Long Term Burial of Phosphorus in Wetlands of the Upper St. Johns River Basin

Phase II

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Executive Summary

Nutrient enrichment threatens the ecology of lakes and wetlands that are adapted to low-nutrient conditions. These changes include broad changes in biological communities, and in some cases, lower overall species diversity. It is therefore critical to determine nutrient loading rates that do not exceed the natural assimilative capacity of the receiving water.

One approach to determining this rate is to compare the nutrient content of surface wetland soils (or lake sediments) to the nutrient content of soil horizons that predate human development. The ratio of these two values is often taken as evidence for the extent to which nutrient loading has increased in the last century. This approach has been used to document increases in nutrient loading in many aquatic ecosystems, including the Upper St. Johns River Basin. However, biochemical processes transform soil organic matter and the nutrients associated with it. These gradual changes need to be considered when comparing soils deposited over broad time scales.

This contract represents Phase II of contract SE157AA. Phase I described the changes in soil phosphorus over a several thousand-year period. This report documents changes in soil organic matter over the same period.

Several approaches were used to characterize the progressive changes that soil organic matter undergoes in Blue Cypress Marsh Conservation Area. They were:

1.) Fractionation of phosphorus into organic and inorganic pools,
2.) Extent of soil humification using fluorometric techniques,
3.) Extent of soil humification using light absorption techniques,
4.) Characterization of classes of organic phosphorus compounds using nuclear magnetic resonance (^{31}P-NMR),
5.) Characterization of classes of organic carbon compounds using nuclear magnetic resonance (^{13}C-NMR) and isotopic analysis of soil lignin.

Organic forms of phosphorus were found to comprise up to 95% of the total phosphorus pool in the soils of BCMCA. Thus it is important to focus on and understand

the biogeochemistry of this phosphorus fraction. The pattern of fluorescence of extracted soil organic carbon suggests that soil organic matter undergoes biochemical conversion to more humified compounds that are progressively less similar to the original plant material. The absorbance data (E4:E6 ratio) indicate an average molecular weight of approximately 2000 daltons. There was a slight increase in molecular weight at the central marsh station with respect to depth, suggesting gradual increase in molecular size with time. This trend was not as clear at the nutrient impacted station. Results from ^{13}C-NMR analysis showed a decline in plant polymers such as lignin and cellulose, and an increase in fatty acids, waxes, and aromatic compounds with increasing soil depth. ^{31}P-NMR results showed increasing organic P with respect to depth and approximately equal monoester and diester forms of P. This is in sharp contract to upland soils where monoester P predominates. Isotopic composition of lignin and carbon-14 dates suggest a major change in plant communities at approximately 1000 AD.

Results from this study suggest that P does not behave as a conservative compound. It is subject to the same biogeochemical processes that dramatically alter soil organic matter. These processes act to transform the original floral and faunal material into inorganic and organic compounds that are progressively less and less similar to the original material. Comparative studies that contrast surficial peat soils to deeper strata need to incorporate the effects that soil forming processes have had on soil properties, particularly in the case of organic soils. This is especially true of studies that base inferences of historical rates of ecosystem nutrient loading on a comparison between surface soils and their deeper counterparts.

1. Introduction

Nutrient enrichment is the third largest cause of impairment to United States surface waters (USEPA, 1998). In Florida, 35% of large lakes do not meet their designated use due to excess nutrients (FDEP, 2004). Eutrophication of historically low-nutrient adapted lakes and wetlands alters trophic structure, favoring species that are better 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 wastewater 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 type, are major determinants of a region's suitability to a crop and the yield of that crop. In a similar manner, soil nutrient status plays a fundamental role in the composition of natural ecosystems. When nutrients are scarce, as in many 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 may be perceived as 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.

The goal of Phase I of this study (FY 2001-2002) was to characterize P according to its recalcitrance and to examine recalcitrance as a function of age and wetland nutrient status. Soil depth is analogous to age; characterizing organic P pools by depth therefore provides insights into how organic P stability changes with respect to time. Also, since a large body of information has been developed for the role that Everglades soils play in determining ecosystem composition, establishing commonalities between the two ecosystems may allow for some direct extension of the Everglades research to the USJRB.

During Phase I, a fractionation scheme that was developed for characterizing organic soil P was applied to soils obtained from Blue Cypress Marsh Conservation Area (BCMCA) and Everglades Water Conservation Area 2A (WCA-2A). Three other organic P extraction techniques were also used: autoclave, enzymatic, and a standardized 10-d P extraction technique. Results showed that the soils of the BCMCA are more enriched with P, at both surficial soil depths and much deeper (>70 cm) than soils in the Northern Florida Everglades. Enriched surface horizons reflect the effects of recent P loading, while greater depths reflect historical conditions. This new data suggests that BCMCA has historically been a higher nutrient status wetland than the Everglades and therefore P loading limits established for the Everglades may not be appropriate for BCMCA. Phase II (this contract) will help to understand the extent to which physical and chemical changes alter soil organic matter as decomposition proceeds.

1.1. Effects of Diagenesis on Estimation of Phosphorus Accretion Rates

Previous research on BCMCA soils indicated recent changes in P accretion and attributed these changes to changes in ecosystem P loading (Brenner et al., 2001). These studies are partly based on the assumption that change in soil P content due to diagenesis is minimal. However, P storage in soils (especially peat) is present in chemical compounds of varying degrees of environmental stability. For instance, Reddy et al. (1998) has 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 percent of total dry weight for standing dead plant litter, to 50 percent for 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 vegetation succession from woody shrubs, to a wetland dominated by soft-tissued herbaceous plants. However, a more likely explanation is the relatively higher loss of the more labile fractions and 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 useful in determining pre-impact P loading rates to these ecosystems.

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 BCMCA. 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 experimental controls in that chronological (depth) changes in P fractions could be expected to be singularly due to the effects of OM diagenesis.

