Characterization of Long-Term Phosphorus Burial in Wetlands and Lakes of the Upper St. Johns River Basin – Phase I / Year 1

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1 Introduction 4

1.1 Soil Phosphorus Characterization and Ecosystem Management 4

1.2 Diagenesis and Estimation of Phosphorus Accretion Rates 5

2 Task 1: Physico-Chemical Fractionation of Organic P 8

2.1 Methods 8

2.1.1 Soil Collection 8

2.1.2 Organic Phosphorus Characterization 10

2.2 Results and Discussion 12

2.2.1 Soil Properties 12

2.2.2 Fractionation Results 13

3 Task 2: Autoclave Extraction of Organic P 27

3.1 Methods 27

3.2 Results and Discussion 27

4 Task 3: Graduated Pyrolysis of Peat 30

4.1 Methods 30

4.2 Results and Discussion 31

5 Task 4: Enzymatic Hydrolysis of Soil Organic Phosphorus 37

5.1 Methods 37

5.2 Results and Discussion 38

6 Task 5: Potentially Mineralizable Phosphorus (PMP) 41

6.1 Methods 41

6.2 Results and Discussion 41

7 References 43

1 Executive Summary

Soil samples were obtained from nutrient impacted and unimpacted sites in Blue Cypress Marsh Conservation Area (BCMCA) and Everglades Water Conservation Area 2A (WCA2A). The samples were analyzed for forms of phosphorus (P) in order to better model the capacity of wetlands in the Upper St. Johns River Basin (USJRB) to assimilate nutrients. The model would then be used to help set pollutant loading goals for USJRB wetlands.

Five P characterization techniques were investigated: organic P fractionation, hot water extraction, graduated thermal Pyrolysis, enzymatic extraction, and a potentially mineralizable P assay.

Highest and lowest total P levels were seen in WCA2A, with stations in BCMCA intermediate between these extremes. Below a soil depth of 40-cm, P levels were greater at both stations in BCMCA then the WCA2A stations, suggesting that BCMCA has not been as historically P limited as WCA2. The principal finding from the organic P fractionation was that inorganic P (Fe/Al and Ca – bound) plays a much lesser role in P biogeochemistry in BCMCA than WCA2A. For both ecosystems, there was a decline in total P with depth in the soil profile for all stations, suggestive of either increasing nutrient loading, loss of P from deeper strata, or both. Phosphorus associated with fulvic acid declined with depth, and highly recalcitrant P increased, suggesting movement out of fulvic acid fraction and into more recalcitrant, un-extractable P fractions.

Hot water extracted much more P from BCMCA than WCA2A samples. This may reflect fundamental differences in the organic matter of the two wetlands, i.e. the soils of WCA2A are more resistant to thermal decomposition. It is also possible that heating of the calcium-rich peat from WCA2A caused precipitation of calcite, which then co-precipitated with P that was released from organic matter. Hot water extracted approximately 85% of the P associated with phytic acid, a synthetic, recalcitrant P compound, suggesting that this extraction liberates more than polyphosphate-P, as previously believed.

Graduated thermal destruction of peat in an oxygen-free environment showed that as heat was applied, more P was liberated from the soil, up to 360^{o}C. After 360^{o}C, P recovery declined dramatically, especially in the BCMCA soils. This effect may be due

to production of reactive charcoal at the highest temperature. Deeper soils in both wetlands required greater heat to extract the same proportion of the total P, suggesting increasing resistance to thermal decomposition of organic matter, and therefore greater recalcitrance with depth in the soil profile.

Application of natural soil P-hydrolyzing enzymes did not result in the extraction of significant P, even when applied in excess. It is possible that the major form of soil P at the study sites was in a diester form. Since the alkaline phosphatase used in this study was a monoesterase, it would have had no effect on diester-bound P.

A short-term soil incubation was used to estimate the capacity of the soils from WCA2A and BCMCA to release P. Surface soil from BCMCA station B4 (unimpacted) released the greatest amount of P (13% of TP), whereas the deepest peat at both stations in WCA2A released the least P (2% of TP).

Results from this phase of the research suggest that phosphorus is not now, nor has it historically been as limiting to primary productivity in BCMCA as it has been to WCA2A in the Northern Everglades. This finding is consistent with another ongoing research study that has demonstrated that P is not currently as limiting in BCMCA as WCA2A.

2 Introduction

2.1 Soil Phosphorus Characterization and Ecosystem Management

Nutrient enrichment in the wetlands and lakes that comprise the Upper St. Johns River Basin (USJRB) is of major concern. Eutrophication of historically low-nutrient adapted lakes and wetlands alters trophic structure, favoring species that are more adapted to higher nutrient conditions. In particular, phosphorus (P) has been shown to be the principal nutrient influencing wetland ecosystem composition (Steward and Ornes, 1975; Davis, 1991; Urban et al., 1993). Agricultural stormwater discharge into wetlands of the USJRB has in some instances lead to changes in local flora that are similar to those observed in the nutrient-impacted regions of the northern Florida Everglades. It is thus critical to the preservation of native flora and fauna to both estimate and maintain the nutrient loading regimes that favored the establishment of the region's long-term biotic community.

Soils (and sediments) play a major role in determining ecosystem composition. Soil properties, such as soil nutrient status and structure, are major determinants of a region's suitability to specific agricultural crops and ultimate yield of that crop. In a similar fashion, soil nutrient status plays a fundamental role in the composition of natural ecosystems. When nutrients are scarce, as in most pristine natural systems, competitive strategies select for unique growth characteristics. These plants then define the long-term composition of the community, not only at the level of primary producers, but at higher trophic levels as well. Similarly, lake sediments can have a profound effect on plant communities, such as phytoplankton levels, though the coupling is not as direct. Anthropogenic nutrient loading, in excess of that to which the ecosystem has been conditioned, invariably leads to community shifts that are perceived to be less desirable than the displaced community. External loading also leads to enrichment in soil nutrient levels that persist until long after cessation of loading, providing a sustained source of nutrients to the newly established community. It is thus of primary concern to ecosystem managers to investigate soil physico-chemical properties when attempting to explain or predict ecosystem behavior.

2.2 Diagenesis and Estimation of Phosphorus Accretion Rates

Recent studies have suggested that the wetlands and lakes of the USJRB are experiencing increased P loading in the last several decades (Brenner et al., 1999). These studies rely on paleolimnological techniques that are partly based on the assumption that material deposited during any time period remains essentially unchanged. However, P storage in soils (especially peat) is present in chemical compounds of varying degrees of environmental stability. For instance, Reddy et al. (1998) have shown that in Water Conservation Area 2A in the Florida Everglades, refractory P increased from 33% of total P in surface soils, to 70% in deeper strata. DeBusk and Reddy (1998) showed that the proportion of refractory carbon along a vertical soil chronosequence (from standing dead litter to deeper soil strata) increases significantly with respect to depth. In their study, lignin content increased from approximately 12 to 50 percent of total dry weight, from standing dead plant litter to peat at a depth of 10-30 cm. It is possible that this reflects the nature of the plant material at the time of deposition, for instance local change in plant

communities from woody shrubs, to soft-tissued herbaceous plants. However, a more likely explanation is the relatively higher loss of the more labile fractions and apparent enrichment of refractory compounds, such as lignin. The P associated with the more labile fractions is thus subject to mineralization, mobilization, and subsequent loss from the soil profile. Thus, previous attempts to estimate the rate of P accretion in USJRB wetlands and lakes may have overestimated the relative increase in P accretion in recent times. A model of P accretion that takes into consideration post-depositional diagenetic processes would therefore be very useful in determining pre-impact P loading rates to these ecosystems. In turn, this information is critical to establishing ecologically "safe" loading of P to the wetlands and lakes of the USJRB.

Similar trends in refractory P as those observed in the Florida Everglades can be seen in soil profiles obtained from the USJRB. Olila and Reddy (1995) showed generally increasing residual (or recalcitrant) P content with respect to depth for soil cores obtained from the Blue Cypress Marsh Conservation Area. Residual P measured in those samples ranged from 20 % (of TP) in surface horizons to approximately 80% at a depth of 35-cm. It is possible that this reflects a changing depositional environment, i.e. increased recent nutrient loading. It may also be due to the loss of labile soil nutrient fractions as a consequence of organic matter mineralization. Pristine wetland regions that have not been impacted by nutrient loading may therefore serve as biogeochemical "reactors" in that chronological (depth) changes in P fractions could be expected to be due only to the effects of OM diagenesis.

Historical (ca. early 1900's) rates of nutrient accretion can be used as one line of evidence to use in establishing nutrient loading thresholds in wetlands and lakes, or pollutant loading goals. Mathematical models that describe pollutant loading to wetlands often include terms for "P settling" or long-term P burial (Kadlec and Knight, 1996; Lowe and Keenan, 1997; Walker, 1995). This term represents the long-term steady-state capacity of the wetland to assimilate P. To this end, knowledge of the stability of P in marsh soils will aid in the establishment of pollutant loading goals in wetlands and lakes of the USJRB. It is important to realize that these models were developed to represent the the removal of nutrients and other constituents in wetlands used for stormwater and wastewater treatment. When the models are used in that context, it is sufficient to derive

the settling term by comparing inflow to outflow concentration and attributing the difference to wetland removal processes. However, when the model is used to set sustainable nutrient loading limitations in natural wetlands, the examination of historical rates of nutrient loading may provide a more realistic estimate of the long-term assimilative capacity of the wetland.

The overall objective of this study is to determine the rate of P sequestration, or P burial, as a function of time, depth, and development in the watershed. The study is broken down into three phases. The transformation of organic phosphorus with time and depth was investigated in Phase I, and the results are presented in this report. In Phase II, the organic matter that constitutes the peat will be examined to determine if any differences in P fractionation observed in Phase I are due to biochemical changes in the peat. Phase III will synthesize the results of the first two phases into a semi-mechanistic model of long-term P burial.

A secondary objective of this study was to compare peat soils of the Everglades to peat of the USJRB. A surface water P standard has been proposed by the Florida Department of Environmental Protection for the Everglades of 10 parts per billion. This standard is based on many years of research and data collection concerning the effects of P enrichment on the flora and fauna of the Everglades. It is possible that some of these results can be applied to the wetlands of the USJRB. However, USJRB wetlands are situated in a different geologic setting, as well as in a different physiographic region (Meyers and Ewel, 1990). The Everglades lies over the shelly marls of the Fort Thompson formation, in the Gold Coast – Florida Bay physiographic region, whereas the USJRB is situated over alluvial marine terrace deposits in the Eastern Flatwoods physiographic region. Thus, extending nutrient standards developed for the Everglades to wetlands of the USJRB may be inappropriate, and comparative studies are needed to determine to what extent research results from the Everglades can be applied to the USJRB. In particular, these studies should focus on the relative degree of P limitation in the two ecosystems in order to develop a phosphorus standard for wetlands of the USJRB. The soil used in Phase I was collected from both WCA2A and BCMCA in order to make these comparisons.

