Estimating the Stability of Organic Phosphorus in Wetland Soils

Millard M. Fisher*

Water Resources Group, LLC
P.O. Box 1978
Olympia, WA 98507

K. Ramesh Reddy

Wetland Biogeochemistry Lab.
Soil and Water Science Dep.
Univ. of Florida
106 Newell Hall
P.O. Box 110510
Gainesville, FL 32611

The distribution of soil P among labile and nonlabile forms can be a major determinant of agricultural and natural ecosystem productivity. Determination of soil P pools is typically performed using operationally defined chemical fractionation methods. Most of the current fractionation techniques were developed for predominately mineral soils, thus they provide only limited information on organic P (P_o), particularly with respect to stability. We hypothesized that the extent to which P could be extracted from organic soils, after exposure to heat, may be related to environmental recalcitrance. We investigated two thermal methods for characterizing P_o stability in organic wetland soils, an autoclave-based and a dry heat technique. Soils from two subtropical wetlands were collected to a depth of approximately 1 m. Autoclave-extractable P was determined by subjecting soils to 128°C and 170 kPa for 90 min in an autoclave. A second set of samples was exposed to dry heat at temperatures of 160, 200, 260, 300, 360, and 550°C. The results were compared with data from a conventional chemical P fractionation scheme. Phosphorus that could be extracted using the hot water technique declined with soil depth, representing 10 to 50% of total P in surficial soils, to 5 to 10% at a depth of 60 cm. Microbial biomass P was correlated with hot water extractable P, and represented approximately 50% of the hot water extract. In the dry heat technique, increasing the extraction temperature resulted in significantly greater extraction of P_o. The 360°C treatment was best able to distinguish between recalcitrant and labile P_o.

Abbreviations: BCMCA, Blue Cypress Marsh Conservation Area; EDTA, ethylenediaminetetraacetic acid; HEP, hot water extractable phosphorus; MBP, microbial biomass phosphorus; NMR, nuclear magnetic resonance; P_o, organic P; WCA-2A, Water Conservation Area 2A.

Wetlands and lakes tend to accumulate material, typically due to their low position in the landscape and high primary productivity (Mitsch and Gosselink, 1993). Nutrients are sequestered into pools of varying environmental stability, and the extent to which they are can be a major determinant of ecosystem productivity. Chemical fractionation of soil P into inorganic and organic pools has been used extensively for estimating the effects of crop management practices and to determine the soil nutrient status in natural ecosystems (Chang and Jackson, 1957; Hieltjes and Lijklema, 1980; Sommers et al., 1972; Hedley et al., 1982; Bowman, 1989; Ivanoff et al., 1998). Numerous procedures have been developed to divide soil P into operationally defined pools, and these tend to be based on solubility characteristics, typically in acid and alkaline fractionation extracts. Fractionation techniques can be broadly grouped according to whether the intention is to better divide P into inorganic (Chang and Jackson, 1957; Hieltjes and Lijklema, 1980) or organic pools (Sommers et al., 1972; Hedley et al., 1982; Bowman, 1989; Ivanoff et al., 1998).

The P_o characterization scheme proposed by Ivanoff et al. (1998) was used to characterize P_o stability in the Florida Everglades. Their technique used a sequential extraction with bicarbonate, HCl, and NaOH to distinguish among labile and nonlabile P_o fractions. Of the total P_o in their soils, approximately 40% was recovered in the NaOH extract, 30% was attributed to microbial biomass, and 17% was nonextractable and believed to consist of refractory P_o. The alkaline-extractable fraction was further divided into two fractions after extraction by acidifying the extract, causing the

precipitation of humic materials. This allowed separate determination of the P contents of humic and fulvic acids. One of the shortcomings of fractionation procedures is that the pools are typically defined by the procedure, and that the extraction does little to identify specific soil P compounds (Chang and Jackson, 1957; Hieltjes and Lijklema, 1980; Hedley et al., 1982). For example, the residue that remains after sequential chemical extraction is considered to be refractory, although the extent to which this fraction is resistant to actual environmental mineralization has not been well documented. Phosphorus-31 nuclear magnetic resonance (NMR) spectroscopy has also been used to characterize organic and inorganic P in wetland peat soils (Robinson et al., 1998; Pant and Reddy, 2001; Turner and Newman, 2005). Sample preparation before ^{31}P-NMR analysis requires extraction with NaOH and ethylenediaminetetraacetic acid (EDTA), and much of the original soil P remains unextracted. For example, in a ^{31}P-NMR analysis of Everglades benthic floc and soil, Turner and Newman (2005) found that only 37 to 58% of the total soil P could be extracted for ^{31}P-NMR quantification. Thus, much of the organic P remains uncharacterized and this unextractable fraction is presumed to be relatively nonlabile.

Characterization of P_o stability is especially important in wetland soils because this tends to be the dominant fraction (Wetzel, 1999). Reddy et al. (1998) found that P_o represented from 56 to 70% of the total P in the peat soils of the Everglades Water Conservation Areas. The P_o mineralization rate can be an important regulator of primary production, especially in P-limited wetlands and other aquatic ecosystems. This rate is determined primarily by the recalcitrance of the P_o pool. The stability of P_o has been shown to increase with soil depth in wetlands. For instance, Reddy et al. (1998) showed that in Water Conservation Area 2A (WCA-2A) in the northern Everglades, refractory P increased from 33% of the total P in surface soils, to 70% in deeper strata. That refractory P increases with increasing soil depth is intuitive because biogeochemical processes have had, in some cases, millennia to either remove labile forms of P_o from the soil P pool or sequester them into more stable soil P compounds.