Historical (ca. early 1900's) rates of nutrient accretion can also be used to calibrate lake and wetland nutrient loading models. Mass-loading, or input-output models often include a loss, or settling term (Kadlec and Knight, 1996; Lowe and Keenan, 1997; Walker, 1995; Vollenweider, 1975). Models such as these are currently used in the USJRB to estimate nutrient transport through lakes and wetlands. The settling coefficient reflects that mass of P that is not transported, i.e. it is lost to the sediment. Input-output models are sensitive to this parameter, therefore it must be chosen carefully. The settling coefficient can be selected from the literature, or it can be experimentally determined. Theoretically, it should be possible to examine soils from a depth (or age) that predates

European settlement, derive a settling rate, and determine a pre-developmental nutrient loading target, since:

$$\frac{\partial M}{dx} = \frac{\partial M}{dt} \text{ if } \partial x = f(t)$$

where M = mass of P at a given soil depth, x = soil depth, t = time

A further assumption is that:

$$\frac{\partial M}{dt}(\text{water}) = \frac{\partial M}{dt}(\text{sediment})$$

This provides the linkage between the settling coefficient in models such as Vollenweider's (1975) and sedimentary P accretion rates.

While this is true at the time of deposition at the sediment water interface, it is less true with time, as soil biogeochemical processes gradually alter soil properties. With time, soil forming factors transform the original plant detrital materials to fibric, hemic, and sapric peat and, given sufficient time, to coal. There is little reason to expect that phosphorus, especially organic P, escapes these transformations, i.e. that it behaves conservatively. Any transformations that involve conversion to soluble organic or inorganic P compounds would likely cause a decline in P at lower soil depths due to diffusional processes, especially in a P-limited ecosystem.

1.2. Characterization of Soil Organic Matter

The development of characterization schemes for organic phosphorus is based partly on efforts to characterize soil organic matter. Late in the 18th century, scientists extracted peat with alkaline extracts and obtained a dark fluid that formed a precipate upon acidification, which was termed "humic acid" (Stevenson, 1994). They observed that more humic acid could be extracted from deeper, older peat. By 1900, it was widely recognized that "humus" was a complex mixture of organic substances that were mostly colloidal in nature and had weakly acidic properties. It was also recognized that soil organic matter was comprised of over 40 compounds in the broad categories of organic acids, hydrocarbons, fats, sterols, aldehydes, and carbohydrates. Early 20th century researchers argued that the terms "humic" and "fulvic" acids should be abandoned, because "these labels designate…certain preparations which have been obtained by specific procedures", rather than identifying specific compounds. Though as Stevenson

(1994) points out “reference to the acid-insoluble material as humic acid is considerably less cumbersome than repeated reference to “alkali-soluble, acid-insoluble fraction””. In fact, many researchers have abandoned the term “humic” materials entirely and refer to this group of compounds as “advanced glycation end-products (AGE’s)”. Soil humus contains most, if not all, of the of the biochemical compounds synthesized by living organisms and many additional compounds that are largely the product of non-biotic secondary synthesis reactions that occur in the soil environment. They are thus dissimilar to the biopolymers of microorganisms and higher plants. Less than 50% of soil organic P compounds have been identified. Identification of the large number of compounds that comprise the organic matter pool (and thus organic P pool) will therefore likely be restricted to identifying classes of compounds based on chemical, biological, or environmental properties.

Organic matter characterization typically involves solubilizing the organic matter with an extracting solution, typically alkaline. Strong bases, such as 0.1 M NaOH, typically extract 80% of the organic matter present. The degree to which organic P is hydrolyzed to simpler molecules (such as the ortho-P monomer) varies and depends in part on the exposure time of the extractant to the solubilized OM. Thus extraction time (and sample storage time) play a large role in P recovery results. The acid soluble fraction of soil OM is composed of amino-sugars, amino-acids, and sugars, whereas organic solvents are necessary to solubilize fats, waxes, and resins.

For this study, we used the following approaches to identifying the organic matter that comprise the peat deposit.

1.) The ratio of absorbance of at 465 and 665 nm, or E4:E6 ratio was used to estimate changes in molecular weight of extracted soil organic matter. Increasing molecular weight has been indicated as a consequence of soil aging and humification.

2.) The pattern of soil extract fluorescence was also used to determine the extent of soil humification, the so-called humification index, or HIX. Soil organic matter contains chromophores that absorb light energy, and re-radiate it at wavelengths other than the incident wavelength.

This re-radiated, or fluoresced light depends on the extent of soil humification.

3.) Changes in the depth distribution of broad groups of soil organic carbon compounds was investigated using solid state ^{13}C nuclear magnetic resonance (^{13}C-NMR).

4.) Solution ^{31}P-NMR was used in a similar way to examine diagenetic changes in soil organic phosphorus.

5.) The preservation of the principal plant polymers, lignin, cellulose, and hemicellulose was determined, as well as the ^{13}C and ^{15}N content of this material. This was used as an indication of changes in floral community composition.

6.) Lastly, intact plant macrofossils (i.e. seed husks, stems) were dated using ^{14}C to establish the chronology of biogeochemical changes investigated here.

1.3. Previous Studies of Soil Nutrient Status in BCMCA

Initial characterization of phosphorus levels in BCMCA soils was performed by Brenner (2001). They conducted their field sampling in September 1992 and found surface soils in the northeast corner of BCMCA (near break in levee; station 3A and 3B) to have very elevated levels of P; approximately 2600 mg kg^{-1} for the surface 0-6 cm layer. Olila and Reddy (1995) returned to this station in winter of 1994 and found lower levels of soil P, approx. 2000 mg kg^{-1} for the same depth interval. Brenner (2001) used this soil P content, coupled with estimates of soil accretion rates from ^{210}Pb studies, to calculate a contemporary P accretion rate of 0.113 mg P cm^{-2} yr^{-1}. Estimated P accretion rates at other less impacted stations were approximately 0.01 to 0.02 mg P cm^{-2} yr^{-1}. These recent rates of P accumulation were compared to historical rates (0.002 – 0.004 mg P cm^{-2} yr^{-1}) to find that at most marsh stations, P accumulation has dramatically increased, from 2 – 17 times greater than accumulations ca. 1920. Brenner (2001) points out that the apparent increase in P accumulation may be due in part to artifacts such as post-diagenetic movement of P through the soil profile.