3 Task 1: Physico-Chemical Fractionation of Organic P

3.1 Methods

3.1.1 Soil Collection

Soils were collected from two sites, Blue Cypress Marsh Conservation Area, and Water Conservation Area 2A (WCA2A)(Fig. 1). Long-term ecological research stations E1 and E5 in Everglades WCA2A and stations C1 and B4 in BCMCA were sampled on July 17 and 18, 2001. Stations E1 and B4 have been impacted by nutrient loading, while stations E5 and B4 are believed to represent pre-developmental, or background conditions. Soil cores were obtained with a 2-m x 7.3 cm dia. stainless steel soil corer. Soil samples were extruded in the field into Ziploc™ bags at 10-cm intervals.

Soil samples were immediately chilled to 4^{o}C and were 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 samples were transferred to rigid polyethylene containers and thoroughly mixed. A 10 – 20 g sub-sample was removed for pH determination. For samples that were insufficiently moist to make a pH determination, deionized water was added until the sample was

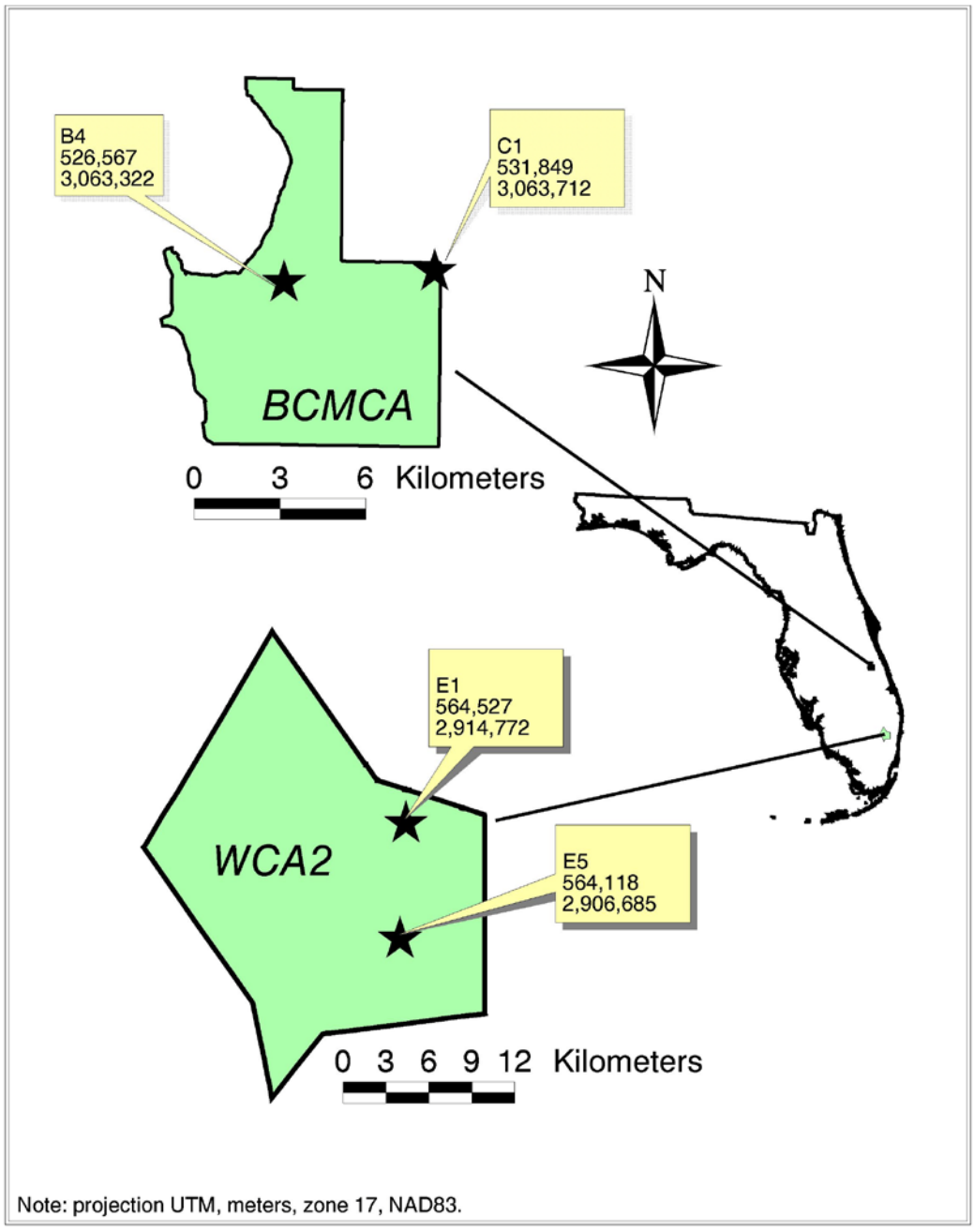

Figure 1. Soil sampling locations used in the "Long-term Phosphorus Burial" project.

sufficiently wet. A separate 50 – 100 g was dried at 80°C to constant weight for % moisture determination.

3.1.2 Organic Phosphorus Characterization

The following analysis was performed on a wet, field moisture content sample. The extraction ratio was 1 (soil) : 50 (extractant). All extraction ratios refer to the ratio of oven-dry mass equivalent of wet sample to extractant volume. The sequential extraction procedure was initiated on July 31, 2001 and was completed on August 16, 2001. Extractions proceeded according to the steps shown in figure 2. A field-wet sub-sample of 0.5 grams dry mass was placed into a 43-ml polyethylene centrifuge tube and 2-mls of $CHCl_3$ was added to each tube. The tubes were left uncapped overnight and extracted the following day with 0.5 M $NaHCO_3$. Twenty-five milliliters of each extractant was added to the tube and it was end-to-end shaken on a reciprocating shaker for the times indicated in figure 2. The samples were centrifuged at 6000 rpm, the supernatant extractant was removed and oxically filtered through 0.45 μm polyethersulfone filters, and the tube was re-weighed. Twenty-five milliliters of the next extractant was then added to the tube and the process was repeated. This procedure was continued in such a manner that with each successive step, less P remained in the soil sample. After the final step, only very recalcitrant P remained. A separate, non-sequentially extracted wet sub-sample was extracted with 0.5 M $NaHCO_3$ and no chloroform was added to the sample. The difference between the P liberated in the chloroformed and the P liberated in the non-chloroformed bicarbonate extraction is believed to represent the P content of the soil microbial biomass. The humic and fulvic acid content of the sodium hydroxide extract was operationally determined in the following manner. After extracting with 1M NaOH and centrifuging, seven milliliters of the supernatant fluid was withdrawn from each tube. Seven drops of concentrated H_2SO_4 was added to each subsample to condense the acid insoluble humic acid fraction. The sample was again centrifuged at 6000 rpm for five minutes, and an aliquot of the supernatant was withdrawn. This aliquot was analyzed for total P by adding 1 ml of 11N H2SO4 and 0.3 g $K_2S_2O_8$ and digesting for 3 hour at 380°C. The P remaining in solution after the acidification step was considered to represent P associated with fulvic acid. The total P content of an un-acidified NaOH

subsample was also determined. Humic acid-bound P was determined as the difference between the TP content of the un-acidified sample and the fulvic acid P. All sequential extractions were done at room temperature.

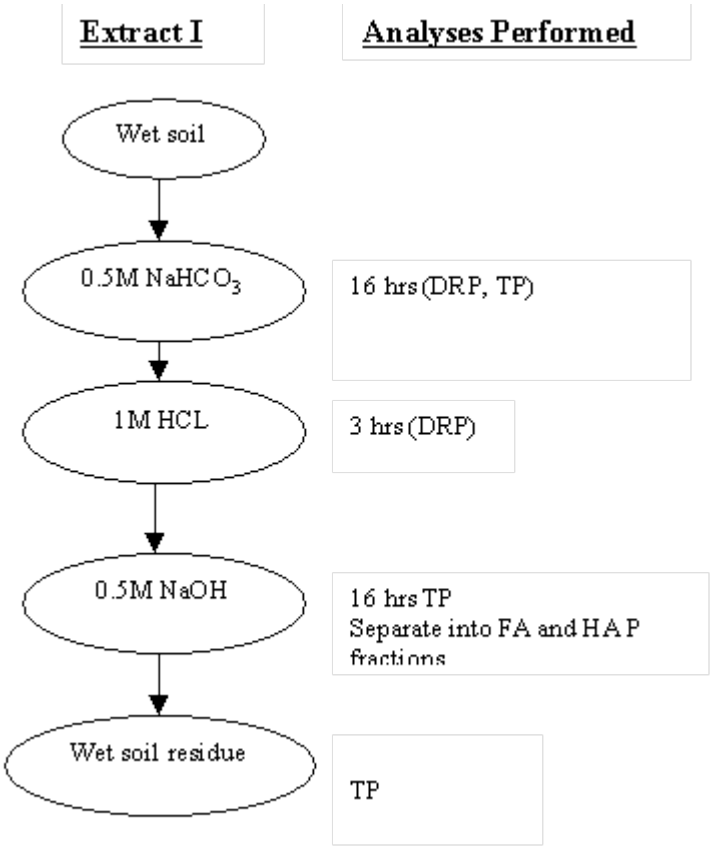

Figure 2. Chemical sequential extraction used to fractionate BCMCA and WCA2A soils into discrete phosphorus pools.

3.2 Results and Discussion

3.2.1 Soil Properties

The samples used in the phosphorus characterization experiments were fibric peats, with the exception of some of the bottom-most samples, which were sands. Sand was encountered at the base of the core retrieved from WCA2A station E1 at a depth of 121-cm. Shelly, clayey, marl was found at the base of the cores taken at E5 at a depth of 153-cm. *Chione sp.* , a marine bivalve, was found at the interface between peat and sand at station E5. Sand was also encountered at the base of the core taken from station C1 at a depth of 260-cm. The peat thickness at station C1 was estimated as approximately 4-m, and seemed to be underlain with clay.

Table 1. Basic physico-chemical properties of surficial (0–50 cm) soils collected in July 2002 from WCA2A and BCMCA.

	BCMCA		WCA2A	
Parameter	B4	C1*	E1*	E5
TN, %	3.21	3.50	2.93	2.42
TC, %	47.4	48.3	45.1	45.7
Dry Weight, %	8.7	10.3	10.0	10.8
pH	5.70	5.88	7.59	7.30
Bulk Dens., g/cm^3	0.081	0.101	0.095	0.111
Ash, %	5.8	8.8	7.3	11.1
TP, mg kg^{-1}	327	367	546	197

* nutrient impacted soils

The soils of WCA2A and BCMCA were similar with respect to most of the soil physical properties (Table 1). The pH of soil from BCMCA was considerably more acidic; almost 2 units lower. Total nitrogen was slightly greater in BCMCA than WCA2A. Total P at the nutrient impacted station in WCA2A was greater than the nutrient impacted station in BCMCA, though TP at the unimpacted station in WCA2A was lower than the unimpacted station in BCMCA.

Three main comparisons were made in this study:

1. How does phosphorus chemistry change with respect to depth, or time? (temporal effects)
2. How do the soils of the Everglades compare to the soils of the USJRB, with respect to phosphorus geochemistry (Inter-ecosystem effects)?
3. How does nutrient enrichment effect the distribution of soil P (enrichment effects)?