The extent to which soil P_o has become resistant to further mineralization is useful for determining how soil and sediment may influence surface water quality in wetlands and lakes. It may also be useful for determining current and historical soil nutrient status in natural wetlands and this may aid in the development of site-specific nutrient loading targets. For example, wetlands that are located downgradient from naturally fertile upland soils will not require as stringent a nutrient loading target as more oligotrophic wetlands. Analysis of the distribution of soil P among labile and refractory forms in deeper soil strata may thus provide some insight into historical predevelopment nutrient loading regimes.

Current soil P fractionation techniques provide only limited information on P_o stability. New techniques are needed to provide further resolution of the extent to which P_o has become immobilized and to confirm the results from conventional soil and sediment fractionation studies. There is some evidence from earlier studies that resistance to thermal breakdown of organic matter in peat soils is related to the extent of decomposition, and

this might also be true of the stability of P_o. For example, Levesque and Dinel (1978) used thermal methods to characterize the extent of decomposition of peats. They extracted humic and fulvic compounds from peat and subjected them to thermogravimetric analysis. Fulvic compounds were completely destroyed at temperatures below 400°C, whereas the presumably more recalcitrant humic materials required temperatures in excess of 400°C to completely oxidize. The objective of this study was to characterize the stability of P_o in peat wetland soils using two thermal techniques and then compare those results with results obtained from conventional sequential chemical fractionation. We hypothesized that as P_o becomes increasingly recalcitrant, more thermal energy is required to liberate it from the organic compounds with which it is associated.

MATERIALS AND METHODS
Study Sites

Field sampling was conducted in two subtropical wetlands, WCA-2A in the northern Florida Everglades and Blue Cypress Marsh Conservation Area (BCMCA) in east central Florida (Fig. 1). The soils in both wetlands are comprised of peat. The Everglades soils are derived from sawgrass (*Cladium jamaicense* Crantz) (Davis, 1943). They are approximately 2 m in thickness and the basal deposits were formed approximately 5000 yr ago (Gleason and Stone, 1994). They thus present a vertical profile that progresses from recently deposited plant litter that can be presumed to be relatively labile, to deeper organic deposits that are presumed to be composed of relatively recalcitrant P_o. Two sampling locations, E1 and E5, were selected in WCA-2A, a peatland in the northern Florida Everglades. These two stations coincide with two long-term South Florida Water Management District (SFWMD) research stations of the same name. Station E1 is located in a nutrient-impacted region, approximately 1.4 km south of SFWMD inflow structure S-10C. This inflow structure discharges relatively high-nutrient water to WCA-2A. Station E5 is located approximately 10 km south of this structure, in the mostly unimpacted central marsh. The vegetation at station E1 is predominantly cattail (*Typha domingensis* Pers.), and at Station E5, sawgrass.

Soils were also collected from the BCMCA. The BCMCA is a 116-km^2 shallow marsh in the headwaters of the St. Johns River. It is also underlain by peat soils that vary from 1.5 m in the eastern marsh to >4 m thick near the center. The vegetation is a mosaic of open slough communities (*Nymphaea odorata* Aiton, *Eleocharis elongate* Chapm., and *Utricularia* spp.), maidencane flats (*Panicum hemitomon* Schult.), broad expanses of sawgrass, and tree islands [*Acer rubrum* L., *Taxodium distichum* (L.) Rich., and *Myrica cerifera* L.]. Agricultural discharges have created a region of elevated soil P in the northeastern area of the marsh, although those discharges ceased in 1994. One sampling location was selected from this region of the marsh, Station C1. Vegetation at this site was a mix of cattails (*Typha latifolia* L.) and willow (*Salix caroliniana* Michx.) A second station (Station B4) was selected from the interior, unimpacted marsh, in an area dominated by maidencane and sawgrass. Vertical rates of soil accretion in interior regions of both marshes have been found to be quite similar: approximately 0.3 cm yr^{-1} (Reddy et al., 1993; Brenner et al., 2001).

Soil samples were collected in triplicate from all stations in both marshes on 17 and 18 July 2002. Soil cores were obtained with a 2-m-long by 7.3-cm-diameter stainless steel soil corer. The corer had a ser-

rated and sharpened leading edge, with a "T" handle affixed to the upper part. The corer was pushed into the soil while slowly twisting, thus cutting into the peat. A comparison of the soil depth inside and outside the core barrel was made during the core drive, and when significant compression (>10 cm) was noted, the corer was withdrawn from the bore hole, a second corer was inserted into the hole, and the sample was extruded. The corer was then reinserted into the hole and coring resumed. This typically resulted in two to three soil sections per station. The total average compression throughout the approximate 2-m core length was 25.3 (±5.3 SD) cm. The samples were extruded in the field into Ziploc bags at 10-cm intervals. The samples were immediately chilled to 4°C and kept on ice for transport to the laboratory. Laboratory processing was performed at the University of Florida's Wetland Biogeochemistry Laboratory. Wet weight was recorded for each sample and the samples were transferred to rigid polyethylene containers and thoroughly mixed. Total P was determined on all samples using the ashing technique (Anderson, 1976). Loss-on-ignition was performed by igniting samples in a muffle furnace at 550°C for 4 h. Total C and N were determined on a dried, ground sample by dry combustion (Nelson and Sommers, 1996) using a Carlo-Erba NA-1500 CNS analyzer (Haake-Buchler Instruments, Saddlebrook, NJ).

Fig. 1. Location of soil sampling stations in Blue Cypress Marsh Conservation Area and Water Conservation Area 2A.

