Published online September 8, 2005

Phosphorus Cycling in Wetland Soils: The Importance of Phosphate Diesters

Benjamin L. Turner* and Susan Newman

ABSTRACT

Productivity in P limited peatlands is regulated in part by the turnover of organic phosphates, which is influenced by the chemical nature of the compounds involved. We used solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy to quantify organic and inorganic phosphates in benthic floc (a mixture of plant detritus and algae) and underlying soil from sites along P gradients in hard water and soft water areas of the northern Florida Everglades, USA. Phosphorus-enriched sites were dominated by cattail (*Typha* spp.), while unenriched sites included sawgrass (*Cladium jamaicense* Crantz) ridges and open-water sloughs. Phosphorus extracted in a solution containing 0.25 *M* NaOH and 50 m*M* EDTA (ethylenediaminetetraacetate) included phosphate, phosphate monoesters, DNA, and pyrophosphate. Signals from phosphate monoesters were consistent with those from alkaline hydrolysis products of RNA and phospholipids formed during extraction and analysis, whereas phytic acid (*myo*-inositol hexa*kis*phosphate), the most abundant organic phosphate in most soils, was not detected. Phosphorus composition was similar among sites, although neither DNA nor pyrophosphate were detected in extracts of benthic floc from a calcareous slough. DNA was a greater proportion of the P extracted from soil compared to benthic floc, while the opposite was true for pyrophosphate. Research on the cycling of organic phosphates in wetlands focuses conventionally on the turnover of phosphate monoesters, but our results suggest strongly that greater emphasis should be given to understanding the role of phosphate diesters and phosphodiesterase activity.

Nutrient availability in highly organic wetlands is controlled in part by regeneration from organic matter, a process that is at least as important as external nutrient inputs (Verhoeven et al., 1988). In wetlands where productivity is limited by P availability, the turnover of organic P is therefore a key regulator of productivity (Wright and Reddy, 2001a; Newman et al., 2003). This involves a range of diverse compounds that vary markedly in their solubility and bioavailability in the environment (Turner et al., 2005b). In most soils, organic P occurs mainly as phosphate monoesters, a group of compounds that includes inositol phosphates, sugar phosphates, and mononucleotides. Much of the organic phosphate in plant and microbial residues is phosphate diesters such as phospholipids and nucleic acids, but these compounds typically constitute a relatively small proportion of the soil organic phosphate. Phosphonates, in which C and P are directly bonded, are detected sometimes in acidic soils.

B. Turner, Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancón, Republic of Panama. S. Newman, Everglades Division, South Florida Water Management District, 3301 Gun Club Road, West Palm Beach, FL 33406. Received 17 Feb. 2005. *Corresponding author (turnerbl@si.edu).

Published in J. Environ. Qual. 34:1921–1929 (2005).
Technical Reports: Wetlands and Aquatic Processes
doi:10.2134/jeq2005.0060

677 S. Segoe Rd., Madison, WI 53711 USA

In terms of their behavior, inositol phosphates react strongly in soil to form insoluble complexes and there is little evidence of their uptake by plants (Turner et al., 2002). Other compounds, such as nucleic acids and their breakdown products, are less stable and may contribute to plant nutrition (Bowman and Cole, 1978). Phosphate is the main inorganic form, although condensed inorganic phosphates (pyro- and polyphosphate) that require hydrolysis before plant uptake may be also present.

Given the diversity of P compounds present in soils, structural information is a fundamental prerequisite to understanding the biogeochemistry of soil P. Yet the P composition of wetland soils is almost unknown. Most studies focus on inorganic phosphate, with organic P typically measured only as part of a sequential fractionation scheme (Ivanoff et al., 1998), although studies from Florida used solution ^{31}P NMR spectroscopy to analyze recently reflooded organic agricultural soils (Robinson et al., 1998) and benthic floc from a constructed wetland (Pant and Reddy, 2001). We addressed the lack of information on organic P in wetland soils by analyzing samples from nutrient-enrichment gradients in two contrasting wetlands in the Florida Everglades. Our aim was to determine the compounds likely to be involved in P cycling in subtropical wetlands.

MATERIALS AND METHODS

Sites and Sampling

Historically, plant productivity in the Florida Everglades was limited by P availability, but the structure and function of the ecosystem changed dramatically during recent decades in response to pollution from agricultural runoff (McCormick et al., 2001). Before the 1960s, soil P sequestration rates were as low as 0.06 g P m^2 yr^{-1} in unpolluted parts of the marsh, but rates of >1.00 g P m^2 yr^{-1} now occur in nutrient-enriched areas (Reddy et al., 1993; Craft and Richardson, 1998). Much of the sequestered P is organic (Qualls and Richardson, 1995; Reddy et al., 1998).

Samples were taken from two impounded wetlands, termed Water Conservation Areas, in the northern Everglades (Fig. 1). Both wetlands receive approximately half of their water from rainfall and half from surface discharge via culverts and pump stations (calculated from data in Sklar et al., 2002). Water Conservation Area 1 (WCA 1) is the only remaining soft water portion of the Everglades. Established as a wildlife refuge in 1951, it is enclosed within 90 km of levees and canals and encompasses 59 000 ha of the northern-most remnant of Everglades habitat. As a result of canal water input there are distinct P and mineral gradients extending into WCA 1 from the western boundary to the interior of the marsh, although canal-water intrusions are generally restricted to the marsh perimeter (Newman et al., 1997). Soils within WCA 1 are Histosols dominated by Loxahatchee Peat, which is acidic and

Abbreviations: NMR, nuclear magnetic resonance; WCA, Water Conservation Area.

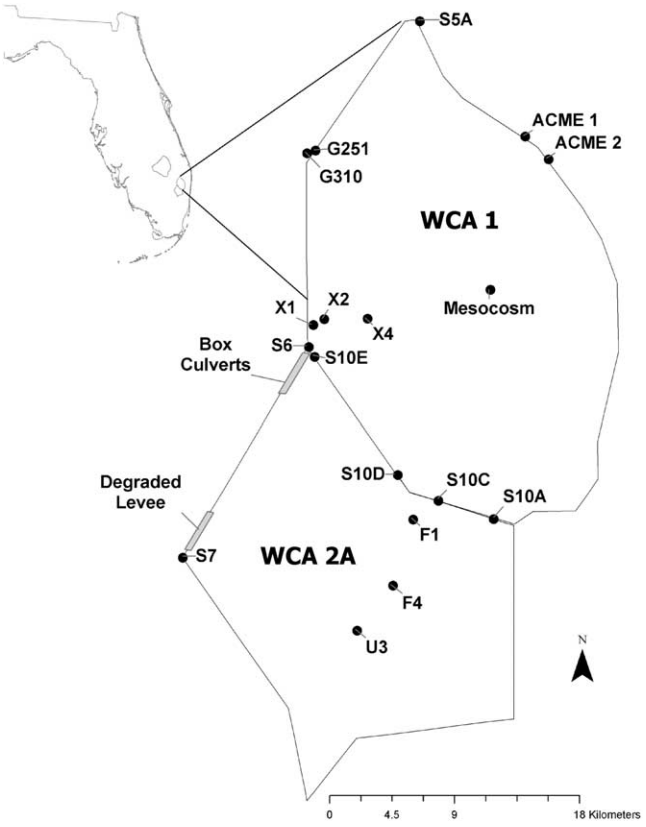

Fig. 1. Map showing locations of sampling sites in Water Conservation Areas 1 and 2A in the Florida Everglades, USA, indicating points of discharge into each of the areas (S5A, G251, G310, ACME 1 & 2, S6, S10s, and S7).

has the lowest ash content of peats in South Florida (Gleason, 1984). Water leaves WCA 1 primarily through culverts along the southern levee (labeled S10s on Fig. 1).

