Organic Phosphorus Sequestration in Subtropical Treatment Wetlands

BENJAMIN L. TURNER,*,[†]
SUSAN NEWMAN,[‡] AND
JANA M. NEWMAN[‡]

*Smithsonian Tropical Research Institute, Apartado
0843-03092, Balboa, Ancón, Republic of Panama, and
Everglades Division, South Florida Water Management
District, 3301 Gun Club Road, West Palm Beach, Florida
33406, USA*

Diffuse phosphorus pollution is commonly remediated by
diverting runoff through treatment wetlands to sequester
phosphorus into soil layers. Much of the sequestered
phosphorus occurs in organic forms, yet our understanding
of its chemical nature is limited. We used NaOH−EDTA
extraction and solution ^{31}P NMR spectroscopy to speciate
organic phosphorus sequestered in a large treatment
wetland (STA-1W) in Florida, USA. The wetland was
constructed on previously farmed peat and was designed
to remove phosphorus from agricultural runoff prior to
discharge into the Everglades. Unconsolidated benthic floc
that had accumulated during the 9-year operation of the
wetland was sampled along transects through two connected
cells dominated by cattail (*Typha dominigensis* Pers.)
and an additional cell colonized by submerged aquatic
vegetation, including southern water nymph (*Najas
guadalupensis* (Spreng.) Magnus) and coontail (*Ceratophyllum
demersum* L.). Organic phosphorus was a greater
proportion of the sequestered phosphorus in the cattail
marsh compared to the submerged aquatic vegetation
wetland, but occurred almost exclusively as phosphate
diesters and their alkaline hydrolysis products. It was therefore
markedly different from the organic phosphorus in
mineral soils, which is dominated typically by inositol
phosphates. Phosphate diesters are readily degradable in
most soils, raising concern about the long-term fate of
organic phosphorus in treatment wetlands. Further studies
are now necessary to assess the stability of the sequestered
organic phosphorus in response to biogeochemical and
hydrological perturbation.

Introduction

Pollution of water bodies from diffuse agricultural sources
is currently one of the most pressing and contentious issues
facing United States agriculture. Numerous examples now
exist of water bodies suffering severe water quality problems
linked to diffuse agricultural pollution, most alarmingly in
the Chesapeake Bay where nutrient enrichment is implicated
in the growth of the neurotoxin-producing dinoflagellate
Pfiesteria piscicida (*1*).

The construction of treatment wetlands is an effective
means of remediating diffuse pollution (*2*). In Florida, for

example, more than 16000 ha of farmland have been
converted into treatment wetlands to sequester phosphorus
in runoff from agricultural land around Lake Okeechobee
(*3*). Similarly, restoration of river flood plains and isolated
wetlands is now considered a valid landscape-scale strategy
to minimize phosphorus transport to adjacent water bodies.

Diversion of polluted runoff through treatment wetlands
allows macrophytes and other organisms to immobilize
phosphorus in their tissue, which is then incorporated into
the underlying soil as vegetation decays. We term this process
"phosphorus sequestration", defined as the removal of
phosphorus from the water column through physical,
chemical, and biological processes, and its retention in stable
forms in the soil. Phosphorus is sequestered in a variety of
forms in wetlands, although organic compounds appear to
be of particular significance. For example, most of the
phosphorus stored in deep peats is organic (*4*, *5*), while
accretion of organic phosphorus is the dominant process
involved in phosphorus sequestration in nutrient-enriched
parts of the Florida Everglades (*6*).

Despite the importance of organic phosphorus in wetland
soils, there is little information on its long-term stability.
This is unsatisfactory because organic phosphorus seques-
tered in treatment wetlands during high-pollutant loading
may be destabilized following changes in nutrient status or
hydrological regime (*7*). The stability of soil organic phos-
phorus depends in part on its chemical nature because the
various compounds behave differently in soil (*8*). For example,
inositol phosphates, which are the predominant group of
organic phosphates in most mineral soils, form stable
associations with soil components and may persist for years
(*9*). In contrast, phosphate diesters such as nucleic acids and
phospholipids are relatively labile, with turnover times of
days to weeks (*10*, *11*).

Understanding the long-term stability of phosphorus in
treatment wetlands is therefore dependent on a thorough
understanding of the chemical nature of the sequestered
organic phosphorus. However, information is limited to a
few isolated reports (*12−14*) because most studies have
focused on inorganic phosphate. Organic phosphorus is
typically measured only as part of a sequential fractionation
scheme (*15*), yet these provide no structural information and
can substantially overestimate the organic phosphorus
component (*16*).

The lack of information on organic phosphorus in
treatment wetlands clearly undermines our ability to predict
their long-term effectiveness in remediating diffuse pollution.
Here, we report the first detailed information on the chemical
nature of organic phosphorus sequestered in constructed
treatment wetlands. We used alkaline EDTA extraction and
solution ^{31}P NMR spectroscopy to identify inorganic and
organic phosphates in two contrasting types of wetland. Our
aim was to derive information on the chemical nature of the
sequestered organic phosphorus and, by comparison with
similar data from nearby natural sites, to determine whether
phosphorus-rich treatment wetlands store organic phos-
phorus in a manner similar to phosphorus-limited natural
wetlands.