Sequential Chemical Fractionation

The soil samples were sequentially chemically fractionation on 31 July 2002, resulting in a sample holding time of 10 d. The soils were extracted at a soil/solution ratio of 1:50, using a technique specifically developed for organic soils (Ivanoff et al., 1998). That method divides the soil P into microbial biomass, fulvic acid, humic acid, inorganic, and recalcitrant (or residual) P. Microbial biomass P was determined by the difference between a soil sample that was extracted with 0.5 mol L^{-1} NaHCO$_3$ for 16 h on a reciprocating shaker and a duplicate, field-moist sample that was also extracted with NaHCO$_3$ to which chloroform had been added (Hedley and Stewart, 1982). Microbial biomass P was not corrected for extraction efficiency, as is sometimes done for mineral soils. It has been suggested that chloroform fumigation leads to overestimation of microbial biomass P due to the lysis of plant root cells, as well as hydrolysis of other relatively labile organic compounds, especially for soils high in organic matter (Reddy et al., 1998). The chloroformed sample was then extracted with 1 mol $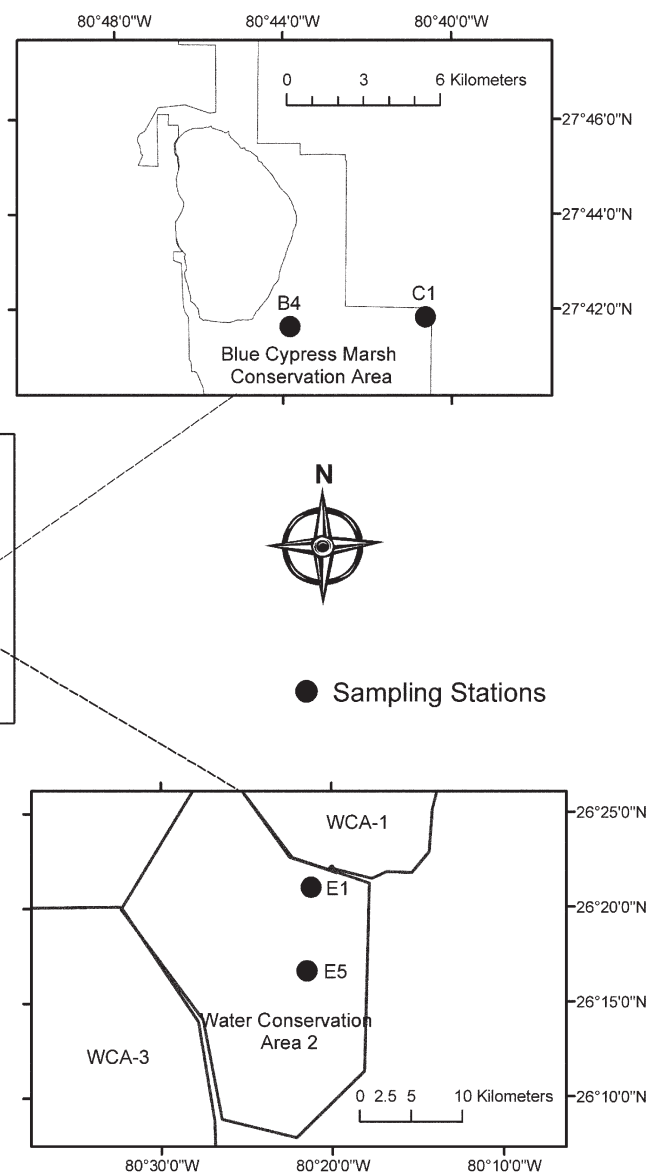L^{-1}$ HCl for 3 h and this fraction was considered to consist of inorganic P. The residue from the HCl extract was extracted for 16 h with 0.5 mol L^{-1} NaOH to extract organic P. Fulvic and humic acids were determined by acidifying an aliquot of this extract to pH <0.2 to precipitate humic materials. The P content of the supernatant was determined and this represented the fulvic-associated P. Humic P was determined by the difference of the total P content of an unacidified aliquot of the NaOH extract and fulvic P. The total P content of the residual material remaining at the end of the sequential fractionation was determined by ashing (Anderson, 1976). The residue is thought to consist primarily of highly recalcitrant organic P. Total organic P was determined by summing the microbial biomass P, fulvic P, humic P, and the total P content of the residue remaining at the end of the sequential fractionation.

Autoclave Extractable Phosphorus

Hot water extraction has been used to determine algal polyphosphate storage of P in algal cultures and lake sediments (Fitzgerald and Nelson, 1966; Kenney et al., 2000); however, this technique has not been

Table 1. Selected physicochemical properties of soils (0–50 cm) collected in July 2002 from Water Conservation Area 2A (WCA-2A) and Blue Cypress March Conservation Area (BCMCA). Values are the means of five 10-cm intervals.

Parameter	BCMCA		WCA-2A	
	B4	C1	E1	E5
Total N, g kg^{-1}	32.1	35.0	29.3	24.2
Total C, g kg^{-1}	474	483	451	457
Dry weight, %	8.7	10.3	10.0	10.8
pH	5.70	5.88	7.59	7.30
Bulk density, g cm^{-3}	0.081	0.101	0.095	0.111
Ash, %	5.8	8.8	7.3	11.1
Inorganic P, mg kg^{-1}	24.3	34.8	120.4	30.7
Microbial biomass P, mg kg^{-1}	67.6	66.0	60.6	37.5
Fulvic P, mg kg^{-1}	81.4	94.7	119.3	36.4
Humic P, mg kg^{-1}	46.3	47.6	33.7	27.7
Residual P, mg kg^{-1}	98.3	92.7	107.0	32.2
Total P, mg kg^{-1}	327	367	546	197