Water Conservation Area 2A (WCA 2A) receives water rich in P and Ca via structures at the northern end of the marsh (labeled S10s on Fig. 1), which moves slowly southward via sheet flow. This has converted the 44 800 ha of WCA 2A from a soft water to a hard water ecosystem (Slate and Stevenson, 2000), with a P gradient that extends 7 km into the interior of the marsh (McCormick et al., 2001). Phosphorus-enriched runoff also enters through the S7 structure on the western boundary. The opening of a constructed treatment wetland in 2000 to remove P from agricultural runoff reduced P inputs to the marsh in discharge from the western levee, which enters WCA 2A through box culverts and across a degraded levee (Fig. 1). Water leaves WCA 2A through a series of culverts along the southern boundary.

In both wetlands, P-enriched areas are characterized by dense mono-specific stands of cattail (*Typha* spp.), while interior sites are characterized by sawgrass (*Cladium jamaicense* Crantz) ridges interspersed with open-water sloughs. The periphyton community in WCA 1 is an assemblage of desmids (unicellular green algae) and diatoms adapted to the soft water conditions, whereas the sloughs in WCA 2A are dominated by calcareous periphyton mats comprised of Ca-precipitating cyanobacteria and diatoms (McCormick et al., 2001). Other key distinctions between the soft water and calcareous sloughs are that calcerous sloughs contain a more cohesive benthic mat and fewer emergent macrophytes.

Samples for NMR analysis were all collected on a single day in June 2003. Sites were selected to represent P enriched (F1, X1) and unenriched (U3, X4) sites in both conservation areas. In addition, samples were collected from sites considered to represent the transition between P-enriched and unenriched conditions (X2, F4). The distinct soft water nature of WCA 1 was assessed by collecting samples from an additional site in the heart of the marsh where experimental mesocosms are located (Mesocosm).

At each site, three replicate cores (10-cm diameter) were taken to 10 cm depth in the organic soil layer. Benthic unconsolidated flocculent material (floc), which included plant detritus and algae, was separated from underlying soil in the field. Samples were transported on ice to the laboratory where they were immediately frozen at −80°C to halt possible nutrient transformations. Time from sampling to freezing was 2 d. Frozen samples were lyophilized and ground to pass a 2-mm sieve. Replicate samples from each site were analyzed separately to provide information on field variability.

Determination of Chemical Properties

Total C and N were determined by combustion and gas chromatography using a Flash EA1112 CNH analyzer (CE Elantech, Lakewood, NJ). Soil pH was determined in a 1:20 ratio of lyophilized soil to deionized water (approximate 1:2 on a wet weight basis). Total Al, Ca, and Fe were determined by digestion of a 0.5-g sample in concentrated HNO_3 (8 mL) and $HClO_4$ (5 mL) (Olsen and Sommers, 1982), with detection by inductively coupled plasma–optical emission spectrometry (ICP–OES). Total phosphorus was determined by automated molybdate colorimetry following ashing at 550°C for 3 h and digestion in 6 *M* HCl. Surface water samples were filtered through 0.45-μm polyethersulfone membranes (Environmental Systems, Ann Arbor, MI) and analyzed for total P (EPA 365.4), reactive P (EPA 365.1), nitrate plus nitrite (EPA 353.2), organic carbon (EPA 415.1), and calcium and iron (EPA 200.7) by standard procedures (USEPA, 1983). Surface water chemistry of the sampling sites is shown in Table 1.

Solution Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy

Phosphorus was extracted by shaking 5 g of lyophilized soil or floc with 100 mL of a solution containing 0.25 *M* NaOH and 0.05 *M* EDTA (ethylenediaminetetraacetate) for 4 h at 20°C (Cade-Menun and Preston, 1996). Each replicate sample was extracted individually and centrifuged at 10 000 × *g* for 30 min, and an aliquot was taken for determination of total P by ICP–OES following a 25-fold dilution. Equal volumes of the remaining replicate extracts were then combined, frozen immediately at −80°C, lyophilized, and ground.

For solution ^{31}P NMR spectroscopy, each freeze-dried extract (approximately 100 mg) was redissolved in 0.1 mL of deuterium oxide and 0.9 mL of a solution containing 1 *M* NaOH and 0.1 *M* EDTA, then transferred to a 5-mm NMR tube. The deuterium oxide provided an NMR signal lock and the NaOH raised the pH to >13 to ensure consistent chemical shifts and optimum spectral resolution. Inclusion of EDTA in the NMR tube reduces line broadening by chelating free Fe in solution (Turner and Richardson, 2004).

Solution ^{31}P NMR spectra were obtained using a Bruker (Billerica, MA) Avance DRX 500 MHz spectrometer operating at 202.456 MHz for ^{31}P and 500.134 MHz for ^{1}H. Samples were analyzed using a 6-μs pulse (45°), a delay time of 1.0 s, and an acquisition time of 0.2 s. Between 48 000 and 69 000 scans were acquired depending on the P concentration of the lyophilized extract, and broadband proton decoupling was used for all samples. Chemical shifts of signals were deter-

Table 1. Selected chemical characteristics of filtered (<0.45 μm) surface water from sites in Water Conservation Areas 1 and 2A in the Florida Everglades, USA. Data are monthly means ± standard error of samples collected between 1997 and 2003 unless indicated otherwise.

Location	Distance from inflow	Total P	Reactive P	Fe	$NO_3 + NO_2$	NH_4^+	Ca	Organic C
	km	μg L^{-1}				mg L^{-1}		
Water Conservation Area 1								
Enriched cattail (X1)	0.5	16 ± 3	10 ± 2	11 ± 1	80 ± 56	0.07 ± <0.01	76 ± 2	35 ± 1
Transitional sawgrass (X2)	1.3	7 ± <1	5 ± <1	8 ± <1	43 ± 36	0.03 ± <0.01	59 ± 3	31 ± 1
Unenriched sawgrass (X4)	4.4	6 ± <1	5 ± <1	39 ± 9	10 ± 2	0.03 ± <0.01	24 ± 3	23 ± <1
Unenriched slough (Mesocosm)†	8.7	4 ± <1	4 ± <1	52 ± 3	5 ± <1	0.05 ± 0.02	5 ± <1	20 ± <1
Water Conservation Area 2A								
Enriched cattail (F1)	1.8	53 ± 11	38 ± 11	13 ± 2	10 ± 3	0.07 ± 0.02	91 ± 3	47 ± 1
Transitional sawgrass (F4)	6.8	12 ± 4	8 ± 3	9 ± 1	8 ± 1	0.03 ± <0.01	71 ± 2	35 ± 1
Unenriched sawgrass (U3)	10.8	5 ± <1	5 ± <1	12 ± 1	11 ± 2	0.06 ± 0.01	60 ± 2	36 ± 1

† Monthly means ± standard error for samples collected between March 1997 and February 1999 (Newman et al., 2001).

mined in parts per million (ppm) relative to an external standard of 85% H_3PO_4. Signals were assigned to individual P compounds or functional groups based on literature reports (Turner et al., 2003b) and signal areas calculated by integration. Spectra were plotted with a line broadening of 8 Hz, although additional spectra were plotted with a line broadening of 1 Hz to preserve fine resolution in the phosphate monoester region.

