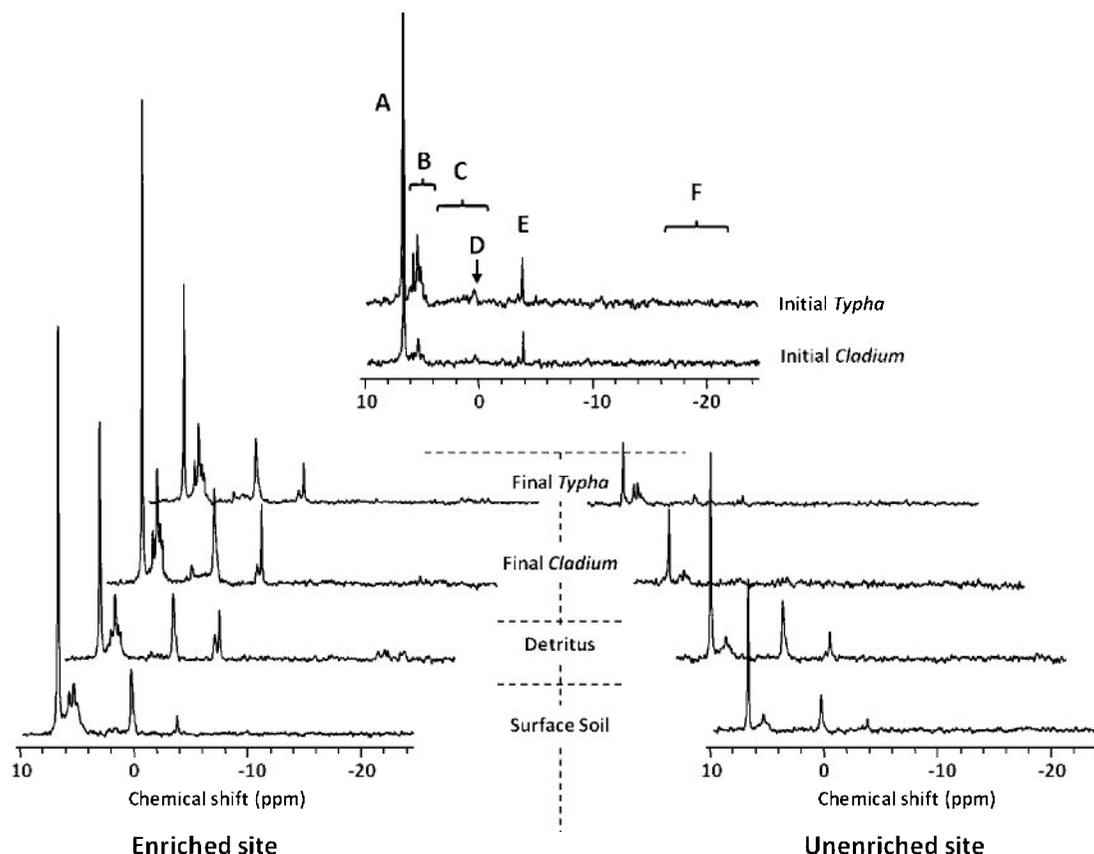


FIGURE 2. Solution  $^{31}\text{P}$  NMR spectra of enriched material through the decay continuum at enriched and unenriched sites. From initial litter material, through to litter material after 445 days in the field, to site detritus and surface soils. Spectra plotted using 15 Hz line broadening and scaled using height of internal standard MDP ( $\delta = 17.46$  ppm). Peaks were assigned to the following: **A** = orthophosphate, **B** = phosphomonoesters, **C** = phosphodiester, **D** = DNA, **E** = pyrophosphate, **F** = midchain polyphosphate residues.