used for wetland soils. Hot water extractable P (HEP) is assumed to be of labile biogenic origin, such as storage of "luxury P" in algal cells, although the actual source of the extracted P has not been identified. Twenty-five milliliters of deionized water was added to 0.5 g of oven-dried equivalent wet soil. The soils were shaken at room temperature for 3 h, centrifuged, and the supernatant water was removed and vacuum filtered through 0.45-μm pore size polyethersulfone filters. The residual soil sample was then placed into an autoclave for 90 min at 128°C and 170 kPa (1.7 atm). The sample was removed from the autoclave, 20 mL of deionized water was added, and the sample was equilibrated for a 1-h, end-to-end shaking period. The sample was then vacuum filtered through 0.45-μm filters. The extracts were analyzed for dissolved reactive P using the automated ascorbic acid method (Murphy and Riley, 1962; Method 365.1, USEPA, 1993). Triplicate samples of the model organic P compounds glycerophosphate and phytic acid (Products G-6501 and P-8810, respectively, Sigma-Aldrich, St. Louis, MO) were also extracted using the above procedure to determine the extent of P_o mineralization in relatively labile (glycerophosphate) and recalcitrant (phytic acid) organic compounds. The difference between the initial deionized water extraction and the post-autoclave extract was termed *hot water extractable P*.

Dry Heat Extractable Phosphorus

The soil samples used in this experiment were subsamples of those used in the previous extraction. Three soil depths, 0 to 10, 40 to 50, and 90 to 100 cm, were used for this experiment. Extraction of P_o was performed in a furnace that was constantly purged of O_2 with N_2 gas. Purging with an inert gas was required to prevent combustion and subsequent loss of temperature control. The oven consisted of a 7.6-cm-diameter galvanized steel pipe with two threaded end caps. A 120-V heating element was spiral-wound around the pipe and connected to a temperature controller. The end caps were drilled and threaded to accept a gas inlet and outlet port, and the entire assembly was placed into an insulated metal box. One end cap was removed and a batch of samples was placed into the furnace.

The samples were first extracted at room temperature with 1 mol L^{-1} HCl on a reciprocating shaker for 3 h to obtain an estimate of inorganic P. Oven-dried samples were weighed in triplicate onto 5-cm squares of aluminum foil. The foil squares were placed onto a tray, the tray was in-

serted into the oven, and the oven was sealed. The chamber was purged for approximately 10 min and the temperature was then increased to the set point temperature. It remained at this temperature for 1 h for each treatment temperature. Treatment temperatures were 160, 200, 260, 300, 360, and 550°C. The samples were removed from the oven and transferred to 43-mL centrifuge tubes. They were then extracted with 1 mol L^{-1} HCl at a 1:50 ratio for 3 h on a reciprocating shaker, then filtered through 0.45-μm filters. The P that was extracted at room temperature with 1 mol L^{-1} HCl was subtracted from each value to yield a net dry heat extractable P.

Statistical Analyses

Statistical comparisons between dry heat treatments were performed using JMP statistical software, version 4.0.4 (SAS Institute, Cary, NC). Statistically significant differences among soil depths for each temperature in the dry heat extraction were determined using the Tukey–Kramer honestly significant difference test, with $\alpha = 0.05$. Regression analysis was performed using Microsoft Excel 2003.

RESULTS AND DISCUSSION
Soil Physicochemical Properties

The soils of WCA-2A and BCMCA were similar with respect to most physical properties (Table 1). The pH of the BCMCA soil was more acidic, averaging 5.8, whereas WCA-2A soils averaged 7.4. Bulk density was similar in both wetlands, increasing from approximately 0.090 g (dry) cm^{-3} (wet) at the soil surface, to 0.110 g (dry) cm^{-3} (wet) at a depth of 80 cm. Below 100 cm, bulk density increases dramatically at the two WCA-2A stations due to increasing sand content. Loss-on-ignition for the upper 100 cm of soil (all stations combined) averaged 87% (±12% SD), indicating the highly organic nature of these peat soils.

Total P at the nutrient-enriched (impacted) sites of WCA-2A was greater than at the nutrient-enriched site in the BCMCA, although the total P at the nutrient-poor (unimpacted) site in WCA-2A was much lower than the unimpacted site in the BCMCA. Total P was highest in the surface soils (0–50 cm) of WCA-2A Station E1 (546 mg kg^{-1}), and declined in the order E1 > C1 > B4 > E5 (Fig. 2). As expected, the nutrient-impacted stations showed higher total P content in the 0- to 50-cm soil layer than the unimpacted stations. The difference in total P between the impacted and unimpacted stations was not nearly as dramatic at the BCMCA stations, however: 367 vs. 327 mg kg^{-1}. Total P at the unimpacted station in the BCMCA was 66% greater than the unimpacted Everglades station. This suggests that the BCMCA has not been under the same degree of P limitation as the Everglades, and that nutrient enrichment was more pronounced at the impacted WCA-2A station. Other studies have shown that the surface soils of the BCMCA appear to be higher in P than those in WCA-2A (Grunwald et al., 2007) and that the BCMCA is less P limited (Prenger and Reddy, 2004). Inorganic P constituted a much greater proportion of total P at the impacted station in WCA-2A. This has also been found by other researchers and has been shown to be due to high loading of both Ca and P near inflows (Reddy et al., 1993).
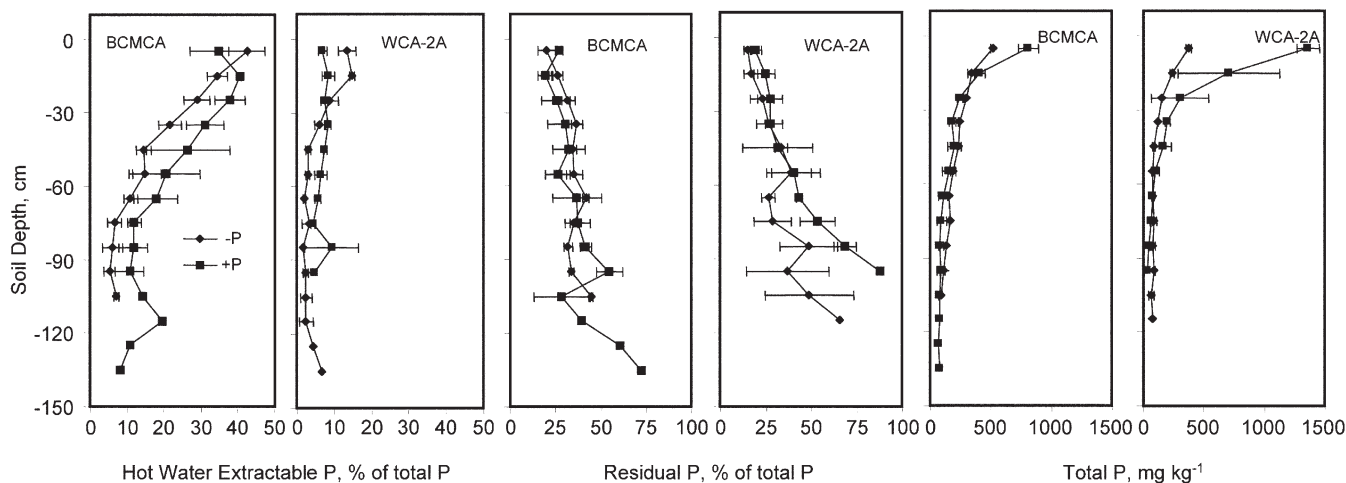
Fig. 2. Hot water extractable P (HEP), residual P (from sequential chemical extraction), and total P of Blue Cypress March Conservation Area (BCMCA) and Water Conservation Area 2A (WCA-2A) soils. Error bars represent one standard deviation.