Statistical Analysis

Data are reported as means ± standard deviation of three replicate cores, which indicates spatial variability at each site. The statistical significance of the difference between means along the nutrient gradients was determined using one-way analysis of variance, with least significant difference of means (5%) shown in Table 2.

RESULTS

Total Element Concentrations along Nutrient Enrichment Gradients

In WCA 1, P concentrations in benthic floc and soil decreased markedly with distance along the enrichment gradient ($p < 0.001$). Concentrations in benthic floc decreased from 1.38 ± 0.11 g P kg^{-1} dry wt. close to the canal inflow to 0.43 ± 0.05 g P kg^{-1} dry wt. in the marsh interior (Table 2). Concentrations in soils were highest at the transitional site (0.56 ± 0.12 g P kg^{-1} dry wt.) and lowest at the unenriched acidic slough (0.22 ± 0.03 g P kg^{-1} dry wt.).

In WCA 2A, changes in P concentrations along the enrichment gradient were only significant in the benthic

Table 2. Total elements in benthic floc and soil along nutrient gradients in Water Conservation Areas in the Florida Everglades, USA. Data are mean ± standard deviation of three field replicates.

Location	pH	C	N	P	Al	Ca	Fe	C to N ratio	N to P ratio
		g kg^{-1} dry wt.							
Water Conservation Area 1									
Benthic floc									
Enriched cattail (X1)	7.10	473 ± 10	33.9 ± 1.3	1.38 ± 0.11	2.3 ± 0.1	40.0 ± 2.7	6.1 ± 0.5	14.0 ± 0.3	25 ± 2
Transitional sawgrass (X2)	7.24	490 ± 7	32.6 ± 2.1	0.83 ± 0.05	0.8 ± 0.1	27.0 ± 1.1	2.4 ± 0.4	15.1 ± 1.3	39 ± 1
Unenriched sawgrass (X4)	6.84	473 ± 7	33.4 ± 3.9	0.54 ± 0.06	1.5 ± 0.2	22.4 ± 0.4	5.0 ± 0.3	14.3 ± 1.7	62 ± 2
Unenriched slough (X4)	6.66	478 ± 5	44.9 ± 1.3	0.49 ± 0.02	1.7 ± 0.1	18.0 ± 0.7	2.9 ± 0.3	10.6 ± 0.3	92 ± 7
Unenriched slough (Mesocosm)	5.80	494 ± 4	45.8 ± 0.2	0.43 ± 0.05	2.2 ± 0.1	11.1 ± 0.2	6.9 ± 0.4	10.8 ± 0.1	108 ± 12
Least significant difference (5%)		10	3.0	0.09	0.2	1.9	0.5	1.4	9
Soil (0–10 cm)									
Enriched cattail (X1)	6.83	495 ± 20	28.1 ± 2.1	0.44 ± 0.08	2.1 ± 0.4	34.9 ± 2.0	4.6 ± 0.7	17.6 ± 0.8	66 ± 12
Transitional sawgrass (X2)	6.75	485 ± 3	35.2 ± 0.4	0.56 ± 0.12	1.8 ± 0.3	28.8 ± 0.5	4.7 ± 0.3	13.8 ± 0.1	65 ± 13
Unenriched sawgrass (X4)	6.44	498 ± 11	33.7 ± 1.4	0.36 ± 0.04	2.4 ± 0.2	23.1 ± 1.8	3.7 ± 0.7	14.8 ± 0.8	94 ± 13
Unenriched slough (X4)	6.47	510 ± 1	41.2 ± 1.9	0.27 ± 0.04	2.1 ± 0.3	18.8 ± 0.7	2.7 ± 0.4	12.4 ± 0.6	156 ± 19
Unenriched slough (Mesocosm)	5.66	514 ± 12	43.7 ± 0.5	0.22 ± 0.03	2.0 ± 0.3	11.9 ± 1.5	5.8 ± 0.8	11.8 ± 0.3	204 ± 23
Least significant difference (5%)		17	2.0	0.10	0.4	2.0	0.9	0.8	23
Water Conservation Area 2A									
Benthic floc									
Enriched cattail (F1)	7.42	446 ± 8	30.3 ± 2.0	1.26 ± 0.17	1.2 ± 0.1	62.4 ± 7.3	4.7 ± 0.6	14.7 ± 0.7	24 ± 2
Transitional sawgrass (F4)	7.93	440 ± 18	42.0 ± 1.6	1.62 ± 0.13	0.7 ± 0.1	45.8 ± 7.4	1.9 ± 0.2	10.5 ± 0.4	26 ± 3
Unenriched sawgrass (U3)	7.37	440 ± 7	29.7 ± 0.7	0.56 ± 0.04	1.5 ± 0.1	34.0 ± 0.3	7.6 ± 0.2	14.8 ± 0.5	53 ± 3
Unenriched slough (U3)	7.91	302 ± 32	25.1 ± 3.7	0.31 ± 0.13	0.7 ± 0.2	204.6 ± 31.8	1.4 ± 0.7	12.1 ± 0.5	86 ± 20
Least significant difference (5%)		25	3.0	0.17	0.2	22	0.6	0.7	14
Soil (0–10 cm)									
Enriched cattail (F1)	7.60	427 ± 41	29.8 ± 2.9	0.67 ± 0.52	1.6 ± 0.5	49.5 ± 25.5	4.5 ± 1.0	14.3 ± 0.3	62 ± 33
Transitional sawgrass (F4)	7.72	355 ± 4	28.8 ± 0.7	0.58 ± 0.19	1.2 ± 0.6	161.3 ± 13.4	3.3 ± 2.6	12.3 ± 0.2	53 ± 15
Unenriched sawgrass (U3)	6.99	477 ± 4	35.4 ± 0.8	0.46 ± 0.07	1.6 ± 0.2	26.6 ± 0.9	8.8 ± 0.6	13.5 ± 0.3	78 ± 13
Unenriched slough (U3)	7.53	480 ± 35	41.1 ± 3.1	0.43 ± 0.16	2.0 ± 0.1	31.6 ± 1.0	12.3 ± 1.1	11.7 ± 0.1	104 ± 31
Least significant difference (5%)		36	2.9	0.38	0.6	18.9	2.0	0.3	32

floc ($p < 0.001$). The highest P concentration was detected at the transitional site (1.62 ± 0.13 g P kg^{-1} dry wt.), decreasing to 0.31 ± 0.13 g P kg^{-1} dry wt. in the unenriched calcareous slough (Table 2). In the soil of WCA 2A, P concentrations ranged from 0.67 ± 0.52 g P kg^{-1} dry wt. at the inflow, to 0.43 ± 0.16 g P kg^{-1} dry wt. in the unenriched slough, but were not significantly different ($p = 0.73$) due to the marked variability at the enriched site. At all sites in both Water Conservation Areas, P concentrations were smaller in the soil than the benthic floc ($p < 0.05$), although the exception was the unenriched calcareous slough in WCA 2A.