3.2.2 Fractionation Results

3.2.2.1 Comparisons Among Stations and Wetlands for the Upper 50-cm Soil Layer

Total P was highest at WCA2A station E1 (546 mg kg^{-1}), and declined in the order E1>C1>B4>E5 (Fig. 3). As expected, the nutrient impacted stations showed higher total P content in the 0 – 50 cm soil layer than the unimpacted stations. However, the difference in total P between the impacted and unimpacted stations was not nearly as dramatic at the BCMCA stations, 367 vs. 327 mg kg^{-1}. Of course, this comparison is based on a large depth interval, so any differences in P loading between the two stations would have diluted by inclusion of relatively deep soil in the sample. Also, total P at the un-impacted station in BCMCA was 66% greater than the unimpacted Everglades station. This suggests that BCMCA has not been under the same degree of P limitation as the Everglades, and that nutrient enrichment was more pronounced in WCA2A.

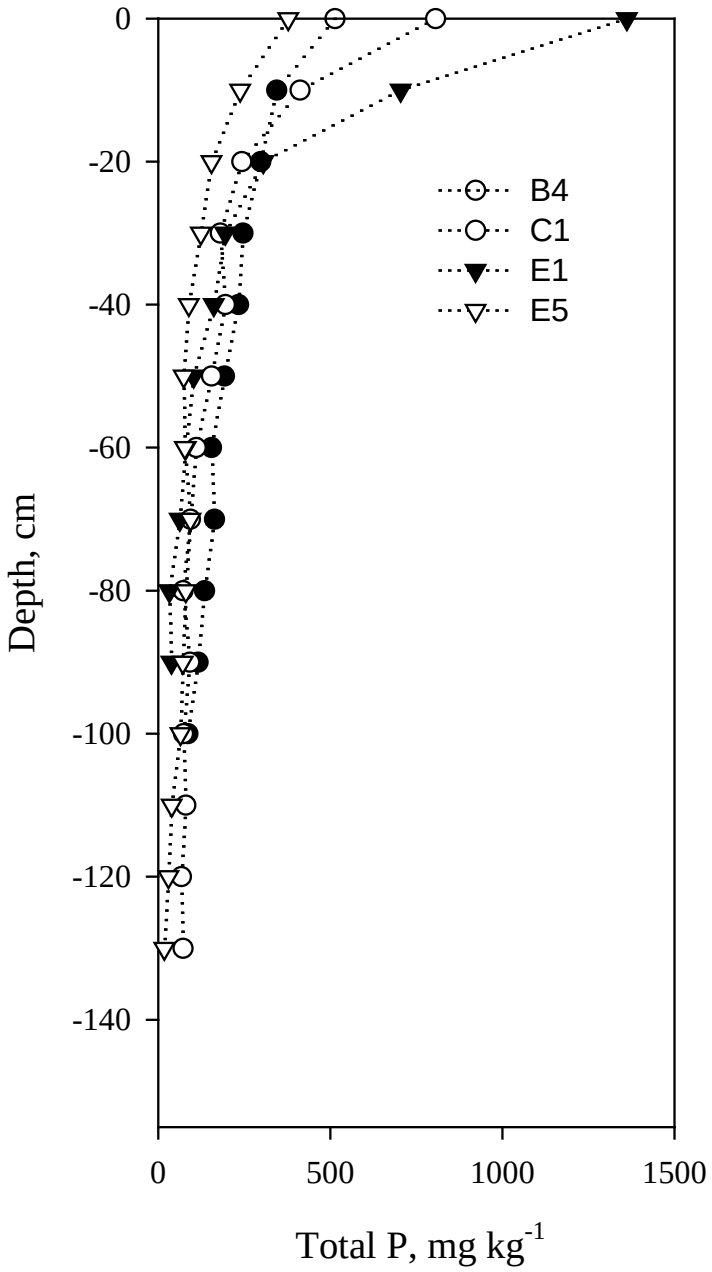

Figure 3. Total phosphorus concentration of soil samples from Blue Cypress Marsh Conservation and Water Conservation Area 2A in July 2002.

The distribution of total P with respect to depth in the soil profile indicates that WCA2A station E1 had elevated TP levels, relative to the other stations, for the upper 30-cm soil layer. Below this depth, total P was lower at both WCA2A stations than the BCMCA stations.

Fulvic acid P represented the dominant form of extractable P at all stations, comprising approximately 30% of total P (Fig's 4 – 7). Generally, un-extractable (or residual) P accounted for one third of the soil total P at all stations. That fraction represents a source of very slowly available, or recalcitrant P, that is mostly unavailable to the biotic community. Relatively little P (4%) was recovered in the labile (bicarbonate extractable) pool and this was consistent among all stations. At the more pristine BCMCA station (B4), bicarbonate P was actually proportionally greater than at the nutrient impacted station, representing 9% of TP. (Fig's. 4 - 5). Previous studies have found relatively high levels of porewater P in this region of the marsh. The cause for high labile P in the interior marsh is not known, but may be another indication of the overall nutrient status of the marsh, i.e., that it is a relatively high nutrient status wetland. One-molar HCl-extractable P comprised approximately 10% of TP at both BCMCA stations. Overall, there were only minor differences in P distributions between the two stations in BCMCA.

Total P at station WCA2A station E1 was approximately 3-fold greater than the central WCA2A station. Humic acid P at station E5 was double that found at E1 (Fig's. 6 – 7). This increase in the proportion of humic acid extractable P came at the expense of the HCL-P fraction at station E5. This could mean that as the peat ages, P is slowly moved out of the calcium-bound forms and sequestered into stable humic compounds.

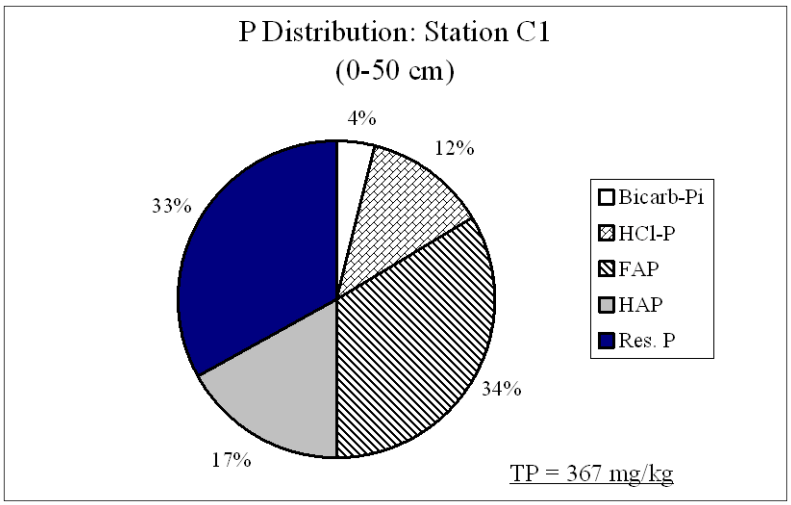

Figure 4. Phosphorus distribution at BCMCA station C1.

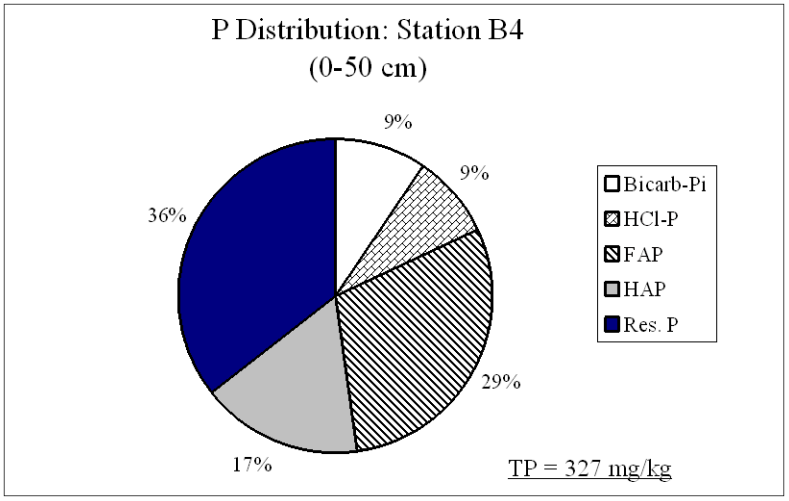

Figure 5. Phosphorus distribution at BCMCA station B4.

The principal difference between the two wetlands was the proportion of P in the HCl-extractable fraction. The HCl-extractable P in BCMCA was approximately 10%, compared to 27% in WCA2A. There are several related reasons for this. BCMCA is a slightly acidic, soft-water wetland, underlain by sand, whereas WCA2A is a slightly alkaline, hardwater wetland, underlain by sand and marl, and therefore rich in calcium. It would therefore be expected that calcium would play a larger role in regulating P mobility in WCA2A. This is one of the major differences between these two wetlands, perhaps explaining many of the differences in vegetation and higher trophic levels observed between the two ecosystems. For instance, the interior regions of BCMCA are dominated by maidencane and sawgrass (*Panicum hemitomon, Cladium jamaicense*), whereas interior regions of WCA2A are characterized by sloughs with periphyton communities, interposed with short stands of sawgrass.

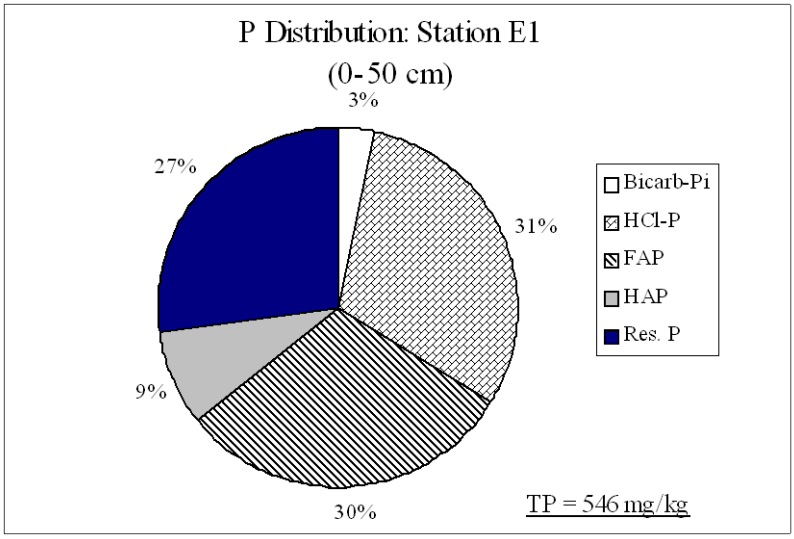

Figure 6. Phosphorus distribution at WCA2 station E1.