Autoclave Extractable Phosphorus

Hot water extractable P accounted for approximately 40% of the total P in surface soils, declining to 10% at a depth of 100 cm in the BCMCA (Fig. 2). The HEP declined by 69% throughout a 1-m depth interval at the nutrient-impacted station in the BCMCA and by 87% at the unimpacted BCMCA station. Both BCMCA stations showed steep declines in HEP with depth, indicating increasing resistance to thermal extraction. Decreasing recovery of P with respect to increasing depth in the HEP was accompanied by increasing residual (or unextractable) P in the sequential chemical fractionation. Residual P increased approximately twofold across the 1-m depth interval at both BCMCA stations. The HEP of WCA-2A soils was considerably lower than the BCMCA soils, ranging from 8 to 15% of total P at the soil surface, declining to 2 to 5% of the total P at a depth of 60 cm.

Differences in HEP between the two wetlands may be due to fundamental differences in water chemistry between the wetlands. Water Conservation Area 2A is a Ca-rich, hard-water system, particularly near inflow sources (Gleason and Spackman, 1974; Reddy et al., 1993), whereas the BCMCA is a low-pH, soft-water system. Turner and Newman (2005), using ^{31}P-NMR spectroscopy, found a general lack of polyphosphates in benthic floc and soil samples from WCA-2A and Water Conservation Area 1 (WCA1), a soft-water wetland immediately north of WCA-2A. They did, however, find pyrophosphate, an inorganic polyphosphate containing two phosphate groups. Pyrophosphate was greater in benthic floc samples from WCA1 than in similar samples from WCA-2A. They attributed this difference to reduced P availability in the more calcareous sloughs of WCA-2A, fostering greater demand for P from both organic and inorganic substrates. Their results are consistent with the differences observed between the two wetlands in this study, i.e., the soft-water wetland (BCMCA) had significantly more HEP than WCA-2A.

Another possible reason for low recovery of HEP in the WCA-2A samples is precipitation of calcium phosphate in the WCA-2A samples during the HEP procedure. Modifying the HEP procedure for Ca-rich systems such that extractions are performed under acidic conditions or with a chelating agent, such as EDTA, may prevent this potential artifact. Further discussion of experimental results will focus only on the BCMCA samples.

Hot water extractable P explained 90% of the variability in microbial biomass P (Fig. 3). Microbial biomass P consists of nucleic acids, phospholipids, and intracellular storage compounds, such as polyphosphates. Even though HEP is a good predictor of microbial biomass P, the slope of the relationship (0.46) implies that HEP is an overestimate of the microbial biomass P, as determined by chemical fractionation. It is possible that in addition to these microbial sources of P, the HEP procedure extracted P from moderately labile, non-living organic compounds such as P associated with fulvic acids. For example, the best model fit of HEP results to microbial biomass P (MBP) is obtained when it is assumed that 80% of the fulvic P (determined by sequential extraction) is also extracted in the HEP procedure. If this quantity is subtracted from the HEP, the model fit improves ($r^2 = 0.95$) and the slope is nearly unity.

Approximately 80% of both glycerophosphate and phytic acid was mineralized at the experimental temperature and pressure (Fig. 4). This indicates that even at the relatively low temperature used here, there was considerable breakdown of P_o that was incorporated into phytic acid. This further suggests that hot water extractions
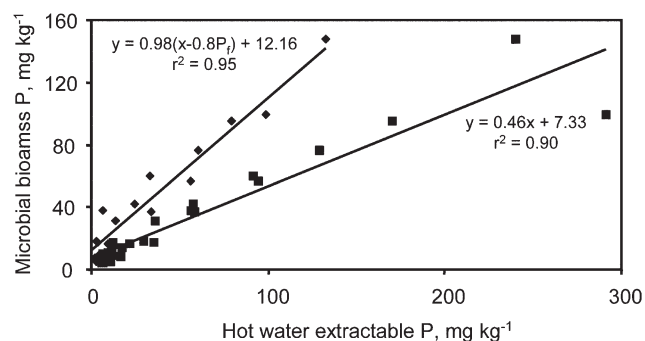