TABLE 2. Phosphorus Forms As Determined by Solution  $^{31}\text{P}$  NMR Spectroscopy in Decomposing Leaf Litter, Site Detritus, and Surface Soils Sampled on the 10/20/03 at Both Enriched and Unenriched Sites within WCA-2A<sup>a</sup>

site	sample	time in the field (days)	total P ( $\mu\text{g P g}^{-1}$ )	% total P								
				phosphonate	orthophosphate	phosphomonoesters	DNA	other phosphodiester	pyrophosphate	polyphosphate	residual	
enriched	<i>Cladium</i>	0	171		35	13	3	1	3		46	
		16	104		27	26	2	2	1		42	
		454	795		20	16	10	5	4		45	
	<i>Typha</i>	0	261		39	23	5	4	6		25	
		16	237	<0.5	18	33	2	12	1		2	35
		454	1257		12	15	8	4	4		57	
detritus	-	899		16	12	7	2	5		4	58	
	soil	-	1311		18	10	5	1	1		64	
unenriched	<i>Cladium</i>	0	171		35	13	3	1	3		46	
		33	67		33	15	1	9	14		28	
		454	85		29	29	7	5	nd		30	
	<i>Typha</i>	0	261		39	23	5	4	6		25	
		33	195		19	30	1	5	4		41	
		454	239		16	18	4	1	2		59	
detritus	-	221		27	9	13	3	5		56		
	soil	-	421		18	7	6	1	2		66	

<sup>a</sup> nd = not detected.

S5). In contrast, at the unenriched site the mass of P in both enriched *Cladium* and unenriched *Typha* did not change significantly with time. The P content of unenriched *Cladium* increased slightly yet significantly ( $0.037 \mu\text{g P g}^{-1}$  initial material day<sup>-1</sup>;  $p = 0.001$ ) during decomposition, whereas in enriched *Typha* there was a significant ( $p < 0.001$ ) decrease in the mass of P.

**Phosphorus Forms.** Analysis of initial enriched leaf litter demonstrated a range of P forms present within both *Cladium*

and *Typha* (Figure 2, Table 2). In both cases, NaOH-EDTA extractable P was dominated by orthophosphate (35 and 39% of P in *Cladium* and *Typha*, respectively). In addition, considerable amounts of phosphomonoesters (13 and 23% total litter P), phosphodiester (4 and 9% total litter P), and inorganic pyrophosphate (2 and 6% total litter P) were identified. It should be noted that there was probably an inherent bias in the analysis, because some phosphodiester (i.e., RNA and some phospholipids) decompose to phosph-

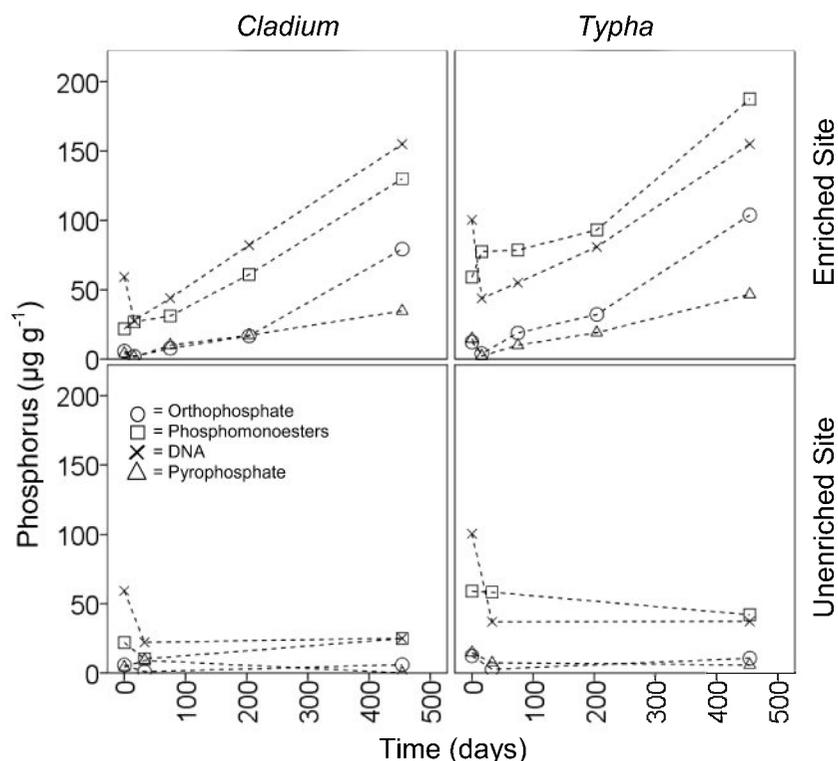


FIGURE 3. Changes in the proportions of major P pools identified by solution <sup>31</sup>P NMR in enriched macrophyte leaf litter over the course of 454 days of decomposition in WCA-2A.

omonoesters in alkaline solution during extraction and analysis (33).