1.4. Site Description and Physical Characteristics of BCMCA Soils

The study location was Blue Cypress Marsh Conservation Area, a 12,000 ha peat marsh (Fig. 2.1). The eastern extent of the marsh is characterized by open-water sloughs and tree islands. Typical vegetation in the sloughs is a *Utricularia sp./Nymphaea odorata* community, while the tree islands are characterized by *Taxodium distichum*/*Acer rubrum/Salix caroliniania.* community. The western region of the marsh is predominately a *Cladium jamaicense/Panicum hemitomon* prairie, with some *Cephalanthus occidentalis, Pontederia cordata,* and *Sagittaria lancifolia.*

The soil at the site consists of well-decomposed peat (Terra Ceia series, Euic, hyperthermic Typic Haplosaprists) ranging from 2-m thick on eastern side to over 5-m thick in the marsh's center. The peat is underlain by coarse sand on the east, and sandy clay in the center. The soils are highly organic, with typical loss on ignition of 94% (Table 1.1). Surface water in the marsh is characterized as soft-water and slightly acidic, with pH of approximately 6.5. Nutrients are low, though not as low as the Everglades, with median phosphorus concentration in the central region of the marsh of 50 $\mu g\ L^{-1}$. The focus of this study is on two stations in BCMCA, stations C1 and B4 (Fig. 1.1). Station C1 is located in a region of the marsh that was impacted by nutrient discharges from farms located immediately north of the marsh. This discharge lead to increased soil P concentration, as well as encroachment of cattails (*Typha latifolia*). Station B4 is located near the marsh's center and is relatively unimpacted by nutrient loading.

The hydrology of the marsh is highly constrained by a network of water control structures. Inflows are primarily from rainwater, a small stream on the western side (Padgett Branch), and three water control structures on the southern side. Outflows occur primarily through the water control structure, S-96C, on the northern end of the marsh and to a lesser extent through the S-250 culverts. The marsh surface elevation is approximately 7-m above mean sea level (MSL). Water level in the marsh typically varies from 7-m (dry) to 9-m MSL.

Table 1.1. Basic physico-chemical properties of soils collected in March 2004 from BCMCA. Each row represents the average of five 10-cm soil samples. Values in parentheses represent one standard deviation.

Depth	LOI	Bulk Density	Total-C	Total-N	Total-P
	%	g cm^{-3}	g kg^{-1}	g kg^{-1}	mg kg^{-1}
Station B4					
0-50	95 (± 1)	0.079 (± 0.017)	501 (± 29)	32 (± 3)	267 (± 97)
50-100	94 (± 2)	0.114 (± 0.019)	518 (± 12)	27 (± 1)	119 (± 29)
100-150	95 (± 2)	0.106 (± 0.015)	541 (± 10)	26 (± 3)	59 (± 7)
Station C1					
0-50	93 (±3)	0.097 (±0.011)	492 (±27)	32 (±3)	367 (± 251)
50-100	94 (±2)	0.097 (±0.022)	531 (±17)	26 (±2)	80 (±37)
100-150	78 (±18)	0.109 (±0.054)	434 (±106)	24 (±6)	59 (±10)

2. Fluorometric Signature of Extracted Organic Matter

2.1. Introduction

Natural dissolved organic matter and extracted soil organic matter contain molecules that are capable of absorbing light energy and promoting an electron to an excited state. Upon returning to the ground state, the molecule emits light, i.e. it fluoresces. The pattern of fluorescence depends on the nature of the organic molecule. It has been shown that as organic matter ages, the fluoresced light is longer in wavelength, or red-shifted. By taking the ratio of the intensities of the higher fluorescence wavelengths to the lower wavelengths, the extent of humification can be approximated (Cox et al., 2000). This index can be used as a measure of the extent of soil diagenesis.

2.2. Methods

Samples were collected from BCMCA at stations C1 and B4. These are the same locations as used in Phase I of this project. Samples were collected by