Fig. 3. Hot water extractable P in Blue Cypress March Conservation Area soils vs. microbial biomass P. Upper regression line adjusts for overestimate of microbial biomass P by assuming that 80% of the fulvic P fraction (P_f) was also extracted by hot water.
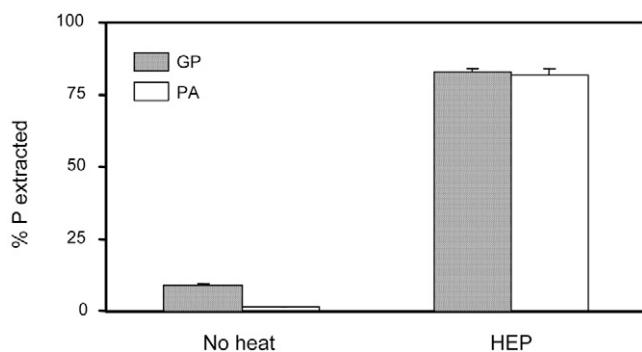
Fig. 4. Hot water extractable P (HEP) of two model compounds, glycerophosphate (GP) and phytic acid (PA). Error bars represent one standard deviation.

Fig. 6. Percentage of total P extracted from dried, ground peat after exposure to selected temperatures. Each bar is the average of three replicate samples for two wetlands. Error bars represent one standard deviation. Bars within a single temperature grouping with the same letter are not significantly different.

may liberate more than microbial and algal stores of polyphosphates, as has been proposed by other researchers (Kenney et al., 2000).

Dry Heat Extractable Phosphorus

When the results from all depths and both wetlands are combined, increasing temperature resulted in greater extraction of total P, with the exception of the 550°C treatment (Fig. 5). At 550°C, the P in the HCl extract declined dramatically. It is not clear what caused the large reduction in dry heat extractable P in the highest temperature treatment; however, one possible explanation is the formation of a material with strongly hydrophobic properties. For example, one method of treating wood for decay resistance is to subject it to a high-temperature, inert atmosphere (Felfli et al., 2005). This treatment apparently eliminates some chemically bound water, and the final product becomes markedly more hydrophobic. This may have prevented the HCl extractant complete access to the soil matrix.

At 360°C, nearly 100% of the P_o was mineralized. The 360°C treatment was the best predictor of sample total P, with lesser temperatures extracting progressively less P. This is expected for these highly organic samples because 360°C approaches the temperature used to destroy organic matter in the loss-on-ignition procedure. The remaining P (y intercept = 61 mg kg^{-1}) consists mostly of the
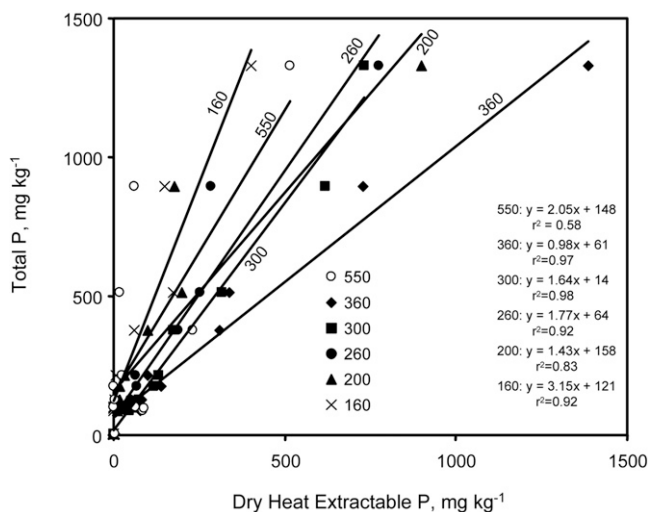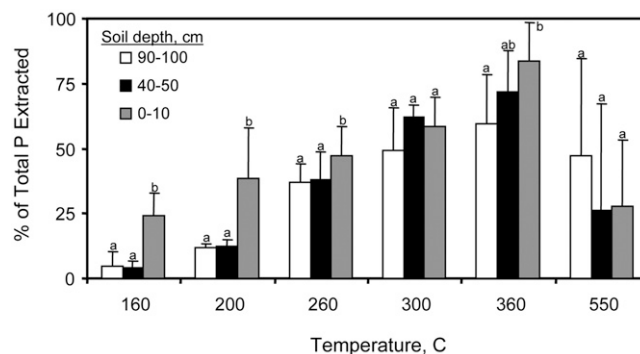

Fig. 5. Effect of dry heat temperature from 160 to 550°C on extraction of total P. Shown are all data from two wetlands and three soil depths combined.

inorganic P that was removed in the pre-HCl extract as well as any residual P_o that remains resistant to thermal breakdown.

Exposing the soils to progressively greater temperatures resulted in corresponding increases in P_o extracted, irrespective of soil depth (Fig. 6). For all temperatures combined, there were significant differences in extractable P among the three soil depths ($P < 0.05$; Table 2). For most of the temperatures used, the surface soils (0–10 cm) released significantly more P than the two deeper soil samples. The pattern of declining thermal extractability with increasing depth coincides with declining lability, as found in the sequential chemical extraction, when labile P_o is defined as the sum of $NaHCO_3$–extractable P_o, MBP, and fulvic P. For example, the labile P_o of the surface soils (0–10 cm) amounted to 43% of the total P ($\pm$19% SD), compared with 1.7% of the total P ($\pm$2% SD) at a depth of 90 to 100 cm. Also, with the exception of the 160 and 550°C treatments, more P was liberated from the mid-depth samples than the deep soil, although the difference was not statistically significant.