Methods

Sites and Sampling. Stormwater Treatment Area 1 West (STA-
1W) is a 2699 ha constructed treatment wetland located 25
km west of West Palm Beach, FL, USA (Figure 1). The wetland
was built on previously farmed peat and was designed to
remove pollutant phosphorus and other nutrients in runoff
from the Everglades Agricultural Area prior to discharge into

* Corresponding author phone: (507)212-8171; fax: (507)212-8148;
e-mail: turnerbl@si.edu.
[†] Smithsonian Tropical Research Institute.
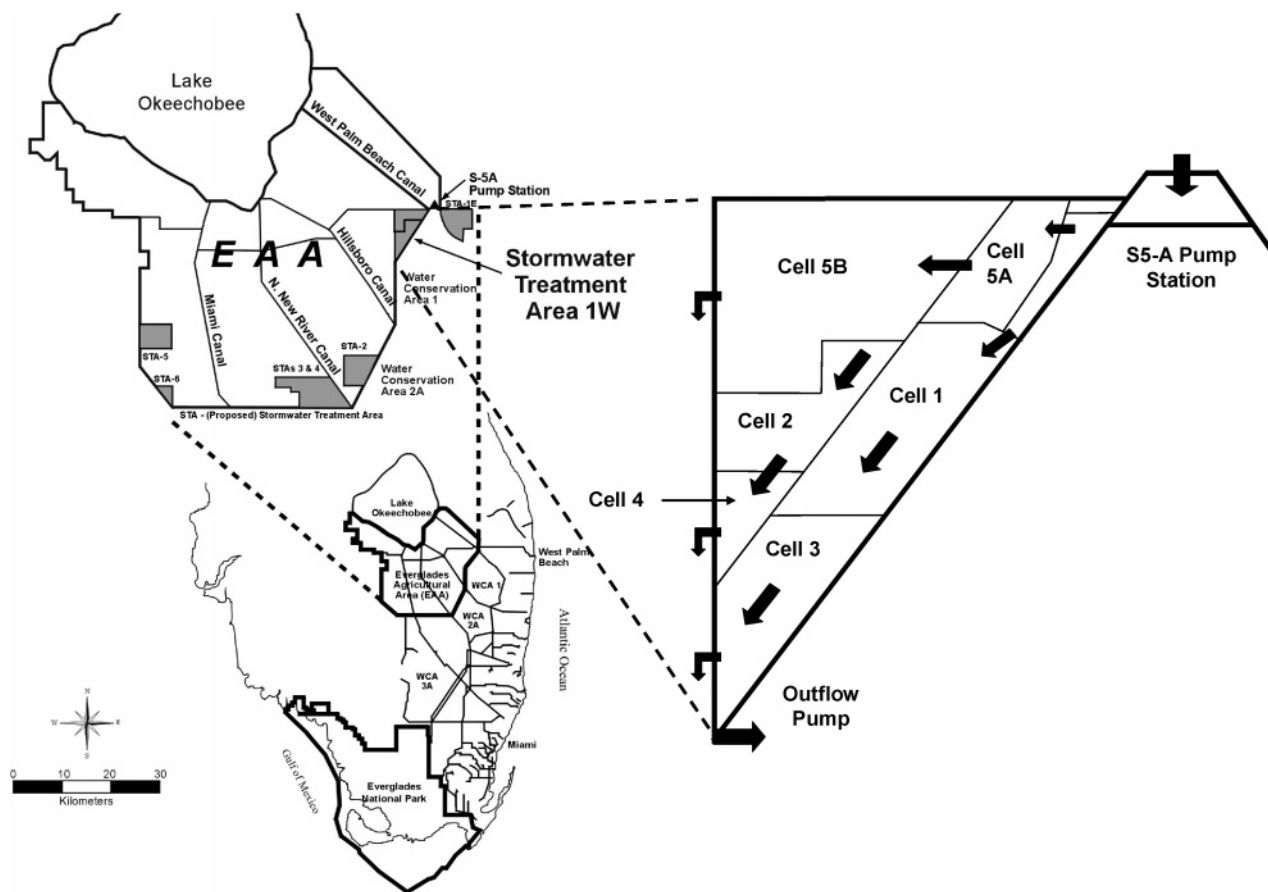[‡] South Florida Water Management District.

FIGURE 1. Map showing locations of the treatment wetland and sampling sites. See text for further details.

the Everglades (Figure 1). Water enters the wetland in the northeast corner and moves into the northern flow way (Cell 5), the eastern flow way (Cells 1 and 3), or the western flow way (Cells 2 and 4), prior to discharge into the northern Everglades. The wetland has been fully operational since August 1994 when the cells were part of the original Everglades Nutrient Removal (ENR) Project (Cells 1−4). Expansion to include Cell 5 occurred in July 2000. Details of the operation and performance of this and other treatment wetlands in the area are reported elsewhere (*17*).