There were significant differences ($p < 0.01$) in concentrations of C, N, Al, Ca, and Fe in benthic floc along the enrichment gradients in both Water Conservation Areas. For soils, there were significant differences in all elements along the enrichment gradients except for C ($p = 0.08$) and Al ($p = 0.40$) in WCA 1 and Al ($p = 0.20$) in WCA 2A. Carbon concentrations were relatively similar among sites, although the concentration in the benthic floc from the unenriched slough in WCA 2A was noticeably lowest (Table 2). Nitrogen concentrations in both Water Conservation Areas were greater in unenriched slough sites for both floc and soil, while for benthic floc in WCA 2A the transitional site contained a high total N concentration (42.0 ± 1.6 g N kg^{-1} dry wt.) compared to the other sites (Table 2). Much of the C in this floc sample almost certainly occurred as $CaCO_3$.

There were significant differences in the C to N and N to P ratios ($p < 0.001$) in both benthic floc and soil along enrichment gradients in both Water Conservation Areas, with the exception of the N to P ratio for the soil of WCA 2A. The C to N ratios generally decreased with distance from the pollutant inflow, while N to P ratios increased. For example, N to P mass ratios in the benthic floc of WCA 1 increased more than fourfold along the enrichment gradient, from 17 ± 2 at the enriched site, to 73 ± 6 in the unenriched slough in the marsh interior (Mesocosm site). Ratios were similar throughout WCA 2A and were greater in soil than benthic floc, with a maximum value of 137 ± 10 in soil of the unenriched slough (Mesocosm site) in WCA 1.

One of the major chemical differences between the two Water Conservation Areas was Ca concentration. A clear Ca enrichment gradient was evident in benthic floc and soil from WCA 1, with Ca concentrations in benthic floc decreasing from 40.0 ± 2.7 g Ca kg^{-1} dry wt. in the enriched site, to 11.1 ± 0.2 g Ca kg^{-1} dry wt. in the unenriched slough (Mesocosm site). The greatest Ca concentration was in the benthic floc of the calcareous slough in WCA 2A (204.6 ± 31.8 g Ca kg^{-1} dry wt.). Aluminum and Fe showed no clear trends, although a high Fe concentration was detected in soil of the unenriched calcareous slough in WCA 2A.

Phosphorus Composition by Solution Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy

Phosphorus recovery was generally greatest from benthic floc in WCA 1 (55–66%), although the highest value was for benthic floc from the transitional site in WCA 2A (74%) (Table 3). Phosphorus recovery was less efficient from soil, but was relatively consistent, being 47 to 58% for soils from WCA 1 and 37 to 46%

Table 3. Recovery of total soil P and concentrations of P compounds determined by solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy in NaOH–EDTA extracts of benthic floc and soil from Water Conservation Areas in the Florida Everglades, USA. Values in parentheses are the proportion (%) of the total extracted P.

Location	Recovery of total soil P	Phosphate	Phosphate monoesters	DNA	Pyrophosphate
	%	mg P kg^{-1} dry wt.			
Water Conservation Area 1					
Benthic floc					
Enriched cattail (X1)	55 ± 3	270 (36)	202 (27)	217 (29)	65 (9)
Transitional sawgrass (X2)	58 ± 6	98 (20)	128 (26)	190 (39)	71 (15)
Unenriched sawgrass (X4)	66 ± 4	82 (23)	116 (33)	101 (29)	52 (15)
Unenriched slough (X4)	65 ± 4	86 (27)	80 (25)	91 (28)	64 (20)
Unenriched slough (Mesocosm)	64 ± 3	53 (19)	74 (27)	108 (39)	39 (14)
Soil (0–10 cm)					
Enriched cattail (X1)	47 ± 6	66 (31)	62 (30)	69 (33)	12 (6)
Transitional sawgrass (X2)	50 ± 2	63 (23)	93 (33)	112 (40)	12 (4)
Unenriched sawgrass (X4)	58 ± 1	48 (23)	42 (20)	100 (48)	20 (10)
Unenriched slough (X4)	55 ± 4	38 (26)	19 (13)	78 (53)	13 (9)
Unenriched slough (Mesocosm)	49 ± 2	30 (29)	16 (15)	51 (48)	9 (9)
Water Conservation Area 2A					
Benthic floc					
Enriched cattail (F1)	48 ± 5	138 (23)	214 (35)	209 (35)	45 (7)
Transitional sawgrass (F4)	74 ± 5	413 (35)	320 (27)	194 (16)	264 (22)
Unenriched sawgrass (U3)	67 ± 4	139 (37)	101 (27)	108 (28)	31 (8)
Unenriched slough (U3)	24 ± 13	25 (38)	40 (62)	ND†	ND
Soil (0–10 cm)					
Enriched cattail (F1)	44 ± 3	82 (29)	91 (32)	98 (35)	12 (4)
Transitional sawgrass (F4)	43 ± 11	99 (38)	91 (35)	57 (22)	14 (6)
Unenriched sawgrass (U3)	46 ± 8	76 (36)	40 (19)	86 (41)	7 (3)
Unenriched slough (U3)	37 ± 13	43 (30)	51 (36)	49 (34)	ND

† Not detected.

for soils from WCA 2A. The lowest P recovery was for benthic floc from the unenriched calcareous slough in WCA 2A (24%).

Chemical shifts detected in solution ^{31}P NMR spectra were similar for extracts of benthic floc and soil from both Water Conservation Areas (Fig. 2 and 3). The strong signal at approximately 6.15 ppm was assigned to phosphate. Concentrations followed the trend in total P, generally decreasing with distance from the pollutant inflow (Table 3). For both Water Conservation Areas, phosphate concentrations ranged between 25 and 413 mg P kg^{-1} dry wt. in benthic floc extracts and between 30 and 99 mg P kg^{-1} dry wt. in soil extracts. Phosphate was the smallest proportion of the extracted P (19%) in benthic floc from the unenriched acidic slough in WCA 1 and greatest (38%) in benthic floc from the unenriched slough and soil from the transitional site, both in WCA 2A (Table 3).