Comparison of initial P composition in the in situ detritus and soil at the two study sites (Figure 2, Table 2) demonstrated distinct differences in the forms and proportions of P identified. Standing detritus and surface soil from both sites contained orthophosphate, phosphomonoesters, phosphodiester (dominated by DNA), and pyrophosphate. However, only detritus from the enriched site contained long-chain inorganic polyphosphate (~3% of total P), indicated by mid-chain phosphate residues at approximately -20 ppm (33). The proportion of total P identified by solution <sup>31</sup>P NMR was higher in detritus than in soil at both sites. There was a distinct shift in the ratio of phosphomonoesters to phosphodiester in material from the enriched and unenriched sites, from 1.35 to 0.58 in detritus and 1.69 to 0.90 in surface soil, indicating a greater proportion of P identifiable as phosphodiester within detritus and at unenriched sites. Signals within the phosphomonoester regions indicative of the phosphomonoester *myo*-inositol hexakisphosphate were not detected in any sample. This is consistent with other studies of calcareous freshwater wetlands (34, 35), although inositol phosphates could have been present at concentrations below the limit of detection by solution <sup>31</sup>P NMR spectroscopy (36).

Over the course of the decomposition study there was a convergence in the proportion of P forms present based upon the site of litter decomposition (Figures S2 and S3). Identifiable P lost during equilibration (presumably by leaching) at both the enriched and unenriched site was dominated by orthophosphate, although in the enriched site this was offset by the accumulation of certain organic P forms. Since concentrations of phosphomonoesters increased or remained relatively stable in all litters between initial material and samples at days 16 and 33 (Figure 3), there was an increase in their proportional contribution to the total P of plant detritus (Table S6).

Recovery of total litter P by NaOH-EDTA extraction averaged 56% across all samples. Residual P was a relatively stable proportion of the total P in *Cladium* litter and an

TABLE 3. Coefficients ± One Standard Deviation of Linear Increases in Concentrations of Major P Forms Identified within Leaf Litter during Decomposition at the Enriched Study Site<sup>a</sup>

	<i>Cladium</i>	<i>Typha</i>
orthophosphate	0.292±0.02 <sup>c</sup>	0.256±0.018 <sup>b</sup>
phosphomonoesters	0.244±0.20 <sup>b</sup>	0.260±0.054 <sup>b</sup>
phosphodiester	0.265±0.39 <sup>b</sup>	0.285±0.057 <sup>b</sup>
pyrophosphate	0.072±0.006 <sup>b</sup>	0.099±0.006 <sup>b</sup>

<sup>a</sup> Units µg P g<sup>-1</sup> day<sup>-1</sup>. <sup>b</sup> *p* < 0.05. <sup>c</sup> *p* < 0.001.

increasing proportion of P in *Typha* material at the enriched site, although in both cases the overall increase in total P resulted in an increase in the concentration of residual P. Trace concentrations of phosphonates and mid-chain polyphosphate were detected in one sample (*Typha* from the enriched site after 16 d) but may have originated from adhering phytoplankton (37). After the initial equilibration, litter at the enriched site accumulated all forms of P identified by <sup>31</sup>P NMR analysis, in direct contrast to the unenriched site, where there was little change in P composition after the initial leaching period (Figure 3). The increase in concentration of P forms at the enriched site showed a significant linear response (Table 3), with most major forms showing a net change of between 26 and 107 mg P kg<sup>-1</sup> y<sup>-1</sup>. Changes in pyrophosphate concentration appeared to be distinct from other major forms, showing a significant (*p* < 0.05) linear response at approximately a third of the rate of change for phosphomonoesters, phosphodiester, or orthophosphate concentrations.

## Discussion

Plant litter decomposition rates determined in this study over the course of 15 months are generally lower than values reported for other herbaceous litters (38) but correspond well with previous data from WCA-2A. For example, Debusk

and Reddy (5) found simple decay rate constants of approximately  $0.003 \text{ d}^{-1}$  for *Typha* decomposing under P-rich conditions. Rates of mass loss were similar to previously observed patterns, with higher litter quality (as determined by structural C and nutrient content) and higher environmental nutrient availability both resulting in an increased rate of mass loss.