STA-1W was designed to treat approximately 219 hm^3 of water annually, which equates to an average hydraulic loading rate of 2.22 cm d^{-1}. The mean design operating depth was 30.5−91.4 cm, with a nominal hydraulic residence time of 10−20 d. The annual total phosphorus design load estimate was 1.55 g m^{-2}, which assumed mean total phosphorus concentrations of 186 μg P L^{-1} at the inflow and 50 μg P L^{-1} at the outflow.

During the 2003 water year (1 May 2002−31 April 2003) the wetland received 730 hm^3 of inflow, equating to a mean hydraulic loading rate of 7.41 cm d^{-1}. This large flow, combined with a flow-weighted mean inflow total phosphorus concentration of 154 μg P L^{-1}, resulted in a loading rate for the year of 4.16 g m^{-2}. Despite this, STA-1W reduced the phosphorus load by 66%, retaining 73.5 metric tons of phosphorus and discharging water with a flow-weighted mean total phosphorus concentration of 53 μg P L^{-1}. Based on flow-weighted mean concentrations (*17*), phosphorus entering the wetland was mainly filterable (<0.45 μm) molybdate-reactive (i.e., inorganic) phosphate (54%) and particulate (>0.45 μm) phosphorus (43%), with a small proportion of filterable organic phosphorus (3%). Phosphorus leaving the wetland was mainly filterable molybdate-reactive phosphate (66%), with smaller proportions of particulate

(19%) and filterable organic (15%) phosphorus. Since August 1994, the wetland has sequestered >207 metric tons of phosphorus, presumably almost all in the accreted benthic floc.

Nine sample sites within three treatment cells of STA-1W (Cells 1, 3, and 4) were selected for this study. Six sampling sites were along a north−south transect within Cells 1 and 3, which comprise the eastern flow train and encompass a total of 1018 ha (603 and 415 ha, respectively) of treatment area. These cells are dominated by cattail (*Typha dominigensis* Pers.) and are known as emergent vegetation or cattail cells. Three sites were similarly located along a transect through Cell 4, the smallest of the internal wetland cells with an effective treatment area of 145 ha. This cell is dominated by submerged aquatic vegetation, including southern water nymph (*Najas guadalupensis* (Spreng.) Magnus) and coontail (*Ceratophyllum demersum* L.).

Samples were all collected within a 24 h period in June 2003 from the inflow, central, and outflow portions of each cell. Three replicate cores (10 cm diameter) were taken at each sampling site at least 5 m apart. The cores were taken to 10 cm in the organic soil layer and the unconsolidated benthic floc was immediately separated by hand from the underlying soil. The floc consists primarily of plant detritus and a smaller amount of algae, although there is also a considerable mineral component in the submerged aquatic vegetation cell. An additional layer, termed the intermediate layer, was also separated at two locations in Cell 1. This more consolidated layer occurred below the floc layer, but was clearly different from the underlying former agricultural soil. Samples were transported on ice to the laboratory, where they were immediately frozen at −80 °C to halt further microbial activity. Time from sampling to freezing was 48 h. The frozen samples were lyophilized (freeze-dried), identifi-

TABLE 1. Total Elements in Benthic Floc along Transects through Three Cells of a Large Treatment Wetland (Stormwater Treatment Area-1W) in Florida, USA[a]

location	pH	carbon	nitrogen	phosphorus	aluminum	calcium	iron	C/N ratio	N/P ratio
		g kg⁻¹ dry wt							
Cattail Cells									
Benthic Floc									
Cell 1 inflow	7.23	306 ± 25	21.5 ± 1.2	1.32 ± 0.09	17.7 ± 0.8	66.9 ± 6.6	37.3 ± 3.1	14.2 ± 0.4	16 ± 2
Cell 1 center	7.39	354 ± 8	23.1 ± 0.5	1.01 ± 0.17	4.4 ± 0.3	129.1 ± 6.5	8.9 ± 0.7	15.3 ± 0.5	23 ± 4
Cell 1 outflow	7.54	394 ± 40	28.4 ± 1.9	0.96 ± 0.21	1.8 ± 0.3	138.1 ± 29.1	4.3 ± 0.7	13.9 ± 0.6	31 ± 8
Cell 3 inflow	6.86	505 ± 12	32.6 ± 1.8	0.70 ± 0.32	2.7 ± 0.6	33.6 ± 3.3	5.9 ± 0.3	15.5 ± 1.1	52 ± 18
Cell 3 center	7.09	507 ± 13	33.6 ± 1.2	0.78 ± 0.18	2.5 ± 0.1	44.7 ± 7.3	5.9 ± 0.3	15.1 ± 0.9	44 ± 9
Cell 3 outflow	6.48	490 ± 13	30.3 ± 2.1	0.82 ± 0.16	1.3 ± 0.4	38.3 ± 5.0	6.3 ± 0.6	16.2 ± 0.8	38 ± 5
least significant difference (5%)		27	2.0	0.25	0.6	16.4	1.7	1.0	12
Intermediate Layer									
Cell 1 inflow	7.02	348 ± 19	23.2 ± 1.7	1.03 ± 0.11	15.1 ± 3.1	49.7 ± 9.9	32.1 ± 5.6	15.1 ± 0.3	23 ± 3
Cell 1 center	7.54	373 ± 30	22.4 ± 4.2	0.49 ± 0.05	2.9 ± 0.1	116.1 ± 6.4	5.7 ± 0.2	16.9 ± 2.1	45 ± 2
Submerged Aquatic Vegetation Cell									
Benthic Floc									
Cell 4 inflow	7.45	310 ± 56	16.7 ± 5.3	0.62 ± 0.08	1.7 ± 0.5	217.4 ± 58.2	4.2 ± 1.4	19.3 ± 3.4	28 ± 12
Cell 4 center	8.01	187 ± 13	8.5 ± 0.8	0.91 ± 0.07	1.7 ± 0.4	294.4 ± 5.4	3.2 ± 0.6	22.0 ± 0.5	9 ± 1
Cell 4 outflow	7.44	409 ± 51	21.0 ± 2.9	0.47 ± 0.11	2.2 ± 0.4	125.1 ± 58.0	4.8 ± 0.8	19.5 ± 0.5	47 ± 15
least significant difference (5%)		89	7.1	0.18	0.9	95.1	2.0	4.0	22