A signal close to −4.4 ppm was assigned to pyrophosphate, an inorganic polyphosphate of chain length n = 2. The largest concentration of pyrophosphate was detected in benthic floc from the transitional site in WCA 2A (264 mg P kg^{-1} dry wt.; 22% extracted P), with much smaller concentrations detected in other samples. At all sites, pyrophosphate concentrations were higher in benthic floc than soil, although none was detected in benthic floc or soil from the unenriched calcareous slough of WCA 2A. With the exception of the transitional site in WCA 2A, pyrophosphate concentrations were larger in benthic floc from WCA 1 (39–71 mg P kg^{-1} dry wt.; 9–20% extracted P) compared to WCA 2A (12–45 mg P kg^{-1} dry wt.; 4–8% extracted P).

Signals between 4 and 6 ppm were assigned to phosphate monoesters, which constituted a large proportion of the extracted P in all samples. Concentrations in benthic floc ranged from 40 mg P kg^{-1} dry wt. in the unenriched calcareous slough in WCA 2A, to 320 mg P kg^{-1} dry wt. from the transitional site in WCA 2A. Concen-

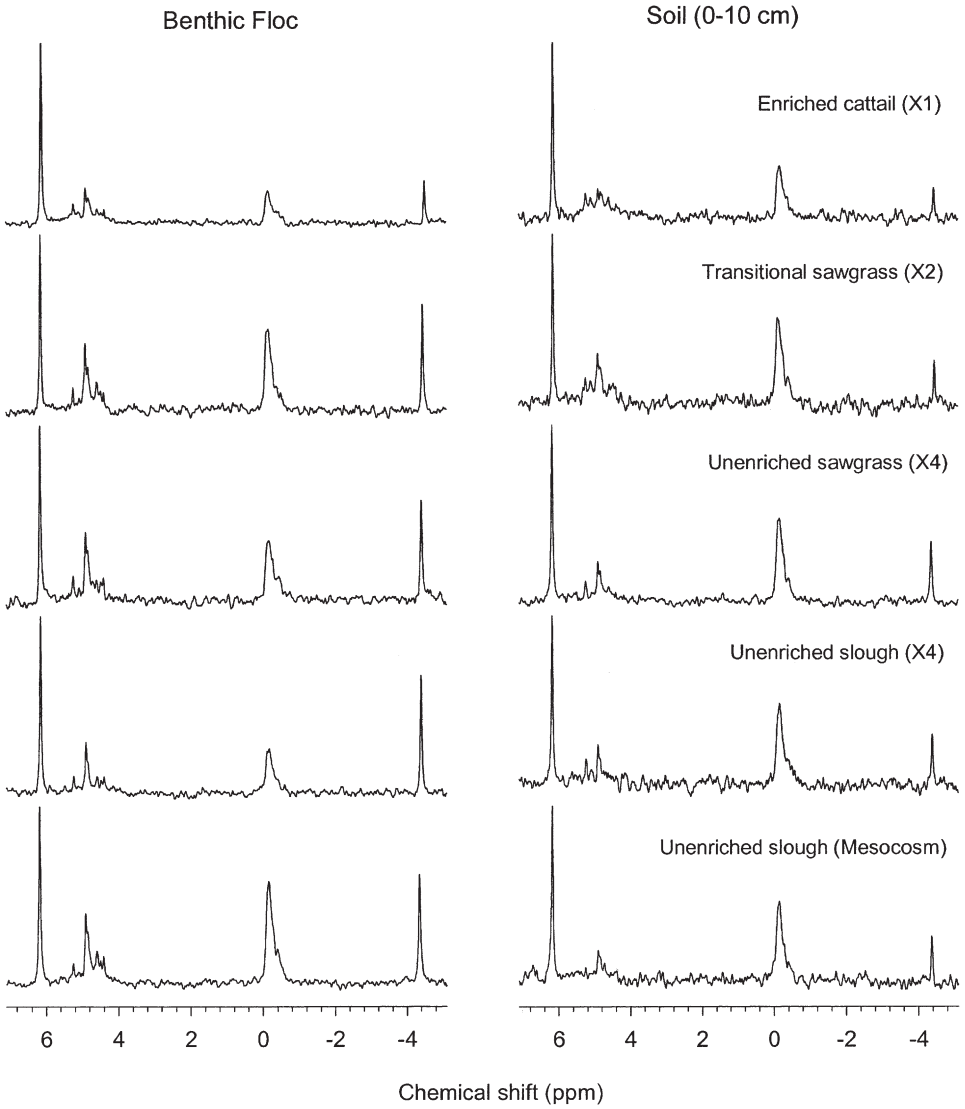

Fig. 2. Solution ^{31}P nuclear magnetic resonance (NMR) spectra of NaOH–EDTA extracts of benthic floc and soil (0–10 cm) from Water Conservation Area 1 in the Florida Everglades, USA. Spectra are plotted with 8-Hz line broadening and scaled to the height of the phosphate signal at 6.1 ppm.

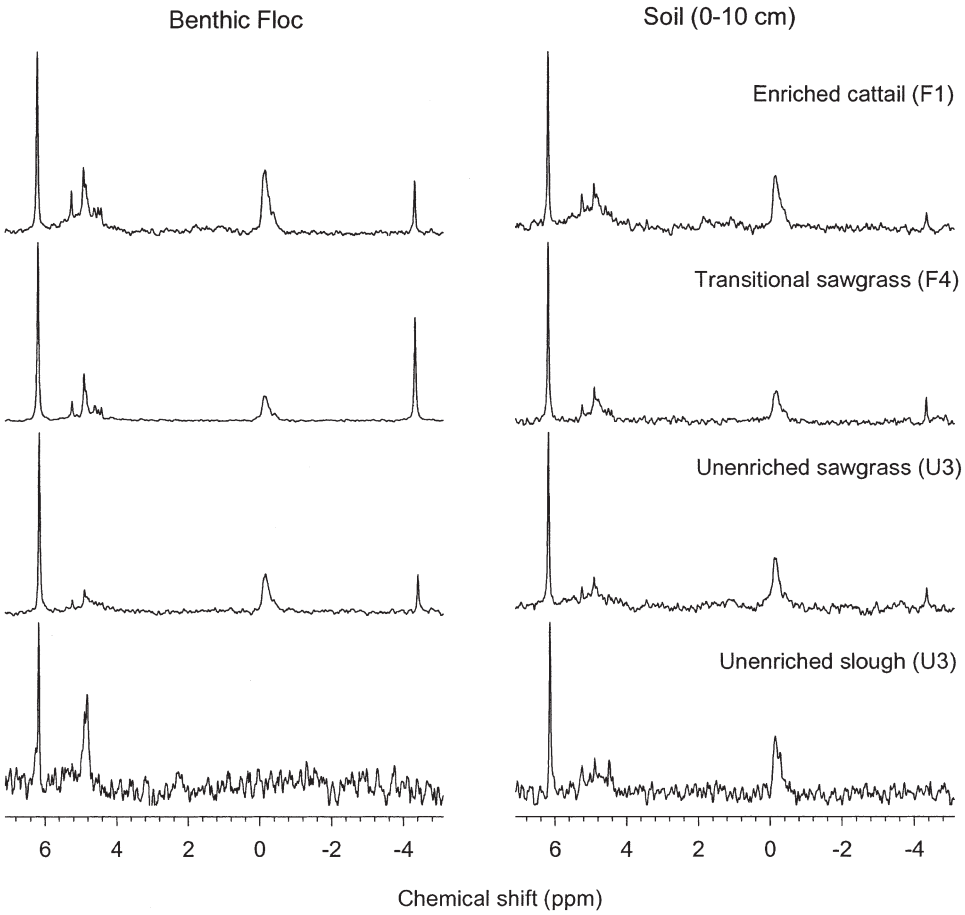

Fig. 3. Solution ^{31}P nuclear magnetic resonance (NMR) spectra of NaOH–EDTA extracts of benthic floc and soil (0–10 cm) from Water Conservation Area 2A in the Florida Everglades, USA. Spectra are plotted with 8-Hz line broadening and scaled to the height of the phosphate signal at 6.1 ppm.