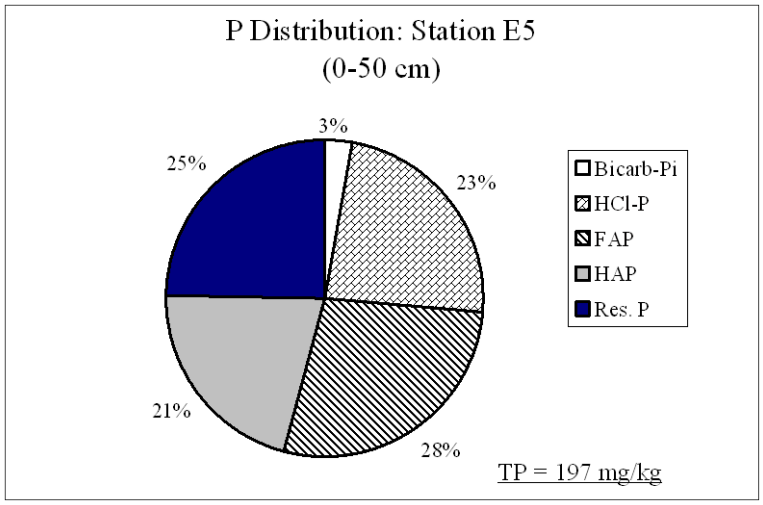

Figure 7. Phosphorus distribution at WCA2 station E5

3.2.2.2 Biogeochemical Changes in Peat Physicochemical Properties, as a Function of Depth, or Time

The peat was thinnest at station WCA2A E1; approximately 120 cm in thickness. However, this likely represents thousands of years of soil diagenetic processes. Therefore, observations of physicochemical properties of this vertical stratigraphic profile represent a temporal investigation of the processes governing the preservation of soil organic matter and long-term P sequestration. The four stations were very similar in bulk density, varying little with respect to depth, changing only when sand was encountered (Fig. 8). Loss on ignition, an indication of the amount of organic carbon, also showed little depth trend. WCA2A stations showed slightly lower loss on ignition, possibly due to increased mineral content. The most unimpacted station, E5, showed the lowest loss on ignition, and the highest bulk density, again reflecting the increased mineral content at this station.

Bicarbonate extractable P was greater (as a proportion of TP) in the surface soils in BCMCA than at the Everglades stations, and was considerably higher at the unimpacted BCMCA station, B4 (Fig. 9). This fraction was nearly constant with respect to depth, showing increases at all stations, except for B4, deep in the profile. Bicarbonate extractable P was approximately 3 – 5% of TP. With the exception of the surface soil samples, this fraction was very similar among stations, and with respect to depth. Because of this, this fraction appears to offer limited possibility of distinguishing between nutrient status of these wetlands, or peat diagenesis.

Total inorganic P (TIP; HCl-P) generally declined with depth in the soil profile, with the exception of WCA2A station E5 (Fig. 10). Total inorganic P was consistently greater in the Everglades. As mentioned above, declining total inorganic P levels may reflect depletion of this pool of P over time, and enrichment of the organic P fraction. The reason for increasing TIP content in the deeper strata may reflect the increasing influence of the calcium-rich marl underlying this region of the marsh. The HCl-extractable P also extracts iron and aluminum-bound P for the sequential fractionation procedure used here. However, WCA2A peat is known to be

low in iron and aluminum, therefore the P liberated in this step is predominately associated with calcium. It is possible that some of the TIP in BCMCA is Fe or Al-bound and further research is needed to determine if Fe or Al plays a role in P mobility in BCMCA.

Microbial biomass P (MBP) generally accounted for 10 to 20% of total P. Highest MBP was seen at BCMCA station C1 and this effect was consistent throughout the soil profile. MBP content showed a slight decline with respect to depth for all stations. The decline is expected and is likely the result of an increase in microbially unavailable refractory organic matter and a shortage of terminal electron acceptors. The values reported here were not corrected for extraction efficiency. Typically, some of the microbial P is either not extracted with the $CHCl_3$, or alternatively, some of the microbial P extracted may be re-incorporated into new biomass. Some researchers divide the value reported here by a factor of 0.4 to account for this, however the true efficiency for these soils is not known so the correction was not attempted. A further possibility is that the $CHCl_3$ extracts P from non-microbial organic matter and may therefore overestimate the magnitude of microbial P.

The proportion of P in the fulvic acid pool declined at all stations, indicating that this is a somewhat labile pool. Humic acid P results were more variable. This fraction increased dramatically at BCMCA station B4, and declined at station E1 in the Everglades. The humic acid-bound P fraction remained mostly constant to a depth of approximately 80-cm for stations C1 and E5. It was expected that this fraction would increase with increasing depth due to increased humification of soil organic matter, however variable results with this fraction proved this to be incorrect. It is possible that the soils were more humified at deeper depths, but the humic materials were not associated with P.

Residual P approximately doubled from surface soils to lower depths, from approximately 20% of TP at the surface, to 40% at 90-cm. The smallest change in this fraction was at BCMCA station B4, increasing from 23% in the 0 – 10 cm interval, to approximately 30% at 1-m. Even though the proportion of P in this pool increased over the sample interval, mass of P in the residual pool declined asymptotically at all stations to approximately 20 mg kg^{-1} at the lower soil depths. The proportional

increase in residual P with respect to depth, combined with a concurrent decline in the total mass of this pool has several implications.

1. There is a gradual loss, or leakage, from the recalcitrant pool,
2. The rate of loss of P is faster from pools other than recalcitrant,

A changing depositional environment is only partly responsible for changes seen in total P content over time, or depth. This is substantiated by observing the increasing P content seen at all stations. The WCA2A station E5 is located in a pristine region of the Everglades, with surface water TP concentration of approximately 5 $\mu g\ L^{-1}$. That station has not yet been impacted by nutrient loading, yet it exhibits an upcore increase in P, though it is not as dramatic as the other three stations. A similar profile can be seen at BCMCA unimpacted station B4. Therefore, even though some of the difference in TP content between surface soils and deeper strata is undoubtedly due to nutrient loading, an unknown proportion of this difference is due to diagenetic transformations of the plant material forming the peat.

For BCMCA, the increase in the un-extractable, residual pool could be accounted for by decreases in fulvic acid P, HCl-extractable inorganic P, and microbial P (Fig. 11). There was little change in the humic acid P fraction (Fig. 9). This suggests that as the peat ages, there is a net mineralization and loss of P from the soil fulvic acid, microbial, and inorganic pool, with subsequent enrichment of the recalcitrant pool. However, in terms of mass, even this pool shows declines with depth and age. The same increasing trends in residual P were repeated for the Everglades stations.

Differences in the fractionation results suggest that the peat soils of BCMCA are higher in total P, and P is distributed slightly differently among P pools. There are several explanations for this, relating to the five soil forming factors of climate, landscape position, biology, parent material and time. Blue Cypress Marsh Conservation Area is at the low point in a relatively confined drainage basin. It is therefore directly connected with the surrounding uplands. Conversely, the Everglades WCA2A is situated at the

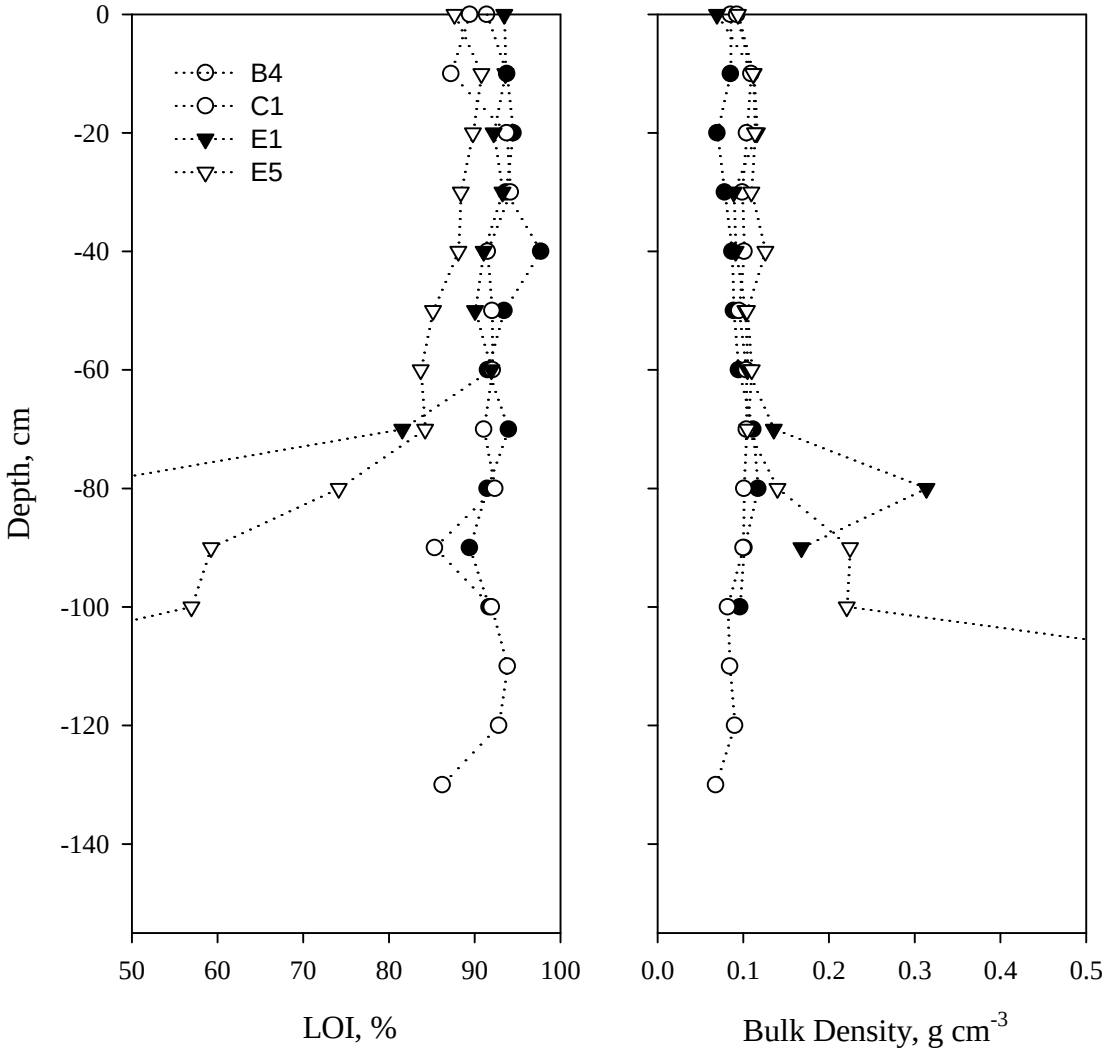

Figure 8. Loss on ignition and bulk density of soil samples from WCA2A and BCMCA.