[a] Data are mean ± standard deviation of three field replicates.

able roots, leaves, and shells were removed by hand, and the samples were then ground gently to pass a 2 mm sieve. Replicate samples from each site were analyzed separately for chemical properties to provide information on spatial variability.

Determination of Soil Properties. Total carbon and nitrogen were determined by combustion and gas chromatography using a FlashEA1112 CN analyzer (CE Elantech, Lakewood, NJ, USA). Soil pH was determined in a 1:20 ratio of lyophilized soil to deionized water (approximately 1:2 on a wet weight basis). Total aluminum, calcium, and iron were determined by digestion of a 0.5 g sample in concentrated HNO_3 and $HClO_4$ (18), with detection by inductively coupled plasma optical-emission spectrometry (ICP−OES). Total phosphorus was determined by ignition (550 °C × 3 h), acid digestion (6 M HCl), and automated molybdate colorimetry (19). All results are reported on the basis of oven-dried material (105 °C × 24 h).

Differences in concentrations of total elements along transects through the cells were determined by analysis of variance. Mean comparisons were made using least significant differences at the 5% level.

Solution ^{31}P NMR Spectroscopy. Organic phosphorus was extracted by shaking 5 g of lyophilized benthic floc with 100 mL of a solution containing 0.25 M NaOH and 50 mM EDTA for 4 h at 20 °C (20). Each replicate sample was extracted individually. Extracts were centrifuged at 10000g for 30 min and an aliquot was taken for determination of total phosphorus by ICP−OES. Equal volumes of the remaining replicate extracts were then combined, immediately frozen at −80 °C, and lyophilized.

For solution ^{31}P NMR spectroscopy, each lyophilized extract (∼100 mg) was redissolved in 0.1 mL of deuterium oxide and 0.9 mL of a solution containing 1.0 M NaOH and 0.1 M EDTA and 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. Solution ^{31}P NMR spectra were obtained using a Bruker Avance DRX 500 MHz spectrometer operating at 202.456 MHz for ^{31}P. Samples were analyzed using a 6 μs pulse (45°), a delay time of 1.0 s, and an acquisition time of 0.2 s. Broadband proton decoupling was used for all samples. Between 49000 and 66000 scans
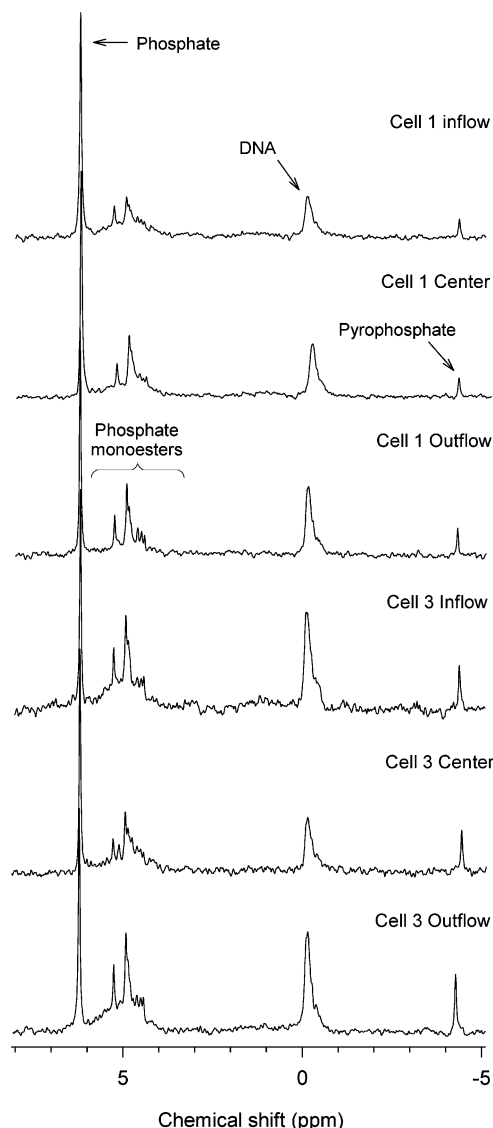
were acquired depending on the phosphorus concentration of the extract.