trations were lower in soils (16–93 mg P kg^{-1} dry wt.), representing between 13 and 36% of the extracted P. Phosphate monoesters were proportionally largest in the unenriched calcareous slough of WCA 2A (62%), although this sample contained a relatively small P concentration.

In all samples, the largest monoester signals were at 5.24 and 4.91 ppm, indicating the presence of phosphatidic acid and β-glycerophosphate, respectively (e.g., in the extract of benthic floc from the transitional site in WCA 2A; Fig. 4). These compounds originate from the hydrolysis of phospholipids, notably phosphatidyl choline, in alkaline solution (Turner et al., 2003b). Further phosphate monoester signals appeared close to 4.85, 4.78, 4.69, 4.51, and 4.42 ppm (Fig. 4), which are characteristic of mononucleotide degradation products of RNA in alkaline solution (Turner et al., 2003b). A further signal at 3.5 ppm in some extracts was assigned to glucose 1-phosphate. Signals from phytic acid (*myo*-inositol hexa*kis*phosphate) were absent in all extracts with the exception of soil from the unenriched sawgrass ridge in WCA 2A, as indicated by a small signal at 5.9 ppm from the phosphate at the C-2 position on the inositol ring (Fig. 3).

A relatively broad signal close to 0 ppm was assigned to DNA, which was present in relatively large proportions in benthic floc and soil from both Water Conservation Areas (Table 3). Concentrations in benthic floc ranged between 91 and 217 mg P kg^{-1} dry wt. (16–39% extracted P), except for the unenriched calcareous slough in WCA 2A, in which DNA was not detected. In soils, DNA concentrations ranged between 51 and 112 mg P kg^{-1} dry wt. (33–53% extracted P) in WCA 1 and between 49 and 98 mg P kg^{-1} dry wt. (22–41% extracted P) in WCA 2A (Table 3). Concentrations followed the enrichment gradient, although the proportion of the extracted P present as DNA tended to increase as total P concentration decreased.

Traces of signals between 0.5 and 2.0 ppm were assigned to phospholipids and RNA that were not degraded during extraction and analysis (see above). Long-chain polyphosphates (signals close to −20 ppm) and phosphonates (signals close to 20 ppm) were not detected in any sample.

DISCUSSION

The most striking aspect of the study was the dominance of phosphate diesters in the organic P fraction. In addition to the large concentrations of DNA, most of the phosphate monoesters were identified as compounds derived from the alkaline hydrolysis of phospho-

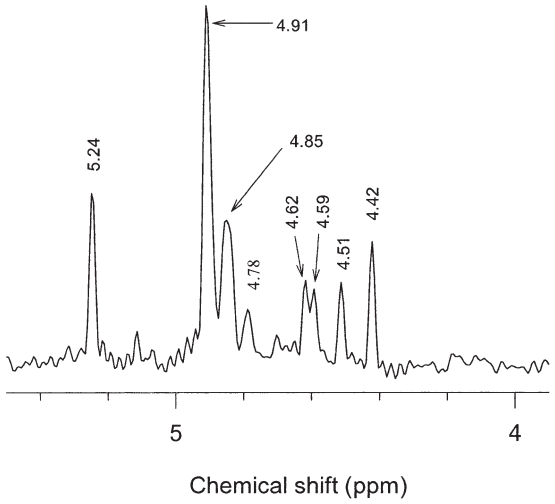

Fig. 4. Solution ^{31}P NMR spectrum of a NaOH–EDTA extract of benthic floc from the moderately enriched transitional site (F4) in Water Conservation Area 2A in the Florida Everglades, USA. Only the phosphate monoester region is shown and is plotted with 1-Hz line broadening to show fine resolution. Signals at 5.24 and 4.91 ppm are degradation products of phospholipids in alkaline solution, while the remaining signals are the mononucleotide degradation products of RNA in alkaline solution (Turner et al., 2003b).

lipids and RNA (Turner et al., 2003b). Consequently, it seems that almost all the extractable organic P was in the form of phosphate diesters before extraction. This is unusual, because extractable organic P in most soils occurs mainly as inositol phosphates, predominantly *myo*-inositol hexa*kis*phosphate, but also a range of lower esters and stereoisomers (Turner et al., 2002). For example, large proportions of *scyllo*-inositol hexa*kis*phosphate can be present (Turner and Richardson, 2004). The absence of detectable concentrations of either of these compounds in the current study was therefore unexpected. The reason for the absence of inositol phosphates is unclear, but may be linked to the limited reactive clay surfaces for stabilization in these organic soils, or to relatively rapid decomposition under anaerobic conditions (Suzumura and Kamatani, 1995). There is little information on inositol phosphates in high organic matter peats, although only small concentrations were present in Scandinavian tundra soils (Turner et al., 2004).

Phosphate diesters are the main input of organic P to soils, but typically constitute only a small fraction of the soil organic P (Anderson, 1967). However, there is little comparable information on the organic P composition of submerged wetland soils. In recently reflooded (1–5 yr) Histosols with a history of cultivation and pH values close to neutral, the organic P in NaOH–EDTA extracts was mainly phosphate monoesters, with diester-to-monoester ratios between 0.1 and 0.2 (Robinson et al., 1998). Alkaline extracts of benthic floc from a constructed wetland near the current study sites was reported to contain large proportions of phosphoenolpyruvate (Pant and Reddy, 2001), although this was almost certainly a mis-assignment of the DNA signal. Subsequent analysis of the water column of this constructed wetland revealed that >70% of the soluble organic P was hydrolyzed by phosphodiesterase and was therefore in the form of phosphate diesters (Pant et al., 2002).

Clearly, the turnover of phosphate diesters must be important in subtropical wetlands, yet the availability of phosphate diesters to organisms in wetlands is relatively unknown. Published data on phosphodiesterase activity in wetlands is rare, although some information is available from P-limited environments elsewhere. In the English uplands, for example, rates of phosphodiesterase activity in aquatic mosses were greater in streams at higher altitude (Christmas and Whitton, 1998). The authors suggested that this was linked to the greater cover of blanket peat at higher altitude, which is rich in phosphate diesters (Turner et al., 2003a). There is evidence for the expression of root phosphomonoesterase activity in some common wetland macrophytes, including cattail and sawgrass (Kuhn et al., 2002), although rates of phosphodiesterase have not been measured.