Over the course of the study, total P concentrations within litter at the two sites showed distinctly different trajectories. At the enriched site, total P concentration in all litters increased, resulting in an average molar C:P ratio of ~488 after 15 months. In contrast, P concentrations at the unenriched site generally did not return to those of the initial material entering the system, with an average molar ratio of ~4150 after 454 days. Although a conventional model of litter decomposition would assume a continued sequestration of P at both sites in response to C:P stoichiometry, analysis of changes in mass of P (Figure 1b) suggests that while microbial activity at the enriched site resulted in the net sequestration of P from the endogenous environment, at the unenriched site there was little net gain. This interpretation assumes that mass loss due to fragmentation is minimal and may represent an underestimation of actual P accumulation in both sites. Yet previous studies have also suggested that oligotrophic systems such as the unenriched Everglades may present an environmental gradient in which P sequestration predicted from detritus C:P ratios is unlikely to occur. Indeed, Qualls and Richardson (2000) (17) demonstrated that an environmental P concentration of  $5 \mu\text{g P L}^{-1}$  in the water column resulted in the net loss of P to the environment from both *Typha* and *Cladium* leaf litter during decomposition.

Initial changes in litter P composition as a result of labile P leaching corresponds to the identification of water extractable forms by He et al. (2009) (39), who showed a predominance of orthophosphate but additional leaching of organic P from plant material. The apparent stability of phosphomonoesters during initial leaching may be due to the recalcitrant nature of phosphomonoesters remaining in plant material after nutrient resorption and senescence of leaves or due to the rapid synthesis of new phosphomonoesters in establishing microbial populations. In addition to leaching from the plant biomass, consideration should be given to the fact that some compounds (e.g., pyrophosphate) may be lost from endophytic and saprotrophic fungi during initial preparation and site equilibration (40, 41). Indeed, the use of standing dead biomass of unknown age leaves us unable to determine alterations of P composition during these initial stages of the decay continuum (41).

Initial abiotic leaching is often considered rapid (42), yet given an inability to interpolate data we drew a conservative breakpoint at 33 days, marking the switch from initial abiotic processes and site equilibration to long-term microbial processes. After 33 days this microbial activity at the enriched site resulted in the accumulation of all forms of P identified by solution  $^{31}\text{P}$  NMR spectroscopy. The estimated rates of change in P concentrations (Table 3) include contributions from both original and newly accumulated compounds. For example, the increase in recalcitrant P concentration (up to 8-fold) is not accounted for by the passive accumulation of initial endogenous recalcitrant P and suggests a mechanism by which additional recalcitrant P is being accumulated. This may represent either recalcitrant biogenic P or alkaline stable (acid-soluble) inorganic P. Given the calcareous conditions of WCA-2A it seems likely that the later accumulated via the precipitation of Ca-phosphate (e.g., hydroxyapatite) (17). It is interesting to note that the proportion of total P determined as 'inorganic' in a previous study (31) of surface material in WCA-2A is quantitatively similar (36–50%) to the residual fraction determined in this study.

Differences in both C quality and total P of initial material appeared to influence the P composition of litter material collected at the enriched site, with *Typha* consisting of more labile forms. However, changes in P composition during decomposition appeared to be independent of initial material. Rather, changes were dependent upon site characteristics, with the most abundant P forms showing similar changes in concentration at the enriched site for both *Cladium* and *Typha* litter. Fungal biomass is a major contributor to heterotrophic decomposition in emergent herbaceous systems (41) and although litter quality can affect the composition of the microfungal community their generalist nature may result in a similar response to the exogenous nutrient availability (43).

Within the enriched study site, the P composition of decomposing litter appears to be on a trajectory toward that determined in standing detritus and surface soil. In contrast, litter material within the unenriched site after 454 days of decomposition did not appear to reflect standing detritus. This could reflect differences in the role of macrophyte litter across the northern Everglades. Within nutrient-impacted portions of WCA 2A, surface substrate originates from *Typha* detritus and a high standing population of heterotrophic microbes, while oligotrophic regions often include only minimal *Cladium* fragments but a significant contribution from the periphyton community (31). Further work would be needed to determine the role of these additional sources of biogenic P in determination of P forms seen within wetland substrates.

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## Supporting Information Available

Three figures and six tables, specifically, change in P concentrations, complete  $^{31}\text{P}$  NMR spectra, ANOVA tables, model coefficients, and additional leaf litter characterization. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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