Chemical shifts of signals were determined in parts per million (ppm) relative to an external standard of 85% H_3PO_4. Signals were assigned to individual phosphorus compounds or functional groups based on literature reports (21−23), with 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 show fine resolution in the phosphate monoester region.

Calculation of Organic Phosphorus Sequestration. To calculate the amount of phosphorus accumulated in the wetland, we assumed that benthic floc represented all the material accreted since the conversion from farmland to wetland. Annual sequestration rates of total and organic phosphorus functional groups were calculated for each cell on an aerial basis (kg P ha⁻¹ y⁻¹) using the number of years of operation of the wetland and average values for bulk density and depth of benthic floc. Total annual sequestration rates were calculated by multiplying these values by the total area of each cell. At the time of sampling the wetland had been in operation for 9 years. Average bulk density values for benthic floc, calculated by measuring the dry weight and depth of the floc layer in multiple 10 cm diameter cores at each location, were 0.08 (0.02−0.18) g cm⁻³ in Cell 1, 0.07 (0.02−0.14) g cm⁻³ in Cell 3, and 0.09 (0.02−0.15) g cm⁻³ in Cell 4. The depth of the benthic floc was 6−40 cm in Cell 1, 4−32 cm in Cell 3, and 10−25 cm in Cell 4.

Results

Total Element Concentrations. In the cattail cells (Cells 1 and 3), total carbon concentrations ranged from 306 g C kg⁻¹ at the inflow of Cell 1 to 507 g C kg⁻¹ at the center of Cell 3, while total nitrogen concentrations ranged from 21.5 to 33.6 g N kg⁻¹ at the same locations (Table 1). Total phosphorus concentrations ranged from 1.32 g P kg⁻¹ at the inflow of Cell 1 to 0.70 g P kg⁻¹ at the inflow of Cell 3 (Table 1). Carbon-to-nitrogen ratios ranged between 13.9 and 16.2, while nitrogen-to-phosphorus ratios ranged between 16 and 52. Relatively large amounts of calcium were present in some samples (up to 138 g Ca kg⁻¹). There were significant differences in the concentrations of carbon, nitrogen,

FIGURE 2. Solution ^{31}P NMR spectra of NaOH−EDTA extracts of benthic floc from two cells dominated by cattail (Cells 1 and 3) in a large treatment wetland (Stormwater Treatment Area-1W) in Florida, USA. Spectra are plotted with 8 Hz line broadening.

TABLE 2. Organic Phosphorus and Pyrophosphate Concentrations in Benthic Floc along Transects through Three Cells of a Large Treatment Wetland (Stormwater Treatment Area-1W) in Florida, USA[a]

location	mg P kg^{-1} dry wt			
	total organic P	phosphate monoesters	DNA	pyrophos-phate
Cattail Cells				
Benthic Floc				
Cell 1 inflow	347 (26)	209 (16)	138 (10)	23 (2)
Cell 1 center	226 (22)	139 (14)	87 (9)	10 (1)
Cell 1 outflow	248 (26)	111 (12)	137 (14)	15 (2)
Cell 3 inflow	269 (38)	149 (21)	120 (17)	15 (2)
Cell 3 center	265 (34)	149 (19)	116 (15)	24 (3)
Cell 3 outflow	294 (36)	174 (21)	120 (15)	30 (4)
Intermediate Layer				
Cell 1 inflow	300 (29)	185 (18)	115 (11)	Tr
Cell 1 center	94 (19)	49 (10)	45 (9)	ND
Submerged Aquatic Vegetation Cell				
Benthic Floc				
Cell 4 inflow	Tr	Tr	ND	Tr
Cell 4 center	117 (13)	62 (7)	55 (6)	ND
Cell 4 outflow	94 (20)	67 (14)	27 (6)	Tr

[a] Phosphorus composition was determined by solution ^{31}P NMR spectroscopy of NaOH−EDTA extracts. Values in parentheses are the proportion (%) of the total soil phosphorus. ND, not detected. Tr, trace.