The assessment of phosphodiesterase activity is confounded by potential use of phosphate diesters by heterotrophic microbes as a source of energy or N rather than P (Heath, 2005). There is some evidence that this occurs in Everglades communities (Wright and Reddy, 2001b), although it is likely to be most important in nutrient-enriched sites where productivity approaches N limitation.

Information on the origins of the extracted phosphate diesters would be useful to infer their potential bioavailability. Some were almost certainly derived from intact microbial cells, because microbes can contain a considerable proportion of the P in wetland soils (Qualls and Richardson, 1995). However, much of the DNA was probably present as part of the nonliving soil organic P (Makarov et al., 2002). Most phosphate diesters are degraded relatively rapidly after addition to soil (Bowman and Cole, 1978), although they accumulate in acidic soils with high organic matter concentrations (e.g., Cade-Menun et al., 2000) and can be stabilized by sorption to clays (Greaves and Wilson, 1969). As soils in the current study were not strongly acidic and contained little clay, further investigation of the mechanisms involved in stabilizing phosphate diesters in wetland soils is required.

Pyrophosphate was detected in considerable proportions in benthic floc but was relatively absent in underlying soil, suggesting that it decomposes rapidly. Despite its common occurrence in soils from a wide range of environments, the origin and function of pyrophosphate in soil remain unclear. It may have been extracted from live microbes, possibly originating as inorganic or organic polyphosphates (e.g., adenosine 5′-triphosphate). However, polyphosphates were not detected in any samples and their absence is not due to degradation during extraction and analysis (Hupfer et al., 1995; Cade-Menun and Preston, 1996). The presence of pyrophosphate in unenriched samples indicates that it is unlikely to be derived exclusively from anthropogenic sources, as suggested in a study of estuarine sediments (Sundareshwar et al., 2001).

The Mesocosm site in WCA 1 was included in this study to compare a true soft water slough with a hard

water equivalent. Differences between the sloughs, including composition of the algal community and the abundance of macrophytes, almost certainly contribute to differences in the forms and stability of organic P. Periphyton in calcareous sloughs mediates the precipitation of insoluble Ca-phosphates or coprecipitation of phosphate with $CaCO_3$. This reduces P availability to organisms (Dodds, 2003) and may intensify the demand for P from organic compounds. Also, the cohesive nature of the periphyton mat in WCA 2A means that nutrients are tightly cycled within the mat structure. Both factors may explain in part the lack of DNA and pyrophosphate in the benthic floc from the calcareous slough, although strong P limitation in the soft water marsh clearly does not preclude the accumulation of large concentrations of DNA.

A large proportion of the total P was recovered from most samples. Values were typical for alkaline extracts of most soils, although high recoveries were reported from high organic matter acidic soils (Cade-Menun et al., 2000; Turner et al., 2003a). For wetland soils, Robinson et al. (1998) reported recovery of between 47 and 64% of the P from recently flooded organic soils in Florida. The NaOH–EDTA extraction procedure is designed to quantitatively recover organic P from soil (Bowman and Moir, 1993; Turner et al., 2005a), so P not extracted in NaOH–EDTA was probably inorganic phosphate, most likely in the form of calcium or magnesium phosphate precipitates (Reddy et al., 1998).

Small amounts of recalcitrant organic P may not have been extracted, although it is impossible to assess this because there is no direct method of determining the total organic P concentrations in soil (Turner et al., 2005a). Total organic P in wetland soils is conventionally assessed by ignition, but this can be inaccurate. Similarly, estimation of organic P by alkaline extraction is compromised by interference in P speciation by molybdate colorimetry, which can markedly overestimate organic P due to the presence of humic–metal–phosphate complexes (Turner et al., 2003a). We therefore consider the combination of NaOH–EDTA extraction with solution ^{31}P NMR spectroscopy to be the optimum method for characterization of organic P in wetland soils.

In summary, phosphate diesters dominated the organic P in alkaline extracts of benthic floc and soils from sites along nutrient gradients in subtropical wetlands. The exception was in benthic floc from a calcareous slough, which contained no detectable DNA. As phosphate diesters are also the dominant organic P compounds in plant and microbial inputs to wetlands, the hydrolysis of phosphate diesters seems likely to be the rate limiting step in soil organic P turnover in the Everglades. This in turn highlights the importance of phosphodiesterase activity in P acquisition by wetland organisms. We recommend that greater emphasis should be placed on understanding phosphodiesterase activity and the turnover of phosphate diesters in wetlands. Emphasis should also be placed on identifying the unextractable fraction of the soil P, which is critical for understanding long-term P sequestration in wetland soils.

ACKNOWLEDGMENTS

The authors thank Dr. Alex Blumenfeld, Susie Hansen, Dr. April Leytem, Dr. Jana Newman, and Dr. Ramesh Reddy for their contribution. B. Turner thanks the USDA-ARS Northwest Irrigation and Soils Research Laboratory and the Soil and Water Science Department, University of Florida, for support during this work. Florida Agricultural Experiment Station Journal Series number R-10526.

REFERENCES

Anderson, G. 1967. Nucleic acids, derivatives, and organic phosphates. p. 67–90. *In* A.D. McLaren and G.H. Peterson (ed.) Soil biochemistry. Vol. 1. Marcel Dekker, New York.

Bowman, R.A., and C.V. Cole. 1978. Transformations of organic phosphorus substrates in soils as evaluated by $NaHCO_3$ extraction. Soil Sci. 125:49–54.

Bowman, R.A., and J.O. Moir. 1993. Basic EDTA as an extractant for soil organic phosphorus. Soil Sci. Soc. Am. J. 57:1516–1518.

Cade-Menun, B.J., S.M. Berch, C.M. Preston, and L.M. Lavkulich. 2000. Phosphorus forms and related soil chemistry of Podzolic soils on northern Vancouver Island. 1. A comparison of two forest types. Can. J. For. Res. 30:1714–1725.

Cade-Menun, B.J., and C.M. Preston. 1996. A comparison of soil extraction procedures for ^{31}P NMR spectroscopy. Soil Sci. 161:770–785.

Christmas, M., and B.A. Whitton. 1998. Phosphorus and aquatic bryophytes in the Swale-Ouse river system, north-east England. 2. Phosphomonoesterase and phosphodiesterase activities of *Fontinalis antipyretica*. Sci. Total Environ. 210/211:401–409.

Craft, C.B., and C.J. Richardson. 1998. Recent and long-term organic soil accretion and nutrient accumulation in the Everglades. Soil Sci. Soc. Am. J. 62:834–843.

Dodds, W.K. 2003. The role of periphyton in phosphorus retention in shallow freshwater aquatic systems. J. Phycol. 39:840–849.

Gleason, P.J. 1984. Environments of South Florida: Present and past II. Miami Geol. Soc., Coral Gables, FL.