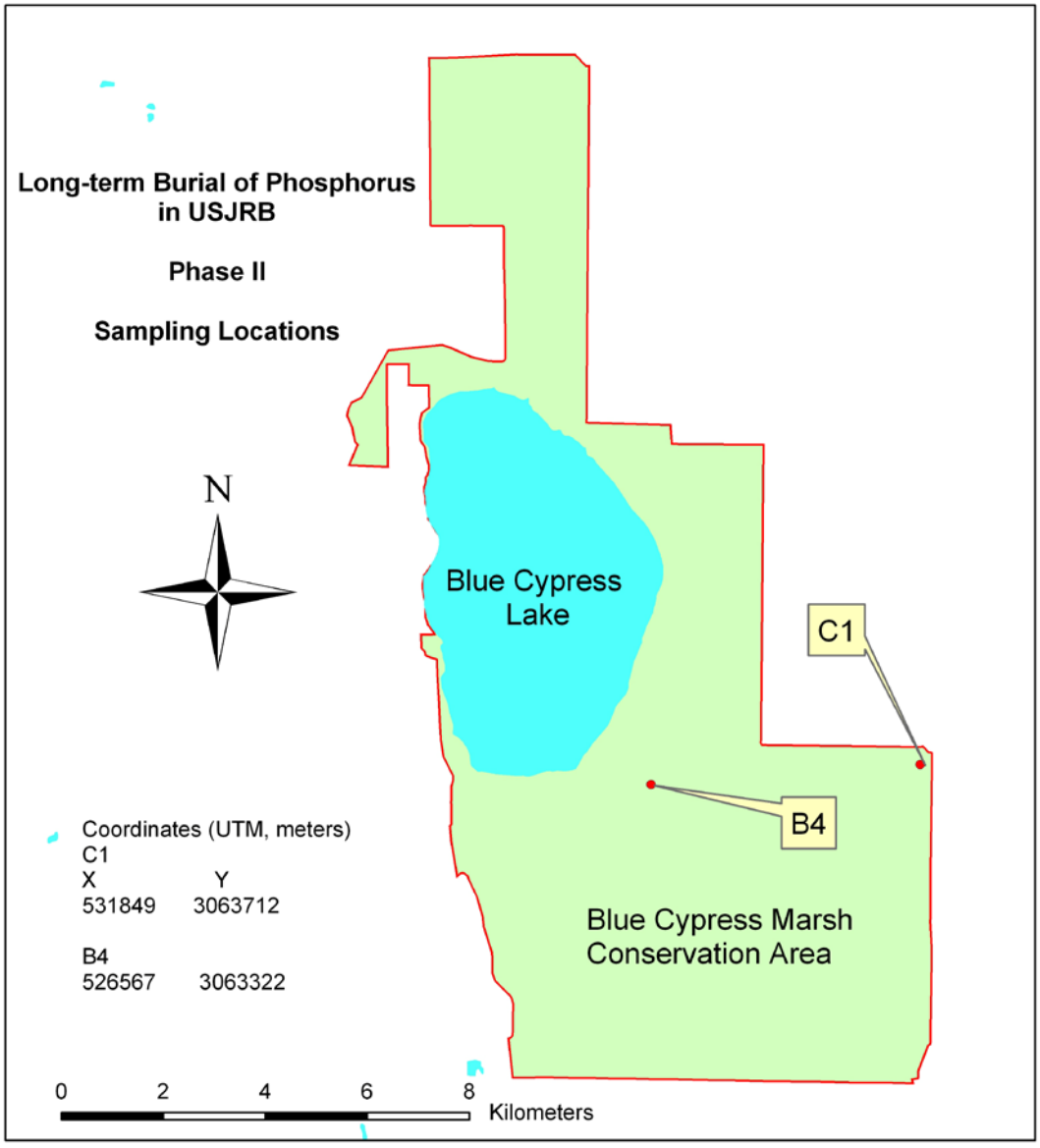


Figure 1.1. Location of sampling stations used in Phase II of the Phosphorus Burial project.

Dr. Benjamin Turner (Smithsonian Tropical Research Institute) and Matt Fisher (SJRWMD) on March 16, 2004. A Clark corer was used to retrieve 2 7/8" inch ID soil cores. This corer is a piston-type corer with a sharpened steel leading edge. Handles on each side allow the device to be rotated, which facilitates cutting through roots and peat fibers. The corer is constructed of steel and employs a thin wall, semi-rigid, polybutyrate core liner. This liner is removed after the core is removed from the ground. The butyrate tubing is sufficiently thin that it can be easily cut with a razor knife. Four cores were collected from each station; three for fractionation and spectral properties of organic matter extracts, and one for lignin fractionation, stable isotope analysis, and ^{14}C analysis. Total core length ranged from 105-cm to 150-cm. All cores consisted of peat or muck.

Soils were sequentially fractionated using 1M HCl extraction to remove inorganic C. This was followed by a 0.5 M NaOH extraction for 17 hours on a reciprocating shaker at room temperature on March 30, 2004. Twenty-five mls of extractant solution was added to approximately 0.5 g of oven-dried soil, for a 1:50 extraction ratio. This solution was filtered through 0.45μm polyethersulfone (PES) filters, diluted 1000X, and pH adjusted to 7 using a 0.05 M $NaHCO_3$ solution that had been pH adjusted to 6.7. This dilution resulted in an optical density at 254 nm of approximately 0.15 (1 cm pathlength). This optical density has been shown to reduce both inner filtering of fluoresced light, and to diminish absorption by the color of the extract (Ohno, 2002).

A subsample of the NaOH extract was acidified to pH<2 to precipitate humic materials, leaving only the acid soluble fraction, or fulvic acid in solution. This was done by adding 5 drops of concentrated sulfuric acid to five mls of NaOH extract. The precipitated humic acid was separated from the supernatant by centrifuging. Fluorescence properties of both samples were determined. A subsample of each soil extract solution was analyzed for phosphorus using EPA Method 365.1.

Fluorescence was determined with a Shimadzu model RF1501 (Shimadzu Corp., Tokyo, Japan) scanning fluorometer during the period May 11, 2004 through May 18, 2004. Fluorometric scans were conducted with an excitation wavelength of 254 nm and a fluorescence emission scan of 260 through 650 nm. Sample vessel consisted of a 1-cm quartz cuvette. The humification index (HIX) was calculated as:

$$HIX = \frac{\sum_{\lambda=435}^{480} FI}{\sum_{\lambda=300}^{345} FI}$$

where FI = fluorescence intensity (Cox, et al. 2000).

2.3. Results and Discussion

2.3.1. Phosphorus Fractionation

Total phosphorus was greatest in surface soils from station C1, as has been cited in previous reports. Total-P at station C1 for the 0-10 cm depth interval averaged 767 mg kg^{-1}, compared to 428 mg kg^{-1} for the surface interval at interior marsh station B4. If it is assumed that the un-extractable portion of soil P is of an organic nature, and that the HCl extraction removed all of the inorganic P, then approximately 95% of soil total P at these two stations is incorporated into organic matter (Fig. 2.1). Thus it is critical to understand processes responsible for cycling of organic matter in order to predict fate of phosphorus in wetlands such as BCMCA.

The un-extractable P fraction comprised approximately 25% of total P at the soil surface, and increased to 40% at a depth of 150 cm. Little is known of the properties of the organic matter (and organic P) that escapes removal by the alkaline extract, except that it is likely refractory. Inorganic P is that P that is associated with iron, aluminum, calcium and magnesium soil minerals, such as gibbsite and goethite. It is also associated with non-crystalline inorganic compounds. This fraction is lower at the surface than inorganic P of Florida Everglades soils, declining from approximately 10% at surface horizons, to 5% at 150-cm depth. BCMCA is a soft water, low calcium system, therefore this fraction would be expected to be relatively low.

Even though total P at station C1 was approximately double that of the interior marsh station, the overall distribution of P in the four fractions investigated here was quite similar between the two stations. There was no trend in distribution of fulvic and humic P with respect to depth. Their proportions were approximately equivalent, each fraction comprising approximately 30% of the total P. A detailed fractionation was

performed in Phase I of this project and the reader is referred to those results for more information on phosphorus fractionation.

2.3.2. Organic Extract Fluorescense

The HIX of the peat extracts showed a steady increase throughout the soil profile (Fig. 2.2). Extracted, live plant tissue had a HIX of approximately 2, detritus 4, whereas peat samples at a depth of 140 cm increased to 10. Both stations had very similar increases with respect to depth, though station C1 had a reversal (declining HIX) at 80 cm. This reversal coincided with a change in physical properties of the peat. At a depth of approximately 80 cm, the texture of the peat at C1 changed from a very decomposed, black muck, to a reddish-brown fibric peat immediately underneath. This more fibric, less decomposed peat likely caused the decline in HIX at station C1.