cell in Figure 3. Spectral resolution was poorer for samples from the submerged aquatic vegetation cell compared to that of the cattail cells, reflecting smaller concentrations of phosphorus in extracts of the former.
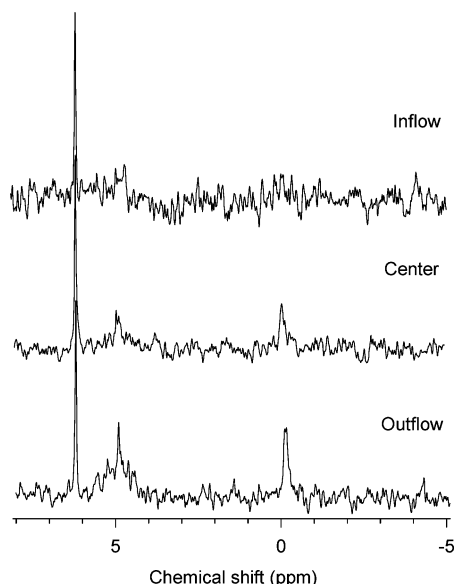
A strong signal at approximately 6.15 ppm was assigned to phosphate. Variable concentrations were extracted by NaOH−EDTA, but are not discussed further. A signal close to −4.4 ppm was assigned to pyrophosphate, an inorganic polyphosphate of chain length $n = 2$. A relatively broad signal at 0 ppm in all spectra was assigned to deoxyribonucleic acid (DNA), while signals between 4 and 6 ppm were assigned to phosphate monoesters.

The phosphate monoester region was dominated by signals at 5.24 and 4.91 ppm, assigned to phosphatidic acid and β-glycerophosphate, respectively (e.g., in the extract of benthic floc from the outflow of Cell 1; Figure 4). These compounds originate from the chemical hydrolysis of phospholipids, notably phosphatidyl choline, in alkaline solution (21). It is unlikely that they were present in the floc prior to extraction because the most common pathway for phospholipid breakdown in the environment is phospholipase C hydrolysis and the release of phosphatidyl choline, but signals corresponding to this compound were not detected in any sample.

The remaining signals in the phosphate monoester region corresponded to the mononucleotide degradation products of RNA in alkaline solution (Figure 4) (21). Consequently, most of the phosphate monoesters detected in all samples were probably phosphate diesters (phospholipids and RNA) prior to extraction. Neither long-chain polyphosphates (most clearly seen as signals around −20 ppm) nor phosphonates (signals around 20 ppm) were detected in any sample.

Concentrations of Phosphorus Species. Total organic phosphorus was calculated by summing the concentrations of phosphate monoesters and DNA determined by solution ^{31}P NMR spectroscopy (Table 2). In the cattail cells (Cells 1 and 3), concentrations ranged between 226 and 347 mg P kg^{-1}, which occurred at the center and inflow of Cell 1, respectively. Organic phosphorus constituted between 22 and 38% of the total P, with values tending to increase with

calcium, and iron with distance from the inflow ($P < 0.001$). There were also significant differences in total phosphorus concentrations ($P < 0.05$) and the ratios of carbon to nitrogen ($P < 0.05$), carbon to phosphorus ($P < 0.01$), and nitrogen to phosphorus ($P < 0.01$).

The submerged aquatic vegetation cell (Cell 4) contained lower concentrations of carbon-and-nitrogen than the cattail cells. The smallest concentrations of these elements occurred in the center of the cell, whereas the smallest concentrations of calcium and phosphorus occurred at the outflow. There were significant differences in the concentrations of carbon ($P < 0.01$), phosphorus ($P < 0.01$), nitrogen ($P < 0.05$), and calcium ($P < 0.05$). Calcium accounted for a considerable proportion of the total weight of the floc in these samples (up to 294 g Ca kg^{-1}). There were also significant differences in the ratios of carbon to phosphorus ($P < 0.01$) and nitrogen to phosphorus ($P < 0.05$). However, there were no significant differences in iron concentrations or the carbon to nitrogen ratio.

Identification of Signals in Solution ^{31}P NMR Spectroscopy. Solution ^{31}P NMR spectra are shown for the cattail cells in Figure 2 and for the submerged aquatic vegetation

FIGURE 3. Solution ^{31}P NMR spectra of NaOH−EDTA extracts of benthic floc from a cell dominated by submerged aquatic vegetation (Cell 4) in a large treatment wetland (Stormwater Treatment Area-1W) in Florida, USA. Spectra are plotted with 8 Hz line broadening.
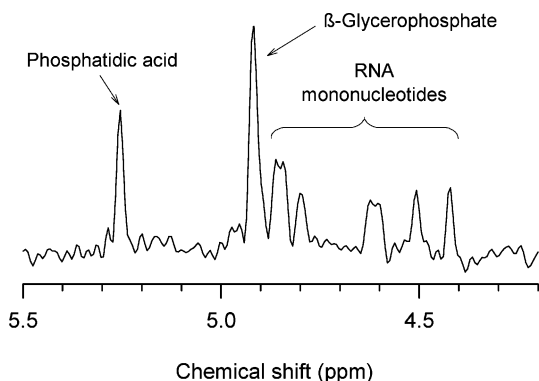
FIGURE 4. A solution ^{31}P NMR spectrum of the phosphate monoester region of a NaOH−EDTA extract of benthic floc from the outflow of a cattail cell (Cell 1) in a large treatment wetland (Stormwater Treatment Area-1W) in Florida, USA. The spectrum indicates the presence of signals derived from alkaline hydrolysis of phosphate diesters.