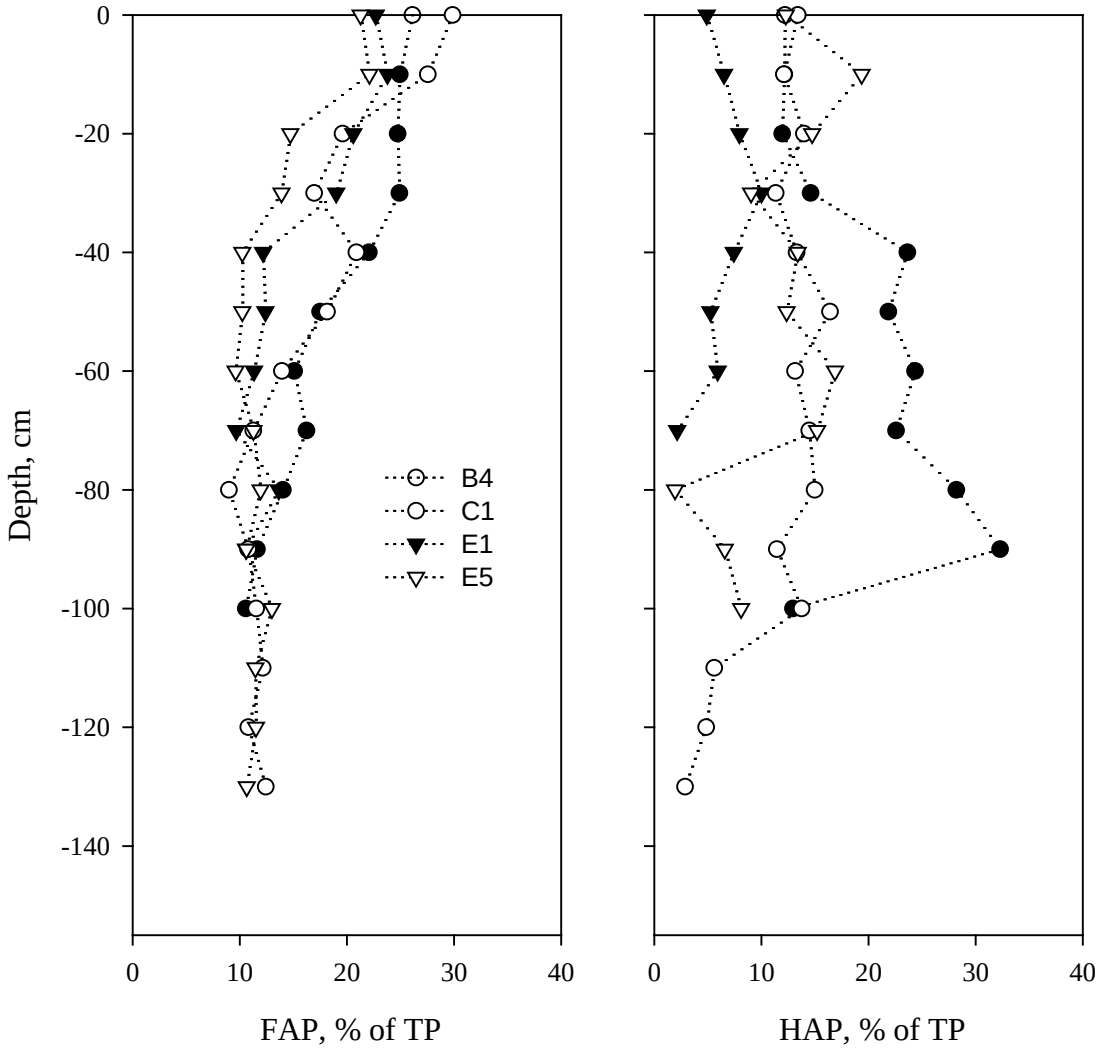

Figure 9. Fulvic and humic acid-bound P in soil samples from WCA2A and BCMCA.

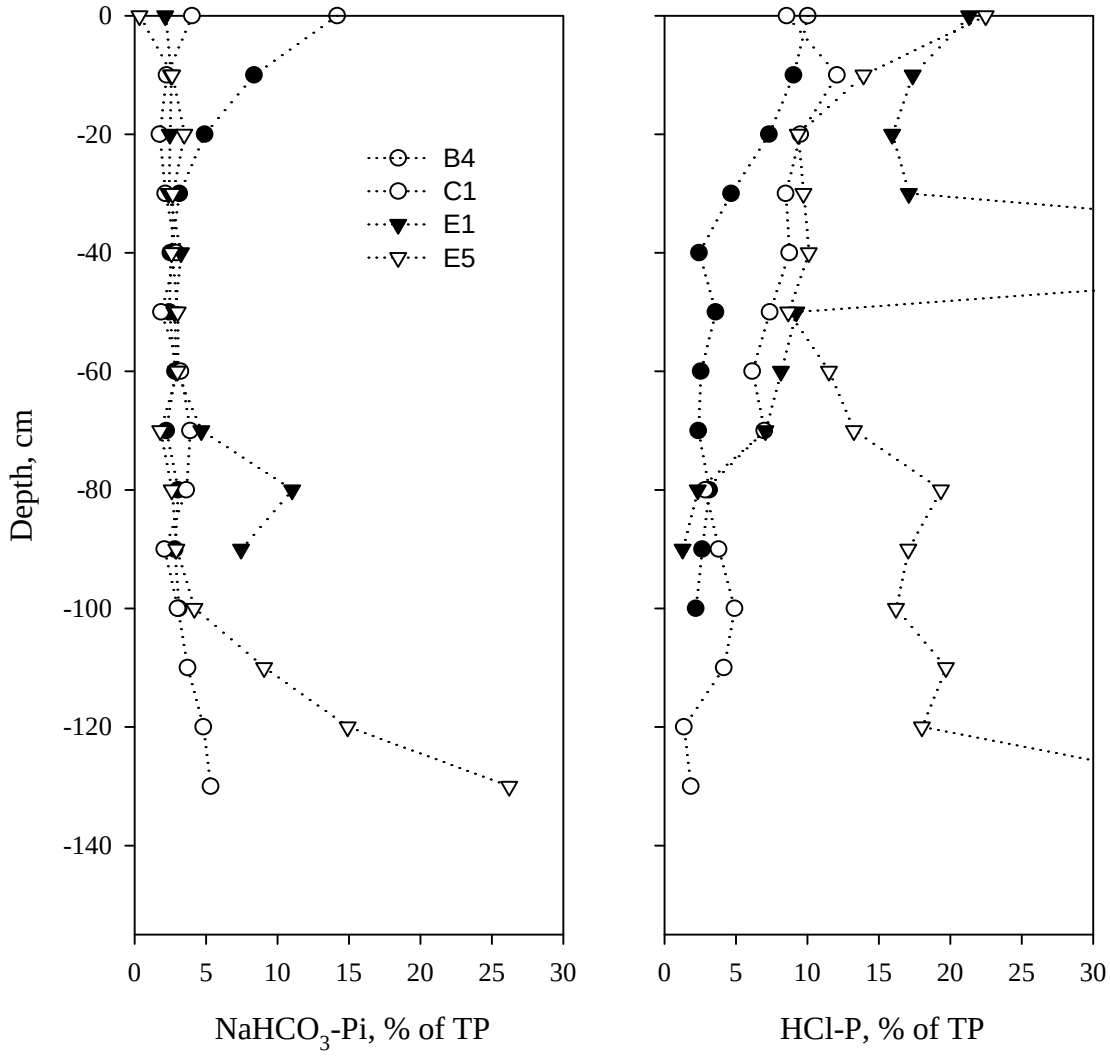

Figure 10. Bicarbonate and 1M HCl-extractable P content of soil samples from WCA2A and BCMCA.

downstream end of the Kissimmee River ~ Lake Okeechobee system. Surface runoff is processed through hundreds of miles of rivers, streams, wetlands, and lakes before entering the Everglades. The upstream ecosystems are a sink for P, reducing nutrient concentrations, and imposing nutrient limitations on the extreme downstream hydrologic units. Farming practices introduced into the region in the early 20th century removed some of this limitation by draining the northern Everglades and causing mineralization of P stored in the peat to be transported to previously nutrient-poor regions of the Everglades. This effect is readily apparent in the surficial (0 – 30 cm) soils at station E1. The effect of parent material is reflected in the difference in the inorganic P pool. Water Conservation Area 2A is underlain by the calcium-rich Fort Thompson formation, whereas BCMCA is underlain by sand. The calcium of WCA2A exerts a strong control on both the biology of the ecosystem and on P mobility.

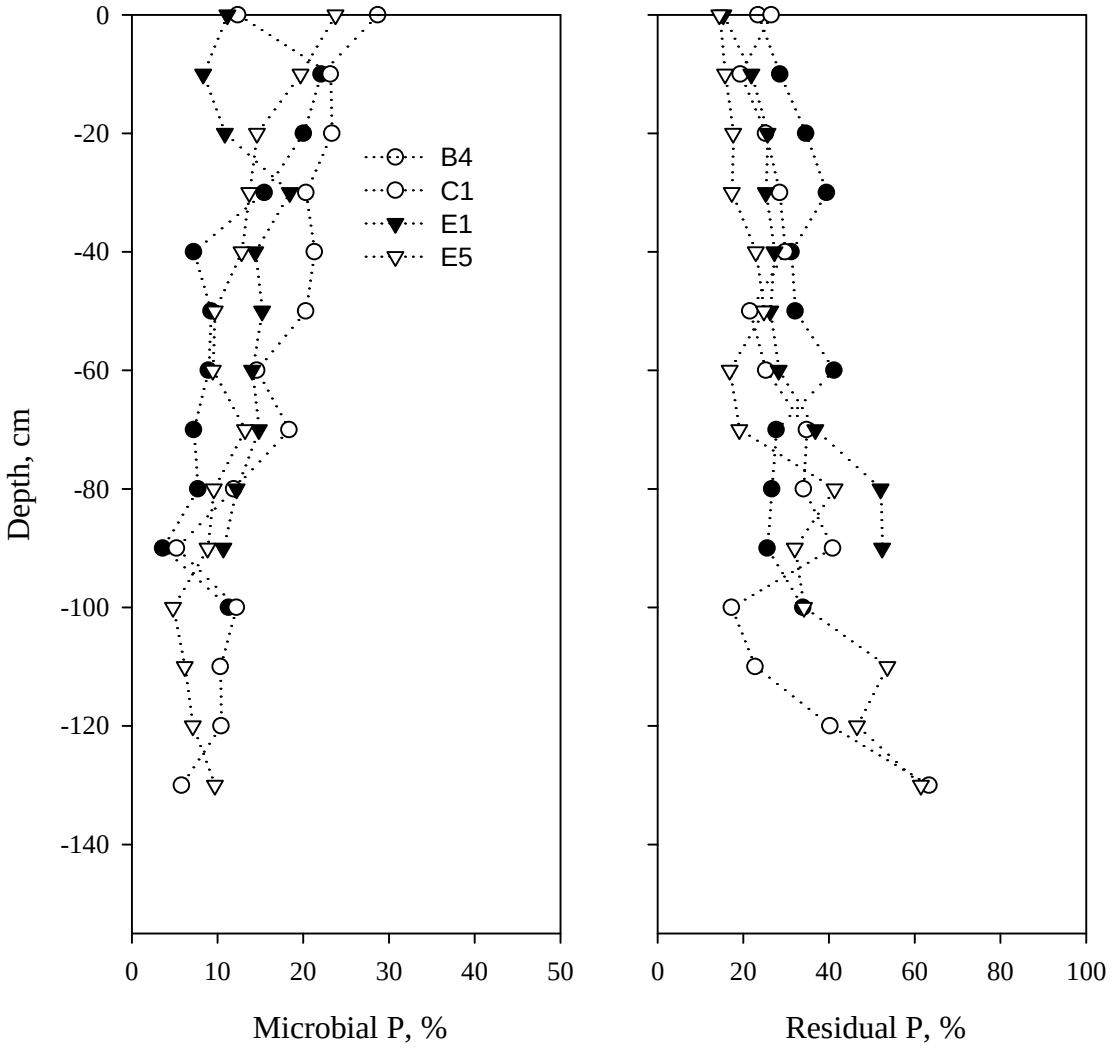

Figure 11. Microbial biomass P and un-extractable, residual P content of soil samples from WCA2A and BCMCA.