Greaves, M.P., and M.J. Wilson. 1969. The adsorption of nucleic acids by montmorillonite. Soil Biol. Biochem. 1:317–323.

Heath, R.T. 2005. Microbial turnover of organic phosphorus in aquatic systems. p. 185–204. *In* B.L. Turner, E. Frossard, and D.S. Baldwin (ed.) Organic phosphorus in the environment. CABI Publ., Wallingford, UK.

Hupfer, M., R. Gächter, and H. Rüegger. 1995. Polyphosphate in lake sediments: ^{31}P NMR spectroscopy as a tool for its identification. Limnol. Oceanogr. 40:610–617.

Ivanoff, D.B., K.R. Reddy, and S. Robinson. 1998. Chemical fractionation of organic phosphorus in Histosols. Soil Sci. 163:36–45.

Kuhn, N.L., I.A. Mendelssohn, K.L. McKee, H. Brix, and S.L. Miao. 2002. Root phosphatase activity in *Cladium jamaicense* and *Typha domingensis* grown under ambient and elevated phosphorus levels. Wetlands 22:794–800.

Makarov, M.I., L. Haumaier, and W. Zech. 2002. The nature and origins of diester phosphates in soils: A ^{31}P-NMR study. Biol. Fertil. Soils 35:136–146.

McCormick, P.V., S. Newman, S. Miao, D.E. Gawlik, D. Marley, K.R. Reddy, and T.D. Fontaine. 2001. Effects of anthropogenic phosphorus inputs on the Everglades. p. 83–126. *In* J.W. Porter and K.G. Porter (ed.) The Everglades, Florida Bay, and Coral Reefs of the Florida Keys. An ecosystem sourcebook. CRC Press, Boca Raton, FL.

Newman, S., H. Kumpf, J.A. Laing, and W.C. Kennedy. 2001. Decomposition responses to phosphorus enrichment in an Everglades (USA) slough. Biogeochemistry 54:229–250.

Newman, S., P.V. McCormick, and J.G. Backus. 2003. Phosphatase activity as an early warning indicator of wetland eutrophication: Problems and prospects. J. Appl. Phycol. 15:45–59.

Newman, S., K.R. Reddy, W.F. DeBusk, Y. Wang, G. Shih, and M.M. Fisher. 1997. Spatial distribution of soil nutrients in a northern Everglades marsh: Water Conservation Area 1. Soil Sci. Soc. Am. J. 61:1275–1283.

Olsen, S.R., and L.E. Sommers. 1982. Phosphorus. p. 403–429. *In* A.L.

Page, R.H. Miller, and D.R. Keeney (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
Pant, H.K., and K.R. Reddy. 2001. Hydrologic influence on stability of organic phosphorus in wetland detritus. J. Environ. Qual. 30: 668–674.
Pant, H.K., K.R. Reddy, and F.E. Dierberg. 2002. Bioavailability of organic phosphorus in a submerged aquatic vegetation-dominated treatment wetland. J. Environ. Qual. 31:1748–1756.
Qualls, R.G., and C.J. Richardson. 1995. Forms of soil phosphorus along a nutrient enrichment gradient in the northern Everglades. Soil Sci. 160:183–198.
Reddy, K.R., R.D. DeLaune, W.F. DeBusk, and M.S. Koch. 1993. Long-term nutrient accumulation rates in the Everglades. Soil Sci. Soc. Am. J. 57:1147–1155.
Reddy, K.R., Y. Wang, W.F. DeBusk, M.M. Fisher, and S. Newman. 1998. Forms of soil phosphorus in selected hydrologic units of the Florida Everglades. Soil Sci. Soc. Am. J. 62:1134–1147.
Robinson, J.S., C.T. Johnston, and K.R. Reddy. 1998. Combined chemical and ^{31}P-NMR spectroscopic analysis of phosphorus in wetland organic soils. Soil Sci. 163:705–713.
Sklar, F.H., K. Rutchey, D. Gawlik, S. Smith, J. Obeysekera, C.J. Madden, M. Darwish, C. Bevier, S. Krupa, S. Newman, G. Crozier, H. Kumpf, and S. Gray. 2002. Hydrologic needs—Effects of hydrology on the Everglades Protection Area (EPA). Everglades Consolidated Report. South Florida Water Manage. Dist., West Palm Beach, FL.
Slate, J.E., and R.J. Stevenson. 2000. Recent and abrupt environmental change in the Florida Everglades indicated from siliceous microfossils. Wetlands 20:346–356.
Sundareshwar, P.V., J.T. Morris, P.J. Pellechia, H.J. Cohen, D.E. Porter, and B.C. Jones. 2001. Occurrence and ecological implications of pyrophosphate in estuaries. Limnol. Oceanogr. 46:1570–1577.
Suzumura, M., and A. Kamatani. 1995. Mineralization of inositol hexaphosphate in aerobic and anaerobic marine-sediments—Implications for the phosphorus cycle. Geochim. Cosmochim. Acta 59:1021–1026.
Turner, B.L., R. Baxter, N. Mahieu, S. Sjögersten, and B.A. Whitton. 2004. Phosphorus compounds in subarctic Fennoscandian soils at the mountain birch (*Betula pubescens*)–tundra ecotone. Soil Biol. Biochem. 36:815–823.
Turner, B.L., B.J. Cade-Menun, L.M. Condron, and S. Newman. 2005a. Extraction of soil organic phosphorus. Talanta 66:294–306.
Turner, B.L., J.A. Chudek, B.A. Whitton, and R. Baxter. 2003a. Phosphorus composition of upland soils polluted by long-term atmospheric nitrogen deposition. Biogeochemistry 65:259–274.
Turner, B.L., E. Frossard, and D.S. Baldwin (ed.) 2005b. Organic phosphorus in the environment. CABI Publ., Wallingford, UK.
Turner, B.L., N. Mahieu, and L.M. Condron. 2003b. Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH–EDTA extracts. Soil Sci. Soc. Am. J. 67:497–510.
Turner, B.L., M. Papházy, P.M. Haygarth, and I.D. McKelvie. 2002. Inositol phosphates in the environment. Philos. Trans. R. Soc. London Ser. B 357:449–469.
Turner, B.L., and A.E. Richardson. 2004. Identification of *scyllo*-inositol phosphates in soils by solution phosphorus-31 nuclear magnetic resonance spectroscopy. Soil Sci. Soc. Am. J. 68:802–808.
USEPA. 1983. Methods for chemical analyses of water and wastes. USEPA, Environ. Monitoring Support Lab., Cincinnati, OH.
Verhoeven, J.T.A., A.M. Kooijan, and G. van Wirdum. 1988. Mineralization of N and P along a trophic gradient in a freshwater mire. Biogeochemistry 6:31–43.
Wright, A.L., and K.R. Reddy. 2001a. Phosphorus loading effects on extracellular enzyme activity in Everglades wetland soils. Soil Sci. Soc. Am. J. 65:588–595.
Wright, A.L., and K.R. Reddy. 2001b. Heterotrophic microbial activities in northern Everglades wetland soils. Soil Sci. Soc. Am. J. 65:1856–1864.