distance from the pollutant inflow. Concentrations of phosphate monoesters in the cattail cells ranged between 111 and 209 mg P kg^{-1} (Table 2) and constituted between 12 and 21% of the total phosphorus. Concentrations of DNA ranged between 87 and 138 mg P kg^{-1} (Table 2) and constituted between 9 and 17% of the total phosphorus. Concentrations of both groups of organic phosphorus compounds showed no clear trends along the transects, although they tended to increase as a proportion of the total phosphorus with distance from the pollutant inflow.

The two more consolidated samples (termed intermediate layer samples) from the inflow and center of Cell 1 contained 300 and 94 mg P kg^{-1} of organic phosphorus, respectively. Phosphate monoesters and DNA were detected in similar proportions to those in the overlying benthic floc, but pyrophosphate was not detected in either sample.

In the submerged aquatic vegetation cell (Cell 4) there were marked differences in the composition of complex phosphates compared to the cattail cells. Only a trace of organic phosphorus was detected at the inflow site, although concentrations were 117 and 94 mg P kg^{-1} at the center and outflow, respectively (Table 2). These values represented 13

TABLE 3. Sequestration of Organic Phosphorus in Three Cells of a Large Treatment Wetland (Stormwater Treatment Area-1W) in Florida, USA, during the 9-Year Operation of the Wetland

	cattail cells		submerged aquatic vegetation cell
	cell 1	cell 3	cell 4
	kg P ha^{-1} y^{-1}		
total P	21.5	11.4	10.2
organic P	5.3	4.1	1.1
	kg P y^{-1}		
total P	12946	4751	1483
organic P	3224	1712	153

and 20% of the total phosphorus. Both phosphate monoesters and DNA were detected. As observed in samples from the cattail cells, phosphate monoesters were all degradation products of phospholipids and RNA. Pyrophosphate was not present at quantifiable concentrations in any sample from Cell 4.

Sequestration Rates of Organic Phosphorus. The phosphorus sequestration rate in Cell 1 of the cattail marsh was 21.5 kg P ha^{-1} y^{-1} of total phosphorus, of which approximately one-quarter was organic phosphorus (Table 3). In Cell 3 the corresponding rates were 11.4 kg P ha^{-1} y^{-1} of total phosphorus and 4.1 kg P ha^{-1} y^{-1} of organic phosphorus. These values equated to organic phosphorus sequestration rates of 3224 kg P y^{-1} for Cell 1 and 1712 kg P y^{-1} for Cell 3. In Cell 4 the phosphorus sequestration rate was 10.2 kg P ha^{-1} y^{-1}, although only 1.1 kg P ha^{-1} y^{-1} was in organic form. This equated to an organic phosphorus sequestration rate for the entire cell of 153 kg P y^{-1}. As phosphate monoesters were mainly degraded phospholipids and RNA, almost all sequestered organic phosphorus in both wetlands was phosphate diesters. Based on our results, the total amount of phosphorus sequestered in these three cells between 1994 and 2003 was approximately 173 metric tons, which compares well with the estimated 207 tons sequestered in the whole wetland (i.e., including Cell 2 and the recently constructed Cell 5) during the same period (17).

Discussion

This study provides the first comprehensive information on the composition of organic phosphorus sequestered by two distinct vegetative communities within a treatment wetland. Organic phosphorus clearly accounted for a considerable proportion of the sequestered phosphorus, although its contribution was greater in the cattail cells. The NaOH− EDTA extraction method quantitatively recovers soil organic phosphorus (24), especially from organic soils (25, 26). Small amounts of recalcitrant organic phosphorus may not be extracted, although this is currently impossible to assess because there is no direct method of determining total organic phosphorus (16). It can therefore be assumed that almost all the phosphorus not extracted by NaOH−EDTA in the samples analyzed here was inorganic phosphate.

The composition of the sequestered organic phosphorus was unusual because it consisted almost entirely of phosphate diesters in the form of DNA and the alkaline hydrolysis products of RNA and phospholipids. This supports previous analysis of the water column of the submerged aquatic vegetation cell (12), which revealed that >70% of the soluble organic phosphorus was hydrolyzed by phosphodiesterase and was therefore in the form of phosphate diesters. Some of the phosphate diesters in the current study were almost certainly derived from intact microbial cells, which can constitute a large proportion of the phosphorus in wetland soils (6). However, phosphate diesters also accumulate under

wet conditions (*27*) and it is likely that a large proportion was present as part of the nonliving soil organic phosphorus (*28*).