4 Task 2: Autoclave Extraction of Organic P

4.1 Methods

The soil samples used in this experiment were subsamples of those used in Task 1. For a description of sampling methods and location, see **Section 2.1.1**. Twenty-five milliliters of deionized water was added to one-half gram oven-dried equivalent wet soil. The soils were shaken at room temperature for 3 hours, centrifuged, and the supernatant water was removed and filtered through 0.45 μM polyethersulfone filters. The sample was then placed into an autoclave for 90 minutes at 128^{o}C and 1.7 atmospheres (25 lbs in^{-2}), similar to the extraction proposed by Kenney et al. (2000). The sample was removed from the autoclave, 20 milliliters of deionized water was added, and the sample was equilibrated with the added water through a one hour, end to end shaking period. The sample was then directly filtered through 0.45 μM filters. Extracts were analyzed for DRP and TP. Triplicate samples of glycerophosphate and phytic acid were also extracted using the above procedure to determine the extent of organic phosphorus mineralization for this procedure. The difference between the two sequential extracts is hot water extractable P (HEP).

4.2 Results and Discussion

Both Everglades WCA2A stations showed much lower amount of HEP than the BCMCA stations (Fig. 12). The HEP at station E1 was almost constant with respect to depth at approximately 10% of TP, showing little effects of recent nutrient enrichment. Station E5 showed slightly elevated HEP at the surface, and declined to approximately 5% throughout the rest of the profile. Otherwise, there were only minor differences between the impacted and non-impacted Everglades stations.

Conversely, HEP accounted for approximately 40 and 50% of TP at the impacted and unimpacted BCMCA stations, declining to approximately 10% in the deeper sampling depths. Unlike WCA2A, there was a gradual decline in % HEP with respect to depth. There was little difference in the extraction results between the nutrient impacted

and unimpacted station. One interpretation of this is that nutrient enrichment in BCMCA has resulted in enrichment of the inorganic P pool, primarily the Fe and Al-bound P forms. Since the HEP extraction liberates P from organic P sources, there would be little evidence of nutrient enrichment in this fraction.

The dramatic difference between the HEP in the two wetlands may relate to quantity of P stored in inorganic, principally calcium-bound, fraction. Calcium carbonate solubility declines with increasing temperature, therefore precipitation of calcite may have occurred during the autoclaving period. This new carbonate may have sorbed any P released during the extraction. If this was the case, it is not surprising that both BCMCA stations showed higher levels of HEP than even the most impacted station, E5. Lower calcium and pH levels in the soft-water BCMCA soils would not have resulted in precipitation to the same extent as the WCA2A stations.

The depth distribution profiles at the two BCMCA stations were quite similar, though the nutrient impacted station showed consistently greater proportion of P in the HEP fraction. This cannot be a function of nutrient loading, since the trend continues to a soil depth of 100-cm, and therefore must be due to spatial differences in peat properties between the two regions of the marsh. One possibility is that P mobility is controlled by inorganic compounds at station B4, and mineral forms of P are not extracted with this technique.

The WCA2A soils should be re-extracted after buffering the pH at a lower value, such as 5. This would prevent any P mineralized during the extraction from co-precipitating with calcium during the autoclaving period or during the final deionized water equilibration period.

Approximately 60% of both glycerophosphate and phytic acid were hydrolyzed at the experimental temperature and pressure (Fig. 13). This indicates that even at the relatively low temperature used here, there was considerable breakdown of recalcitrant organic P that was incorporated into phytin. This further suggests that hot water extractions may liberate more than microbial and algal stores of polyphosphates, as has been assumed by other researchers.

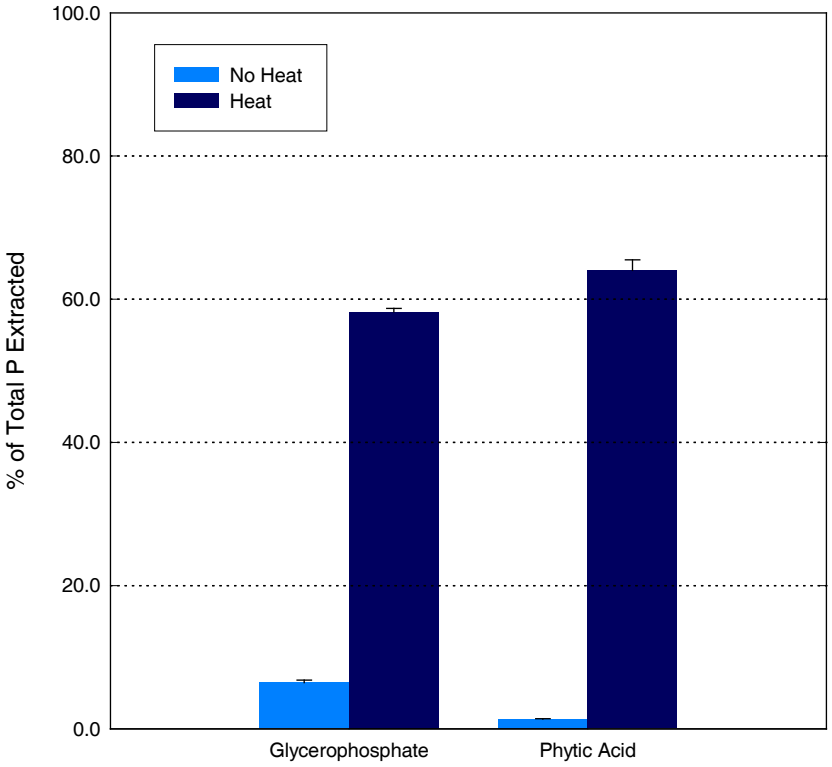

Figure 13. Autoclave extractable P content of glycerophosphate and phytic acid.

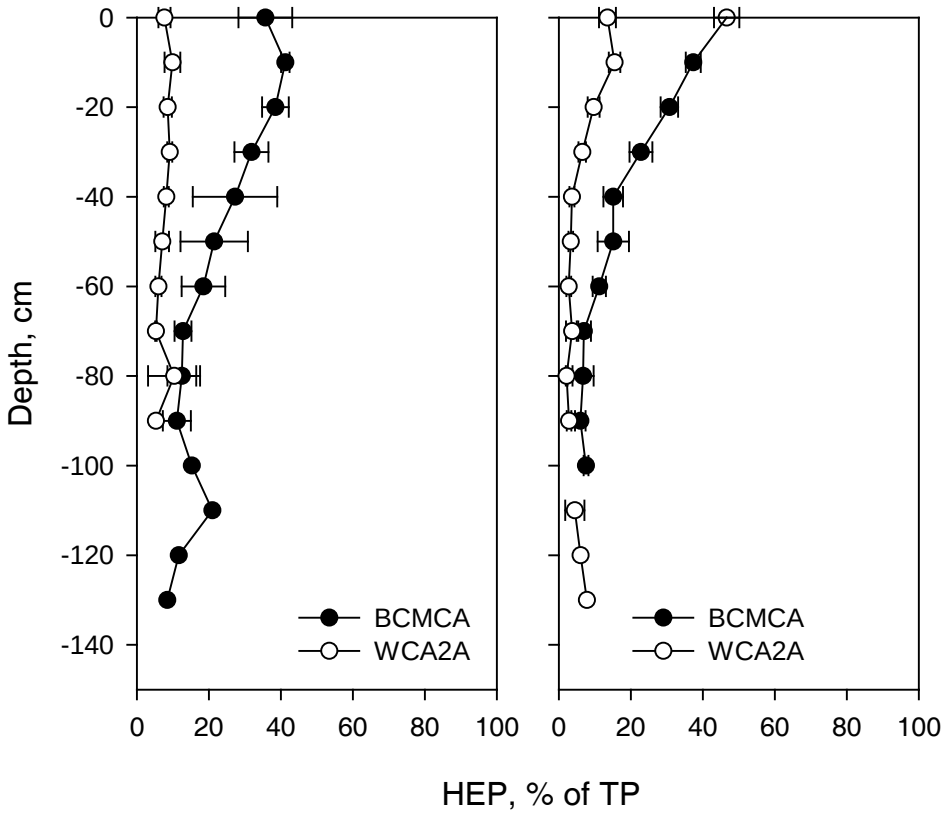

Figure 12. Hot water extractable P content of soil samples from BCMCA and WCA2. Error bars represent ±1 SD.

5 Task 3: Graduated Pyrolysis of Peat

5.1 Methods

The soil samples used in this experiment were subsamples of those used in Task 1. For a description of sampling methods and location, see **Section 2.1.1**. Pyrolysis was performed in a furnace that was constantly purged of O_2 with N_2. The oven consisted of a 7.6-cm (3-in) diameter galvanized steel pipe with two threaded end caps. A 220V 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. Each sample was weighed in triplicate onto 5-cm

squares of aluminum foil. The foil squares were placed onto a tray that was slid into the furnace, which was then sealed. The chamber was purged for approximately 10-min. and was then increased to the set point temperature. It remained at this temperature for one-hour for each treatment temperature. Three soil depths (0 -10, 40 – 50, and 90 - 100 cm) were used for this experiment. Pyrolysis temperatures were 160, 200, 260, 300, 360, and 550^{o}C. The samples were removed from the oven and transferred to 43-ml centrifuge tubes. They were then extracted with 1M HCl at a 1:50 ratio for three hours on a reciprocating shaker, then filtered through 0.45 μM filters. The P that was extracted at room temperature with 1M HCl was subtracted from each value to yield a "net" pyrolyzed P.

5.2 Results and Discussion

The overall effect of temperature on the % of P that could be liberated depended on the sample depth, ecosystem, and temperature. Each of these parameters played a major role in the extractability of the organic P. Some general observations were:

1. There was an increasing mineralization of P with increasing temperature, with the exception of the highest (550^{o}C) temperature,
2. Less of the TP pool was thermally extracted from the lower depths, possibly indicating greater stability of this material,
3. Reduced extraction efficiency at 550^{o}C was most pronounced in surface samples.

Temperature had a dramatic effect on the % of P that could be extracted and this effect was most pronounced in the deep peat ($p<0.0001$) (Fig's. 14-21). If the analysis is repeated on the surficial peat, more P is liberated *at lower temperature*, for instance 75% at 300^{o}C. This suggests that the less decomposed peat is not as resistant to thermal decomposition and that it is less recalcitrant. For the deep peat, only 65% of the P could be liberated at 300^{o}C. The greatest release of P (for both surface and deep peat) occurred at 360^{o}C. For the surface peat, nearly 100% of the P was liberated. For the deep peat, only 71% was liberated. A curious effect occurred in both sample depths: that of a dramatic decline in P recovery at the highest temperature. This temperature may have had the effect of "charcoaling" the sample. Therefore, lower P recovery could be due to adsorption of soluble organic P compounds on the highly reactive organic matter created

during pyrolysis, though the 1M HCl extract should have exchanged any sorbed inorganic compounds. This suggests that changes in phosphorus chemistry at 550^{o}C may have resulted in conversion of the original P stored in the peat into more thermally stable organic compounds. The lower temperatures extracted very little P in the deep peat, approximately 2% at 160C and 10% at 200C, compared to 22% and 38% in the surface peat. This implies that much more thermal energy was needed to break down the organic P in the deeper, older peat.