The temperature that most effectively distinguished between the shallow, middle, and deep samples was 360°C. For the surface soils, exposure to 160°C was sufficient to liberate on average 24.2% ($\pm$8.8% SD) of the total P, whereas soils at a depth of approximately 1 m released only 4.4% ($\pm$5.7 SD) of the total P. At 360°C, surface soils released 83.5% ($\pm$14.9 SD) compared with 63.6% ($\pm$20.5 SD) for the deepest soil samples. This suggests that the less decomposed surface peat is not as resistant to thermal breakdown as the deeper soil. If P_o in deeper, older peat soils can be presumed to be more stable, then there is evidence to support the hypothesis that the amount of thermal energy needed to mineralize P_o is dependent on its recalcitrance.

As each sample was subjected to progressively higher heat, the weight loss of the sample increased, as would be expected.

Table 2. Analysis of variance results for differences in average extractable P among three soil depths for all dry heat temperatures combined; 550°C treatment not included in analysis.

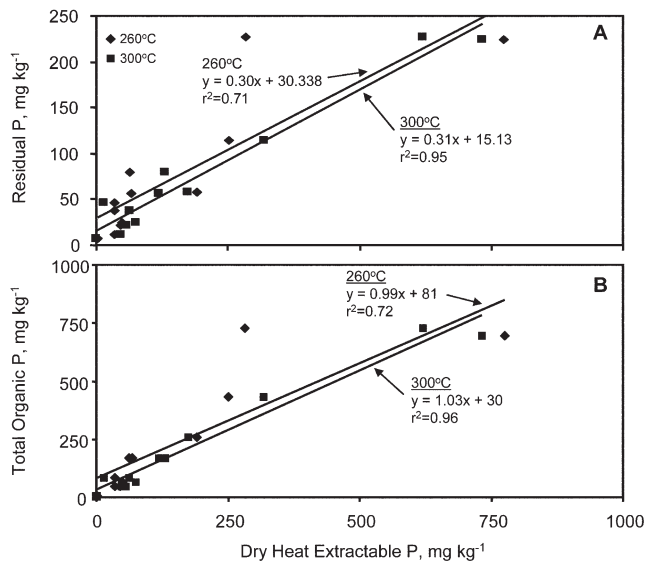| Source	df	Sum of squares	Mean square	F	P
Depth	2	10,705	5353	8.17	0.0004
Error	174	113,961	655		
Total	176	124,666			

Fig. 7. Relationship between dry heat extractable P and (A) residual P from sequential chemical extraction and (B) total organic P for treatment temperatures of 260 and 300°C.

This weight loss was probably due to the loss of gaseous C thermal decomposition products. Bartkowiak and Zakrzewski (2004) subjected extracted lignin to pyrolytic thermal gravimetry and found that the lignin had declining H content, relative to C and O, across the range of temperatures they used. They attributed this to the destruction and loss of methoxyl groups associated with the lignin molecule. At 360°C, the BCMCA samples had lost as much as 40% of their initial dry weight. There was a relationship between the sample dry weight loss and the amount of P released, with 73% of the variance in extractable P accounted for by weight lost during the procedure. For every 10% increase in weight loss there was a 24% increase in extractable P. This suggests that the increase in extractable P was due to the destruction of soil organic matter and subsequent liberation of P from organic compounds.

The two temperatures that best agreed with the sum of the organic fractions and the residual P in the sequential chemical extraction were the 260 and 300°C treatments (Fig. 7). The 300°C treatment was a good predictor ($r^2 = 0.95$) of the residual P in the sequential chemical extraction, although a slope of <1 indicates that the 300°C treatment also removed P from other pools, perhaps from fulvic and humic compounds, as well as microbial sources of P. These probably constituted the remaining 69% of the extracted P. The slope of the relationship between the sum of the organic P fractions (total P_o) in the sequential chemical fractionation and the 300°C treatment was nearly unity, indicating that 300°C is a sufficiently high temperature to liberate P from organic compounds for these samples. The 260°C treatment seemed to be as effective at liberating P_o, although the model fit was not as good ($r^2 = 0.72$).

When both residual P and dry heat extractable P are expressed as a percentage of total P, the significance of the relationship declines (Table 3). Even so, the negative relationship between residual P and dry heat extractable P indicates that as the percentage of P in the residual pool increases, it becomes increasingly difficult to

Table 3. Relationship between the percentage of total P in the residual fraction of the sequential chemical extraction vs. the percentage of P liberated at selected temperatures. Tabulated values are for model y = ax + b, where y is the residual P (%), x is the P liberated in the procedure (%), and b is the y intercept.

Temperature	a	b	r^2
°C			
160	−0.312	29.4	0.127
200	−0.227	30.9	0.146
260	−0.758	57.4	0.423
300	−0.256	40.2	0.129
360	−0.294	47.7	0.271

liberate P from the organic matter using thermal means. This may be taken as further support for the concept that the operationally defined residual P in the sequential chemical extraction represents forms of P that are resistant to breakdown. Ultimately, more P was extracted in the sequential chemical fractionation than in the two thermal techniques. For example, the P content of the residual material from the HEP extraction was approximately fourfold greater than the residue from the sequential chemical extraction (Fig. 8).