There was no significant difference in average HIX between the two stations for the humic + fulvic samples. (One-way ANOVA; $F = 1.45$; $p = 0.232$; MiniTab). This is to be expected, since both stations have been exposed to the same hydrologic regime, and must have accreted peat at a similar rate. Fulvic acid extracted from the soil samples showed very similar trend of increasing humification with respect to depth (Fig. 2.2). In general, the whole NaOH extract (humic + fulvic) had an average HIX value approximately one unit higher than the fulvic sample.

The sudden increase in HIX at station C1 (70-90 cm) coincides with a dramatic increase in OM age. Station B4 also showed local maxima at approximately this same depth. One possible reason for this increase is accelerated decomposition during a long dry period in the marsh (see section 6.3.2). Both stations show increase in HIX at this depth, followed by a slight decline (less decomposed), then a gradual increase to the base of the core.

3. Spectrophotometric Properties of Extracted Organic Matter

3.1. Introduction

The ratio of absorbance at 465 and 665 nm has been shown to be related to the average molecular weight of compounds in solution (Chen, et al., 1977). The objective of

this experiment was to determine if biogeochemical changes in peat lead to changes in molecular weight. It is possible that mostly abiotic chemical reactions are producing humic compounds of progressively greater molecular size, and weight.

3.2. Methods

Spectrophotometric properties were determined with a Shimadzu model UV160 spectrophotometer (Shimadzu Corp., Tokyo, Japan) on April 9, 2004. Absorbance was determined at 465 and 665 nm in a 1-cm path length, flow-through cell. Analyses were performed on the same 0.5M NaOH extract as used for the fluorometric analyses, though these samples were diluted only 40X and adjusted to pH 7 with dilute HCl. This dilution resulted in average absorbance at 465 nm and 665 nm of 0.25 (±0.18 SD) and 0.04 (±0.03 SD), well within the measurement range of the instrument. The ratio of the absorbance at these two wavelengths is reported here; i.e. the E4:E6 ratio.

3.3. Results and Discussion

The E4:E6 ratios observed in this experiment indicate that the soil extract is richer in fulvic than humic acids at both stations for all depths. The ratio E4:E6 ratio of fulvic acid in most soils is typically in the range of 6.0 to 8.5, whereas humic acids tend to be less than 5 (Stevenson, 1994). Chen at al. (1977) found that this range corresponded to fulvic acids with an average molecular weight of approximately 2000 atomic mass units (amu; or daltons). For this study, an average weight of 2000 amu corresponds to the complete soil extract containing both humic and fulvic materials.

The interior marsh station (B4) showed a slight decline in E4:E6 ratio, beginning at approximately 12 in the 0-10 cm soil depth, declining to 7 at a depth of 80 cm, followed by very little change to the base of the core (Fig. 3.1). This suggest relatively constant rate of diagenesis for the surficial 70-cm of peat, with an increase in molecular weight of the extracted organic matter to 70 cm. The E4:E6 ratio at the nutrient impacted station showed a slight decline to 50 cm, increased to a depth of 100 cm, then declined to the base of the core.

The E4:E6 profile at station B4 is characteristic of the diagenesis of a relatively constant source, or quality, of organic matter. The ratio at the soil surface is high, reflecting lower molecular weight, poorly humified organic matter, as would be expected

from recently deposited plant materials. The ratio suggests increasing molecular weight with respect to soil depth to the base of the core, as would be expected from the progressive humification of a constant source of organic matter input. The gradual decline therefore implies that this region has experienced a similar history of vegetation over the time period that this soil profile represents.

The E4:E6 profile at station C1 does not show the same constant decline as station B4. The E4:E6 ratios at station C1 are lower than station B4 for soil depths less than 70 cm, and greater for lower depths. Lower ratios in surface depths (than B4) may be a consequence of two factors; vegetation history and root biomass. Soil organic matter with greater molecular weight at station B4 for soil depths < 70 cm may reflect changes in vegetation community in this area of the marsh. This region of the marsh is known to have experienced nutrient impacts that have altered the vegetation in the northeastern corner of BCMCA. A result of this impact is the conversion of slough to cattail (*Typha latifolia*) and willow (*Salix caroliniana*) marsh. Aquatic plants that characterize slough vegetation include such plants as *Nymphaea spp., Utricularia spp.* phytoplankton, etc. These plants contain less structural tissue, such as lignin, than the emergent vegetation and shrubs mentioned above. Lignin is one of the principal refractory biogenic compounds and is thought to be a precursor of humic materials (Stevenson, 1994), and humic content is a major determinant of the E4:E6 ratio. The nutrient impact may have, by altering the local plant community, lead to alterations in the biochemical characteristics of the recent peat in this area of the marsh. A second cause of greater E4:E6 ratios at station C1 may reflect greater contribution of live root biomass to the carbon pool of the soil extracts. *Panicum hemitomon* is the dominant plant at station B4. Many small roots were noted in the samples during core sectioning, many more so than at station C1, which was dominated by *Typha latifolia* and *Salix caroliniana*. This new carbon would have consisted of lignin, cellulose, and other polysaccharides of much lower molecular weight than humic materials and may have biased the E4:E6 ratio upwards in the surface soil horizons relative to station C1.

Results from E4:E6 ratio study were mixed. At neither station was a dramatic increase in molecular weight with increasing depth observed. In fact, at station C1 very little overall change with respect to peat age was seen. This suggests that peat soil

diagenesis does not involve dramatic changes in molecular weight of extractable soil components.

4. Solid-state ^{13}C-Nuclear Magnetic Resonance (NMR) Spectroscopy

4.1. Introduction

Soil carbon can be categorized based on solubility properties (humic vs. fulvic), on its association with living biomass (organic vs. inorganic), on structural properties, and by molecular identification. These last two classifications require advanced instrumentation, carbon-13 nuclear magnetic resonance and gas chromatography – mass spectrometry, respectively. Solid-state carbon-13 nuclear magnetic resonance (^{13}C-NMR) was used in this study to document changes in broad classes of soil organic compounds. It involves subjecting dry soil samples to an intense magnetic field and is thus minimally disruptive to the original soil sample. This information should help to describe the degradation of major plant-derived organic compounds.