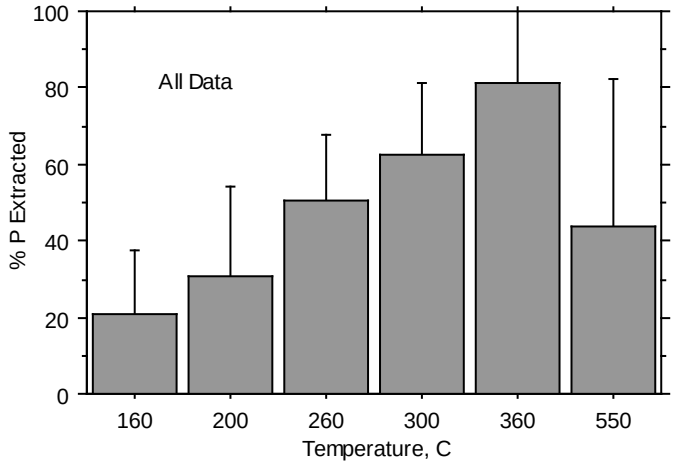

Figure 14. Effect of temperature on P extraction. All data (three depth's, two wetlands, four stations) included. Effect of temperature is significant at $p < 0.0001$. Error bars represent ±1 SD.

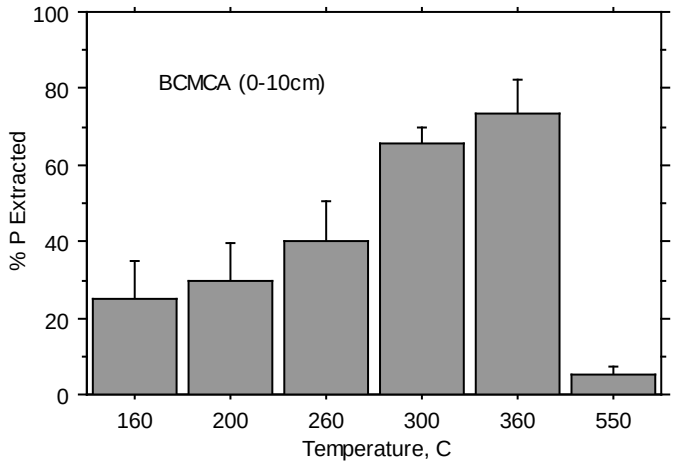

Figure 15. Effect of temperature on P extraction. Surface (0-10cm) of BCMCA stations included. Error bars represent ±1 SD.

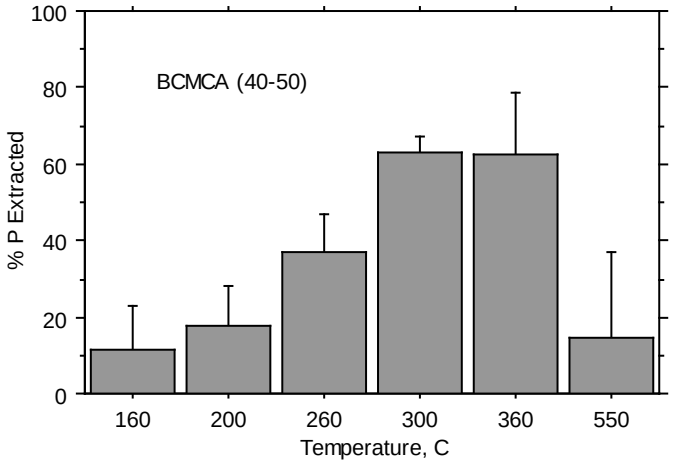

Figure 16. Effect of temperature on P extraction. Mid-depth (40-50 cm) of BCMCA stations included. Error bars represent ±1 SD.

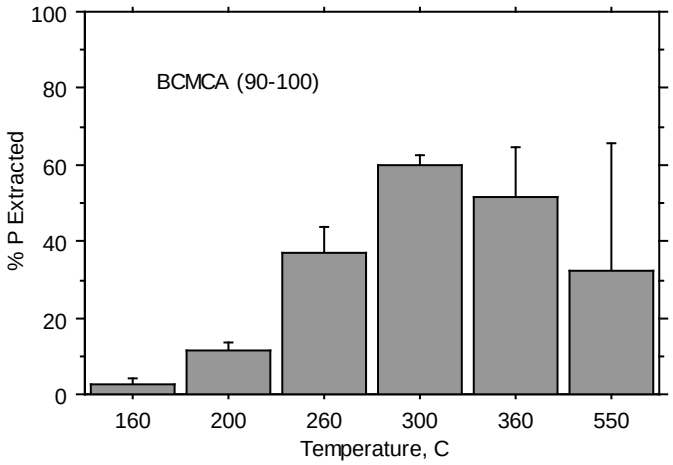

Figure 17. Effect of temperature on P extraction of peat samples. Lower depth (90-100 cm) of BCMCA stations included. Error bars represent ±1 SD.

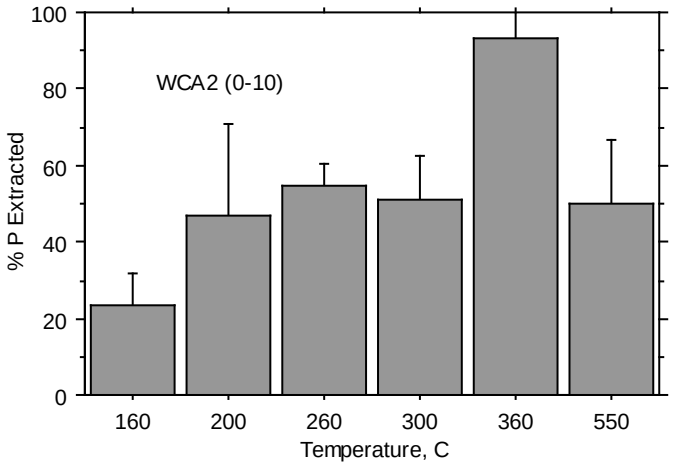

Figure 18. Effect of temperature on P extraction of peat samples. Surface (0-10 cm) of WCA2 stations included. Error bars represent ±1 SD.

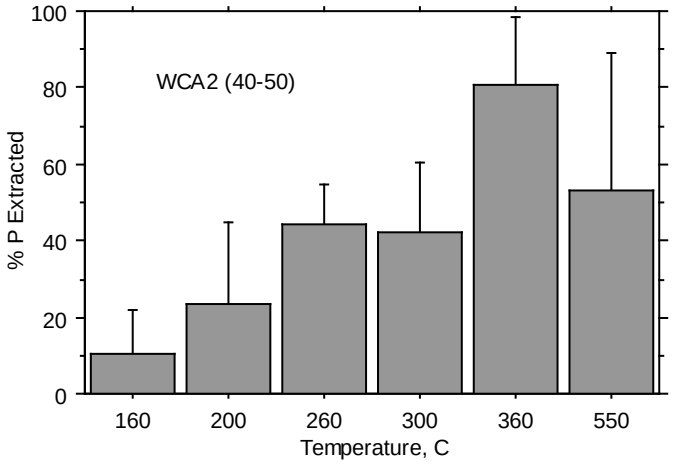

Figure 19. Effect of temperature on P extraction of peat samples. Mid-depth (40-50 cm) of WCA2 stations included. Error bars represent ±1 SD.

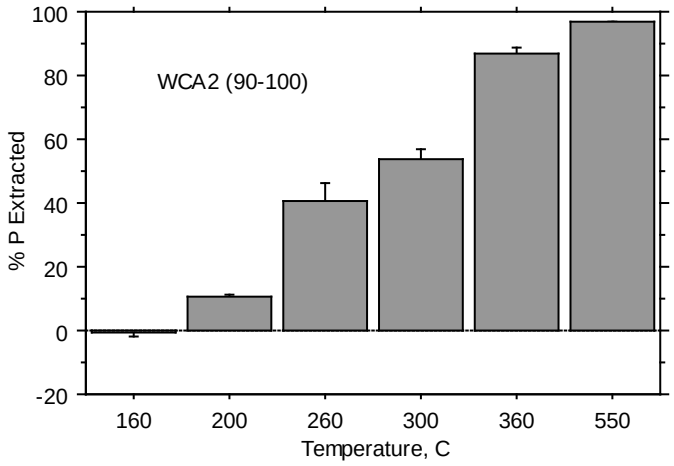

Figure 20. Effect of temperature on P extraction of peat samples. Lower depth (90-100 cm) of WCA2 stations included. Error bars represent ±1 SD.

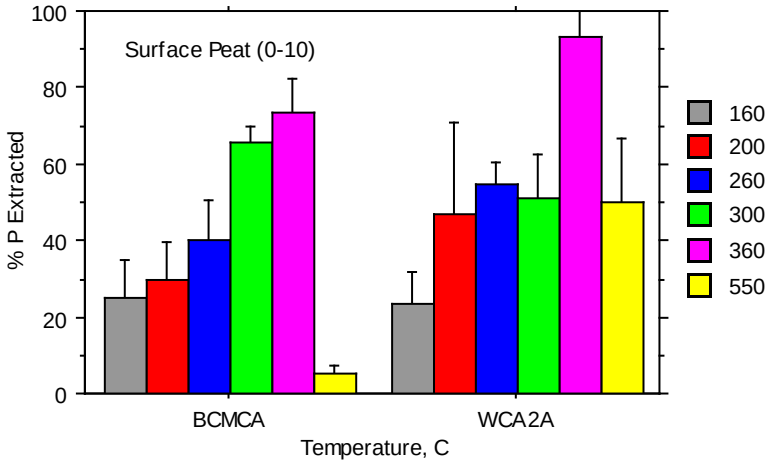

Figure 21. A comparison of pyrolysis-extractable P between BCMCA and WCA2 for the surface (0-10 cm) soil depth. Error bars represent ±1 SD.

6 Task 4: **Enzymatic Hydrolysis of Soil Organic Phosphorus**

6.1 Methods

Organic P that is biologically available via extracellular enzymes was determined using phosphatase enzymes. Soils from only the unimpacted sites in WCA-2A and BCMCA were used in this study. For a description of sampling methods and location, see **Section 2.1.1**. This experiment was conducted on February 13, 2003. One-half gram of oven-dried equivalent fresh soil was weighed into 50 ml centrifuge tubes. Each enzymatically treated tube was amended with 5 mls of modified universal buffer (MUB) containing 25 enzyme units. This enzyme concentration is high, and represents enzyme non-limiting conditions. The enzyme was purchased from Sigma-Aldrich Co. (St Louis, MO), catalog no. P-8361, lot no. 062K1359. The enzyme was alkaline phosphatase monoesterase obtained from recombinant bovine, at a concentration of 20.6 mg protein per milliliter. The enzyme was added to diluted stock modified universal buffer (MUB). Stock MUB consisted of the following, diluted in 1 L of deionized water:

1. 12.1 g THAM
2. 11.6 g maleic acid
3. 14 g citric acid
4. 6.3 g boric acid
5. 488 mls 1N NaOH

Two hundred milliliters of the stock MUB was diluted to 500 mls, and this was pH adjusted with 0.1N NaOH to pH 10, the optimum for this enzyme. The activity of the MUB + enzyme solution was qualitatively examined on a fluorometer and high activity was confirmed.

One set of samples was treated with the MUB + enzyme, and a duplicate set was treated with MUB only. Eight samples were incubated in triplicate to examine the reproducibility of the technique. All samples were incubated at 30^{o}C for 4 hours and then extracted with 0.5M $NaHCO_3$ (pH = 8.24) for 30 min. on a reciprocating shaker. samples were then directly filtered, without centrifuging, through 0.45 μM polyethersulfone

filters. Enzymatically extractable P was determined by difference between the MUB and MUB + enzyme samples.