Classical chemical P fractionation methods have been used extensively to characterize the fertility of agricultural soils and the extent to which soils and sediments influence water quality in natural ecosystems. Those operationally defined techniques are presumed to divide P into discrete pools, such as broad categories of organic or mineral P compounds (Fe–P, Ca–P, humic P, fulvic P, etc.), or into pools of varying environmental stability. The unextractable material remaining after sequential extraction has been assumed to be resistant to further environmental mineralization. The two thermal techniques presented in this study were in agreement with the chemical fractionation results, particularly with respect to P_o stability. Both thermal techniques may be useful in assessing relative differences in P_o stability without the need to fully characterize soil P pools using conventional sequential extractions. Better characterization of organic P stability will potentially lead to refinements in predictions of the rate at which P is immobilized in soils and sediments. Results from this study suggest that (i) the thermal stability of P_o is related to other indirect measures of recalcitrance, such as the residual P
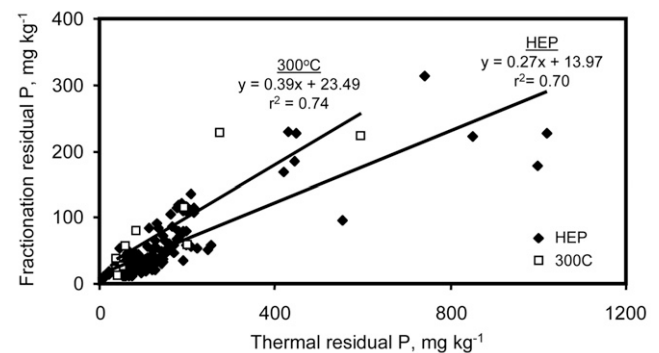

Fig. 8. Relationship between the unextractable (residual) material remaining after sequential chemical extraction of P vs. the residue from the two thermal extraction techniques. HEP = hot water extractable P; 300C = dry heat extracted P at 300°C.

determined by conventional chemical characterization, and (ii) thermal extraction of P_o from wetland soil may provide a useful diagnostic tool for investigating P_o stability.

REFERENCES
Anderson, J.M. 1976. An ignition method for determination of total phosphorus in lake sediments. Water Res. 10:329–331.

Bartkowiak, M., and R. Zakrzewski. 2004. Thermal degradation of lignins isolated from wood. J. Therm. Anal. Calorim. 77:295–304.

Bowman, R.A. 1989. A sequential extraction procedure with concentrated sulfuric acid and dilute base for soil organic phosphorus. Soil Sci. Soc. Am. J. 53:362–366.

Brenner, M., C.L. Schelske, and L.W. Keenan. 2001. Historical rates of sediment and nutrient accumulation in marshes of the Upper St. Johns River Basin, Florida, USA. J. Paleolimnol. 26:241–257.

Chang, S.C., and M.L. Jackson. 1957. Fractionation of soil phosphorus. Soil Sci. 84:133–144.

Davis, J.H. 1943. The natural features of southern Florida, especially the vegetation and the Everglades. Geol. Bull. 25. Fla. Geol. Surv., Tallahassee.

Felfli, F.F., A.A. Luengo, J. Suarez, and P. Beaton. 2005. Wood briquette torrefaction. Energy Sustain. Dev. 9:19–22.

Fitzgerald, G.P., and T.C. Nelson. 1966. Extractive and enzymatic analyses for limiting or surplus phosphorus in algae. J. Phycol. 2:32–37.

Gleason, P.J., and W. Spackman. 1974. Calcareous periphyton and water chemistry in the Everglades. p. 146–181. In P.J. Gleason (ed.) Environments of South Florida: Present and past. Mem. 2. Miami Geol. Soc., Coral Gables, FL.

Gleason, P.J., and P. Stone. 1994. Age, origin, and landscape evolution of the Everglades peatland. p. 149–197. In S.M. Davis and J.C. Ogden (ed.) Everglades: The ecosystem and its restoration. St. Lucie Press, Delray Beach, FL.

Grunwald, S., K.R. Reddy, J.P. Prenger, and M.M. Fisher. 2007. Modeling the spatial variability of biogeochemical soil properties in a freshwater ecosystem. Ecol. Modell. 201:521–535.

Hedley, M.J., and J.W.B. Stewart. 1982. Method to measure microbial phosphate in soils. Soil Biol. Biochem. 14:377–385.

Hedley, M.J., J.W.B. Stewart, and B.S. Chauhan. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. 46:970–975.

Hieltjes, A.H., and L. Lijklema. 1980. Fractionation of inorganic phosphates in calcareous lake sediments. J. Environ. Qual. 9:405–407.

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

Kenney, W.F., C.L. Schelske, and M. Brenner. 2000. Changes in polyphosphate sedimentation: A response to excessive phosphorus enrichment in a hypereutrophic lake. Can. J. Fish. Aquat. Sci. 58:879–887.

Levesque, M., and H. Dinel. 1978. Applicability of thermal methods for characterization of peats and plants. Geoderma 20:201–213.

Mitsch, W.J., and J.G. Gosselink. 1993. Wetlands. 2nd ed. Van Nostrand Reinhold, New York.

Murphy, J., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27:31–36.

Nelson, D.W., and L.E. Sommers. 1996. Total carbon and total nitrogen. p. 961–1010. In D.L. Sparks (ed.) Methods of soil analysis. Part 3. Chemical methods. SSSA Book Ser. 5. 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.

Prenger, J.P., and K.R. Reddy. 2004. Microbial enzyme activities in a freshwater marsh after cessation of nutrient loading. Soil Sci. Soc. Am. J. 68:1796–1804.

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 in wetland organic soils. Soil Sci. 163:705–713.

Sommers, L.E., R.F. Harris, J.D.H. Williams, D.E. Armstrong, and J.K. Syers. 1972. Fractionation of organic phosphorus in lake sediments. Soil Sci. Soc. Am. Proc. 36:51–54.

Turner, B.L., and S. Newman. 2005. Phosphorus cycling in wetland soils: The importance of phosphate diesters. J. Environ. Qual. 34:1921–1929.

USEPA. 1993. Methods for chemical analysis of water and wastes. Environ. Monit. Support Lab., Cincinnati, OH.

Wetzel, R.G. 1999. Organic phosphorus mineralization in organic soils. p. 225–245. In K.R. Reddy et al. (ed.) Phosphorus biogeochemistry in subtropical ecosystems. Lewis Publ., Boca Raton, FL.