Phosphate diesters constitute the majority of the fresh inputs of organic phosphorus to soils, mainly as phospholipids and nucleic acids (*29*), but they degrade within days (*11*) and typically constitute <10% of the soil organic phosphorus (*30*). In contrast, the organic phosphorus in most soils, including recently reflooded wetlands (*13*), is dominated by phosphate monoesters in the form of phytic acid (*myo*-inositol hexa*kis*phosphate) and other inositol phosphates (*9*). These compounds constitute only a small proportion of the organic phosphorus inputs to soil in fresh plant and microbial residues, but accumulate following strong interaction with soil components to form a stable organic phosphorus fraction (*31*).

It is unclear why inositol phosphates have not accumulated in these subtropical treatment wetlands. The paucity of sorption sites on clay minerals may limit their stabilization (*32*), although they would be expected to complex strongly with the abundant calcium and iron present (*33*). The decomposition of inositol phosphates may be accelerated by anaerobic conditions, which occur close to the surface of the benthic floc in nutrient impacted areas of the Everglades (*34, 35*). Anaerobicity leads to the rapid hydrolysis of *myo*-inositol hexa*kis*phosphate in marine sediments (*36*) and submerged rice soils (*37*), presumably by reducing iron complexes that protect the inositol molecule from enzymatic attack (*38*). A caveat is that anaerobic reduction of freshwater sediments was reported to form insoluble iron-phytate rather than solubilizing free inositol hexa*kis*phosphate (*39*), although it seems unlikely that this would preclude extraction in NaOH−EDTA.

The labile nature of phosphate diesters raises concern over the long-term stability of organic phosphorus in treatment wetlands because perturbation may remobilize the sequestered compounds. For example, pollution abatement would be expected to increase the biological demand for phosphorus, leading to the slow remobilization of organic phosphates through synthesis of phosphatase enzymes by plants and microbes. Indeed, there is concern that organic phosphorus in enriched parts of the Everglades, where phosphorus concentrations in benthic floc exceed 1000 mg P kg^{-1} (*40*), will be converted to more labile forms as pollutant concentrations in inflow water are reduced.

Water-level drawdown and soil drainage are commonly used in treatment wetlands to consolidate flocculated material, accelerate soil accretion, and allow access for maintenance operations (*2*). This can release large concentrations of inorganic phosphate to the water column upon reflooding (*41*), but the effects on organic phosphorus are unclear. Drying and rewetting can release organic phosphorus from soil through microbial cell lysis (*42*), while redox changes could destabilize organic phosphorus in complexes with iron. A solution ^{31}P NMR study of the potential decomposition of organic phosphorus in detritus from the inflow site in Cell 1 reported that nucleic acids were preferentially decomposed during drawdown (*43*), although spectral resolution seems too poor to make firm conclusions. Drawdown and reflooding regimes caused a net flux of organic phosphorus from experimental mesocosms designed to simulate cattail cells (*44*), while microbial lysis was suspected to be responsible for an increase in NaHCO$_3$-extractable organic phosphorus following drying and reflooding of benthic floc from a treatment wetland (*41*). Importantly, drawdown and reflooding stimulates the activity of microbes and hydrolytic enzymes involved in organic phosphorus degradation (*45*), suggesting that any destabilized organic phosphorus would be degraded rapidly.

Design of the treatment wetlands used to abate Everglades phosphorus pollution, including those assessed here, was based on rates of phosphorus input and sequestration observed in Water Conservation Area 2A and assumed that biological mechanisms of phosphorus removal would be the same. Our data suggest strongly that these assumptions were valid. Pollutant phosphorus inputs to the natural Everglades in recent decades caused phosphorus sequestration rates to increase from 0.6 kg ha^{-1} y^{-1} in pristine areas to 11 kg ha^{-1} y^{-1} in highly enriched areas (*46−48*), which are similar to those observed in the treatment wetland studied here. The composition of the phosphorus sequestered in the treatment wetland was also similar to that in the natural Everglades (*14*). In particular, the organic phosphorus composition of benthic floc in the cattail cells was similar to that in enriched parts of Water Conservation Areas 1 and 2A supporting cattail, while the composition of benthic floc in the submerged aquatic vegetation cell was similar to that in an unenriched calcareous slough in Water Conservation Area 2A. This suggests that at least some of the organic phosphorus in the treatment wetlands is stable because it was similar in composition to that in unpolluted parts of the Everglades where biological productivity is strongly limited by the availability of phosphorus (*14*). However, the considerable difference in phosphorus concentrations between these areas almost certainly has important implications for organic phosphorus stability in the treatment wetlands following perturbation.

In summary, organic phosphorus was an important component of the phosphorus sequestered in subtropical treatment wetlands, although it was quantitatively greater in a cattail marsh than in a submerged aquatic vegetation wetland. In direct contrast to most mineral soils, inositol phosphates were not detected, and the sequestered organic phosphorus consisted entirely of phosphate diesters and their degradation products. These compounds are considered relatively unstable in soils, which raises concern about the long-term stability of organic phosphorus sequestered in subtropical treatment wetlands. Additional studies are now required to assess the stability of the sequestered organic phosphorus in response to changes in nutrient status and hydrological regime.

Acknowledgments

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