6.2 Results and Discussion

The amount of P that was enzymatically extractable was very low (Fig. 22). There are several possible explanations for this. The microbial community at both stations are functioning in a P-limited environment, though less so at BCMCA station B4. This would have the effect of eliciting enzyme production in those organisms capable of producing it. The small pool of organic P that could be hydrolyzed by the added phosphatase may thus have already been exposed to the native phosphatase, thus reducing the pool that was susceptible to this enzyme . Another possible explanation is that the dominant form of organic P in these wetlands was diester-P, and therefore was not hydrolyzable with the monoesterase used in this study.

Precision of the technique was good. Note that figure 23 essentially shows the quantity of P that was extracted with the 0.5 M $NaHCO_3$, since the amount of P that was extracted via enzyme is the difference between the two plots.

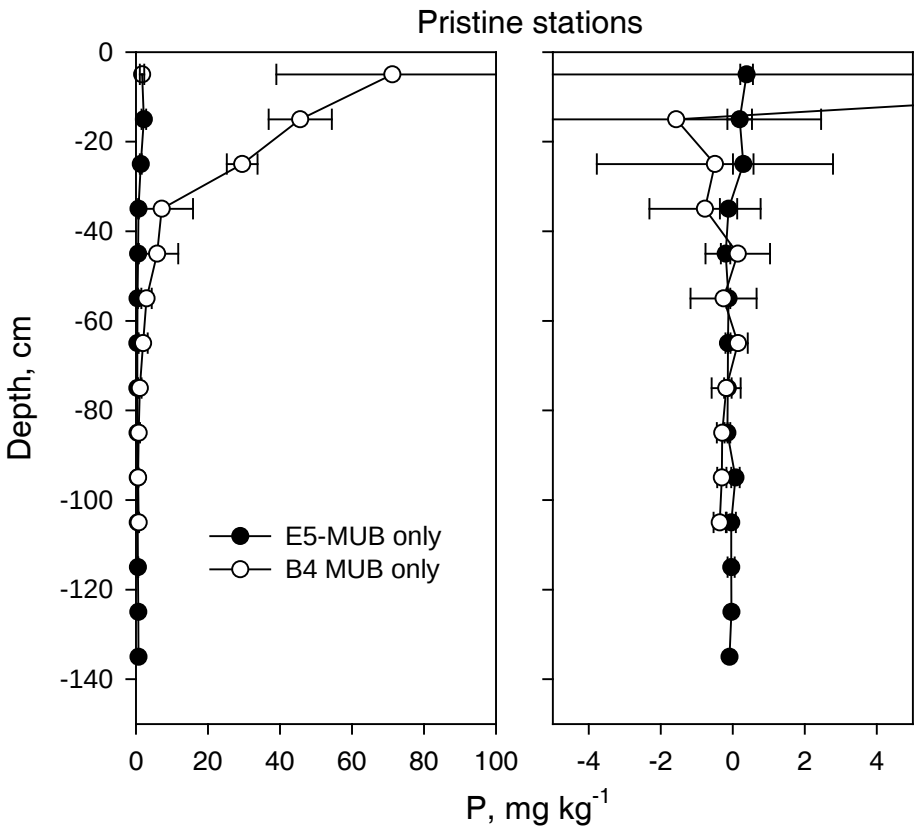

Figure 22. Phosphorus liberated with the 0.5 M $NaHCO_3$ for the MUB-only samples (left panel) and the net release of P due to the enzyme (right panel). Error bars represent ±1 SD.

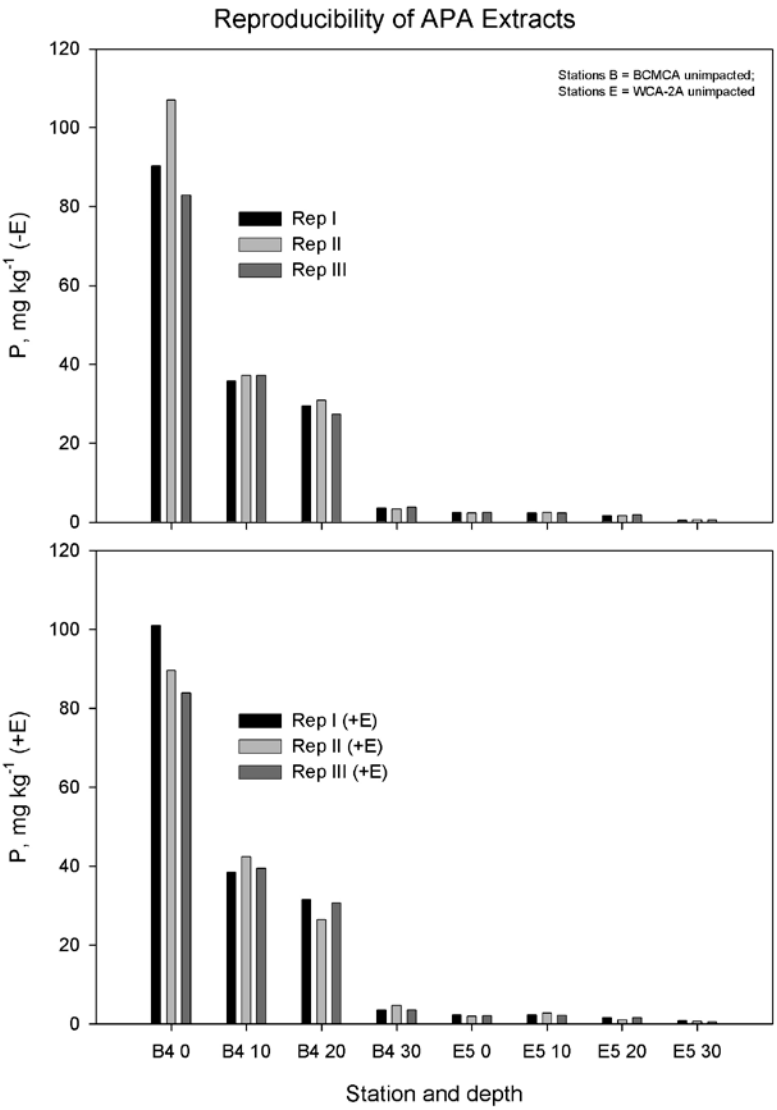

Figure 23. Reproducibility of the enzyme extracts.

7 Task 5: Potentially Mineralizable Phosphorus (PMP)

7.1 Methods

The soil samples used in this experiment were subsamples of those used in Task 1. For a description of sampling methods and location, see **Section 2.1.1**. The stability of organic P was estimated using a short-term anaerobic incubation. Soil depths of 0 – 10, 40 – 50, and 90 – 100 cm were used for the following extraction. The wet soil equivalent of a 0.5 g dry wt. sample was placed into a 50-mL glass serum bottle and 5 mL of distilled deionized (DDI) water was added. Simultaneously, a duplicate set of samples was placed into 50-mL centrifuge tubes for immediate extraction (see Time O Control Extraction below). All bottles were sealed with a butyl rubber stopper and aluminum crimp tops. The bottles were evacuated and purged with O_2-free N_2 gas for 2-5 min. They were then placed into an incubator on September 10, 2002 (in the dark) at $40^oC \pm 2^oC$ for 10 days.

Twenty-five mL of 1.0 *N* HCl was added to the time=0 tubes and they were then placed into a reciprocating shaker for 3 hr. The samples were centrifuged for 10 min. at 6000 rpm and filtered through 0.45-μm polyethersulfone membrane filters into polyethylene scintillation vials.

7.2 Results and Discussion

The amount of P that could be mineralized under the experimental conditions declined at all stations with increasing soil depth. This indicates increasing stability of the organic matter, as a function of soil depth, or age. Mineralizable P ranged from 13% of total P in the surface soil at BCMCA station B4, to approximately 2% in the 90-100 cm depth at the two WCA2 stations. PMP was greater in BCMCA, again suggesting that this marsh is not as P-limited as the Everglades.

As with the results from the previous tasks, there are several possible explanations for declining P levels with soil depth (Fig. 23). It is possible that the material comprising the peat in the deeper soil layers is derived from different original plant material. For instance, it is possible that there have been major changes in the plant community over the history of the marsh. The peat at a depth of 1-m may be derived from a plant community with a higher proportion of upland plants, established during a drier climatic

regime. These plants could be expected to higher in lignin, and therefore more resistant to degradation. A more likely explanation is that the plant community has been mostly constant, and the changes in P chemistry reflect diagenetic changes in organic matter composition. These post-depositional changes of plant material need to be incorporated into models that attempt to describe ecosystem nutrient loading history. Without this information, there will almost certainly be an underestimation of historical nutrient loading rates.

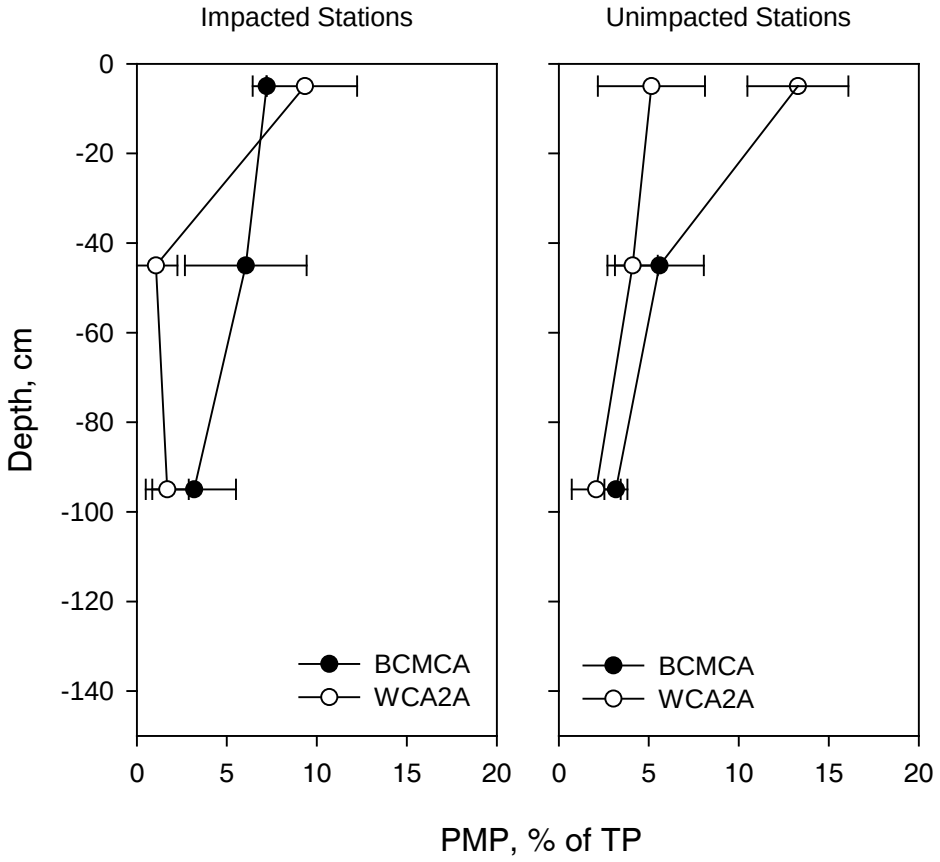

Figure 23. Phosphorus mineralized during a short-term laboratory incubation. Error bars represent ±1 SD.

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