4.2. Methods

The samples used for this analysis were from Phase I of this project. Samples were collected February 11, 2003 from station B4. Oven-dried (70^{o}C) and ground samples were sent to Dr. Sandy Chudek, at the School of Life Sciences Research Biocentre, University of Dundee, Scotland. Solid samples were analyzed at a spectrometer frequency of 75.5 Mhz, contact time = 1 msec, pulse delay = 2 sec, acquisition time = 2 sec, and accumulation of 4000 scans. Raw spectra are included in Appendix A.

Spectra were divided into four principal regions: carboxyls (benzene, carboxylic acid, amides and ethers), aromatics (phenols), O-alkyls (plant polymers such as lignin and cellulose), and alkyls (aliphatic, alkanes, fatty acids, and waxes). The fractions correspond to the spectra peak shift regions of 46-110 ppm, 110-162 ppm, 59-92 ppm, and 0-46 ppm, respectively.

The total area under each region was determined and its proportion in the whole sample was calculated by dividing by the total area of the spectra. It was assumed that the total area under the curve represented the total carbon of the sample. This value was

multiplied by the soil carbon content to determine the actual mass of each fraction in the original soil sample.

4.3. Results and Discussion

Total O-alkyls are associated with plant polymers, such as lignin and cellulose, and are considered relatively labile. This group represented the major fraction of soil organic carbon (SOC), comprising 30-40% of the total carbon pool, although they declined from 225 mg kg^{-1} at the surface, to a minimum of 133 mg kg^{-1} at a depth of 90 cm (Fig. 4.1) As a percentage of total C, these value are slightly higher than reported by Sjögersten et al. (2003) for forest and tundra soils. Concentrations declined throughout the soil profile, with the exception of a slight increase for the bottom two depth intervals. An inflection at 90-cm coincided with a textural change to peat characterized by much finer organic matter. The most likely explanation for this change is a period of greater water depths, favoring soft-tissued plants such as phytoplankton or floating macrophytes. These plants would have contained less refractory carbonaceous tissue (such as lignin), and thus would have been more rapidly mineralized. The more fibric materials underlying this strata visually appeared less decomposed, and this was reflected in an increase in O-alkyl concentration below 90-cm.

Alkyl-C compounds consist of fatty acids, waxes, and alkanes and includes compounds such as acetate, butyrate, and other fermentation end-products, as well as methane. Alkyls constituted the second greatest fraction, comprising 25-30% of the soil organic carbon pool. This fraction increased from 91 mg kg^{-1} at the soil surface, to a maximum of 191 mg kg^{-1} at a depth of 90 cm, or an increase from 20% of SOC, to 38%. This is slightly greater than observed by Sjögersten et al. (2003), probably due to increased production and storage of fermentation products in the flooded organic soils of this study, as compared to their tundra soils.

Total aromatic carbon increased linearly with respect to depth, showing a very similar concentration profile as alkyl-C, suggesting their accumulation at a similar rate. Total aromatics accounted for 20-30% of SOC. Baldock et al. (1997) reviewed the C-MNR results from organic and mineral soils, forest litter, and composts and found that the proportion of aromatic carbon compounds was not a particularly good surrogate for

extent of decomposition. However, their review did not include flooded soils. Organic wetland soils lack the fungi responsible for production of the extracellular enzymes that are capable of hydrolyzing aromatic compounds. This is likely the reason that the soils from BCMCA increase in aromaticity with depth, in contrast to results from Baldock et al. (1997).

The rate of increase in alkyl-C and total aromatic compounds with respect to depth in the soil profile was inversely related to the O-alkyl content, suggesting the biogeochemical transformation of plant polymers to microbial metabolites and resistant organic compounds. The ratio of alkyl to O-alkyl functional groups (A:O-A) has been shown to be a better indicator of the extent of decomposition, especially for organic soils (Baldock et al., 1997). The ratio increases steadily through the soil profile, with a dramatic increase in the region of silty organic matter (Fig. 4.2). The ratio begins at 0.4 at the soil surface, with a maximum value of 1.44 at 80-90 cm. Baldock et al. (1997) reviewed C-NMR data from numerous studies on mineralization of forest, wetland, and agricultural soils and found a range of A:O-A values, from 0.1 to 1.5, with the ratio depending mostly on degree of decomposition and secondarily to plant community and soil mineral content. The ratios found in this study are on the high end of this range, indicating advanced decomposition, especially at the lowest depths.

The steady increase in phenolic and aromatic compounds was very similar to the increase seen in the humification index (HIX) reported on in an earlier section of this report. Both techniques indicate a progressive transformation of plant components to more humified, aromatic, and presumably stable soil organic matter. This is a clear indication that plant litter that constitutes the bulk of peat soil undergoes extensive biochemical change after senescence. Thus, inferences concerning ecosystem history that are based on stratigraphic changes in peat chemistry need to consider that a significant proportion of observed change may not be due to changes in ecosystem history (i.e., plant community, nutrient loading, etc.), but are may be due to biogeochemical transformations in soil organic matter.

5. Solution ^{31}P-Nuclear Magnetic Resonance (NMR) Spectroscopy

5.1. Introduction

Solution ^{31}P-Nuclear Magnetic Resonance Spectroscopy provides information on classes of organic phosphorus. Analytically, it is very similar to ^{13}C-NMR, with the exception that the soil organic matter must first be extracted, typically using a strong base. There are several shortcomings of the technique that relate to the extraction. Firstly, there is some degradation of the organic phosphorus by the extracting reagent. Conversion of diester-P to phosphomonoesters has been documented. Secondly, only the alkaline extract is subjected to analysis, therefore nothing is known of the un-extractable component. Like the ^{13}C-NMR, ^{31}P-NMR provides information on classes of organic phosphorus compounds.

5.2. Methods

Samples were collected July 18, 2002 from station B4 only. Oven-dried (70^{o}C) and ground samples were extracted by shaking the soil: solution ratio of 5:100 with a solution containing 0.25 *M* NaOH and 0.05 *M* EDTA (ethylenediaminetetraacetic acid) for 4 h at 20°C (Cade-Menun and Preston, 1996). Each sample was extracted individually and centrifuged at 10,000 x *g* for 30 min. Equal volumes of the extracts were then frozen immediately at –80°C, lyophilized, and ground to a fine powder.

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

Solution ^{31}P NMR spectra were obtained using a Bruker Avance DRX 500 MHz spectrometer operating at 202.456 MHz for ^{31}P and 500.134 MHz for ^{1}H. Samples were analyzed using a 6 µs pulse (45°), a delay time of 1.0 s, and an acquisition time of 0.8 s. The delay time used here ensured sufficient spin-lattice relaxation between scans for P nuclei (Cade-Menun et al., 2002). Between 48,000 and 69,000 scans were acquired depending on the P concentration of the lyophilized extract, and broadband proton

decoupling was used for all samples. Chemical shifts of signals were determined in parts per million (ppm) relative to an external standard of 85% H_3PO_4. Signals were assigned to individual P compounds or functional groups based on literature reports (Turner at al., 2003) and signal areas calculated by integration. Spectra were plotted with a line broadening of 8 Hz, although additional spectra were plotted with a line broadening of 1 Hz to examine signals in the phosphate monoester region.

5.3. Results and Discussion

Organic phosphorus in the extractant solution, as a proportion of total P, increased throughout the soil profile. Organic P represented approximately 20% of total P at the soil surface, and increased to 40% at a depth of 120-cm (Fig. 5.1). Within the organic P pool there was no significant difference between phosphate monoesters and diesters (Student's paired t-test; $p = 0.321$; $df = 7$). However, it should be noted that the EDTA-NaOH extraction is known to degrade phosphate diesters such as phospholipids and RNA to their phosphate monoester constituents, so the actual proportion of phoshate diesters is likely higher and thus probably constitutes the dominant form of organic P in these soils. Notably absent was *myo*-inositol hexaphosphate (phytic acid), the dominant phosphate monoester in most upland soils (Turner et al. 2002). This form of P is of plant origin, with major concentration in seeds. Its high charge density causes it to adhere to soils, making it less available to microbial attack than other organic phosphates, and therefore more stable. Few studies have documented the long-term fate of these two functional classes of organic P in soils, particularly in wetlands. It appears from this study that both phosphate monoesters and diesters increased slightly with soil depth, and maintained approximately equal proportions. This represents an unusually high proportion of phosphate diesters. In a study by Turner et al. (2003) the ratio of mono- to diester P ranged from approximately 5 to 26 for 29 temperate pasture soils, compared to 1:1 in this study. One of the principal sources of phosphodiester is microbial tissue. The soils in this study appear to be much higher in microbial biomass, or alternatively that diester P is much better preserved under the anaerobic conditions of these flooded soils. Turner and Newman (manuscript in prep.) also found elevated levels of diester P in the Florida Everglades, which they attributed to slowed hydrolysis of plant and microbial inputs of

phosphate diesters. The slight increase in organic forms of P appeared to be at the expense of orthophosphate, which declined throughout the profile. A strong signal from DNA is apparent down to a depth of 80-cm. Since DNA is not well preserved in upland soils, this suggests a prominent microbial community at these depths (See spectra; Appendix A). However, preservation of DNA under flooded conditions in a pond near Titusville, FL was quite good (Doran, 2002). The Windover site is approximately 55 miles northeast of BCMCA and has gained international notoriety as having some of the best-preserved human remains and cultural artifacts (particularly woven fiber) ever recovered. In that study, 8000 year old human brain tissue was recovered from remains found in shallow pond. The DNA from this tissue was intact enough to partially sequence, though the overall DNA yield was low; approximately 1% of that from fresh tissue. Thus, phosphodiesters such as DNA may be better preserved than previously thought and thus may play a more prominent role in long-term phosphorus stabilization.

6. Fiber Analysis

6.1. Introduction

Lignin, cellulose, hemicellulose and soluble carbon are the principal carbonaceous plant polymers deposited at the soil surface after plant senescence. These polymers form the basis for the development of the peat deposit. They also contain an isotopic record of changes in plant communities. Isotopic analysis of the fibric portion of the peat deposit is superior to analysis of bulk peat, as it does not contain inorganic carbon, algal remains, or percolated humic and fulvic compounds.

6.2. Methods

Samples for lignin fractionation were collected on March 16, 2004 from stations C1 and B4 in BCMCA. A single core from each station was split length-wise and one half of each 10-cm interval was used for fiber analysis. Analysis was performed using an Ankom Fiber Analyzer, model 200/220. (Ankom Inc., Fairport NY). Samples were sequentially extracted with a neutral detergent, an acidic detergent, and concentrated sulfuric acid. The mass of each fiber fraction was determined gravimetrically after each extraction step. This technique was originally developed for crop residues, and needed to

be modified in order to characterize plant derived soil carbohydrates. Initial attempts to fractionate soil carbohydrates indicated that much fine organic matter leaked from the 30-μM glass fiber bags used in the analyses. Since the procedure is based on weight loss, an alternate method was developed. The modified procedure was as follows:

1. Place each 10-cm bulk-soil interval into a No. 35, 500 micron brass sieve,
2. Immerse sieve in 0.5% Liquinox solution to disperse fine soil organic matter from fibric material,
3. Gently agitate for approximately 5 minutes,
4. Wash fine particulate material from fibric material remaining in sieve with stream of deionized water until clean,
5. Air dry,
6. Grind through 20-mesh screen in a Wiley mill,
7. Fractionate using Ankom methodology.

Therefore, the results reported here represent the properties of the fibric material in the sample, not the bulk soil.

A subsample of the peat fiber was analyzed for ^{13}C and ^{15}N on a Thermo Finigan DELTA Plus isotope ratio mass spectrometer at the University of Florida Soil and Water Science Department. Purified N_2 and CO_2 were used as internal standards, with a helium carrier gas. Results are expressed in delta notation, relative to the ^{13}C content of PeeDee Belemnite foraminifera, and ^{15}N content of atmospheric N_2. Carbon 14 analysis was performed on plant macrofossils removed from selected peat depths. These macrofossils were subjected to a sequential extraction to remove carbonates and percolated humic acids with 0.1M HCl, 0.5M NaOH, 0.1M HCL, and deionized water. The samples were dried at 70^{o}C. They were then analyzed by Dr. Susan Trumbore at the University of California, Irvine on a National Electrostatics Corporation (NEC 0.5MV 1.5SDH-2) Accelerator Mass Spectrometer. Variations in atmospheric ^{14}C were accounted for using the computer program CALIB, version 5.0.1 (Stuiver and Reimer, 1993).

6.3. Results and Discussion

6.3.1. Lignin

The amount of plant fiber recovered using the wet-sieving technique varied from 5 to 15 grams dry weight, per 10-cm interval. Note that each core was split length-wise, so the actual recoverable fibric material is twice this value. There was no apparent trend down core, though plant fiber declined at depths where fine organic matter increased. The relative abundance of each plant polymer was lignin > cellulose > hemicellulose.

Lignin content increased gradually at both stations, from approximately 40% of the material dry weight in the surface soil, to 80% at the base of the core (Figure 6.1). Lignin was the most abundant plant polymer, from three to five times as abundant as cellulose and hemicellulose. Station C1 showed a pronounced decline in lignin for the 50 to 60 cm depth, coinciding with an increase in fine organic matter, and a sudden increase in organic matter age. This may reflect either a deepening of the marsh, favoring soft-tissued macrophytes and phytoplankton that possess less lignin, or alternatively that a long-term drought reduced much of the lignin to smaller plant polymers. Evidence for a circum-Caribbean dry period that occurred approximately 1000 years before present favors the second hypothesis (Buck, et al, 2005).

Cellulose showed a gradual decline throughout the soil profile, declining from approximately 20% at the surface, to 5-10% at 150 cm. Downcore lignin and cellulose content was very similar to that found by DeBusk and Reddy (1998) for soil samples taken from a peatland in the northern Everglades. They found that the lignin cellulose index (LCI; lignin/(lignin + cellulose)) varied from 0.26 in standing dead plant litter, to 0.81 in peat taken from a depth of 30-cm. The LCI of the samples in this study varied from 0.73 at the soil surface, to greater than 0.90 at the base of the core, indicating a greater degree of decomposition at the lower soil depth. The greater lignin content, as well as greater LCI in this study reflects the greater soil depths investigated in BCMCA. DeBusk and Reddy (1998) examined peat samples from a maximum of 30-cm, whereas in this study peat from a depth of 150-cm was analyzed. Hemicellulose declined somewhat erratically with respect to depth, from approximately 10% at the surface, to 3-5% at the base of the core.

Soil lignin content increased with respect to depth at both locations, while cellulose and hemicellulose declined. Enzymes responsible for lignin decomposition, such as phenol oxidase and lignin peroxidase, are secreted by the white rot fungi such as *Phanerochaete chrysosporium*. These fungi are not active in flooded soil, hence the buildup of lignin. Some of the increase in lignin content with depth is undoubtedly due to the compaction and concentration of lignin as more peat is accreted. However, the predominant mechanism is likely the loss of more labile plant polymers from the separated soil fibric material. Overall, it is clear that as this fibric material ages, it takes on a more purely lignin character. This data seems to conflict with the ^{13}C-NMR results described earlier in this report. The NMR data showed declining lignin throughout the soil profile. However, those results were from a bulk soil sample (not sieved). It is likely that even though lignin degrades slowly under flooded conditions, it was being converted to humic materials and thus it represented a declining fraction of the total soil. In fact, this is one of the principal theories of humic matter formation, that lignin is a precursor to humic matter (Stevenson, 1994). The sieved material contained only the fibric material of macrophytes. That the lignin content of this material increased with depth reflects the loss of other plant polysaccharides from the fiber, such as cellulose, hemicellulose, and sugars, thus leaving only lignin. In summary, even though the lignin content of the soil declined with respect to depth, the plant fiber that remained became more purely lignin.

6.3.2. Isotopic Composition of Lignin

Live plant biomass at both stations was enriched in both ^{13}C and ^{15}N, relative to the underlying plant litter (Fig. 6.2). Plant litter was submerged at the time of sampling and therefore had probably become colonized with epiphytic algae and enriched in microbial biomass. The difference in composition between plant and litter was most dramatic for ^{15}N, with plant litter containing essentially an atmospheric ^{15}N content ($\delta^{15}N = 0$). This may reflect extensive colonization of the detrital material by N_2-fixing cyanobacteria. The stations showed distinctly different isotopic signatures to a depth of approximately 50-cm, for ^{13}C and 80-cm for ^{15}N. This very likely reflects a long-term difference in plant communities at the two stations. The method of analysis used in this study, i.e. analysis of intact plant fiber, has the advantage of revealing the isotopic

signature of only the principal vascular plants that colonized the site at that depth interval. Thus, extraneous carbon from carbonates, percolating organic acids from other depths do not substantively contribute to the signature. Currently, the community at station B4 is predominantly *Panicum haemotomon/Cladium jamiacense* whereas the C1 station is *Utricularia sp./Typa latifolia/Nymphaea odorata* (slough community). At both stations, there is a reversal in both isotopes approximately mid-core, perhaps reflecting a dramatic shift in plant communities. Carbon-14 dates at both stations indicate that this change occurred approximately 1000AD (Fig. 6.3). As mentioned previously, this period was characterized by a long-term tropical drought that may have precipitated a major change in marsh plant communities.

An extended dry period, perhaps several hundred years, would have led to a dramatic loss of soil organic matter and a lowered marsh surface elevation. Upon reflooding, the shallow water adapted community would have been replaced by deeper water adapted plants, such as *Nymphaea* and others. An abrupt increase in age of plant macrofossils is apparent at a depth of between 70 and 90-cm. This sudden increase in carbon age has been observed at other locations in BCMCA (Fisher, unpublished). There are two plausible explanations for this sudden increase in the age of BCMCA peat: little or no accretion for almost a millennia, or (more likely) loss of carbon due to an extended dry period. This marsh dryout would have been accompanied by greatly accelerated aerobic peat decomposition, a peat fire, or both. In any case, once normal rainfall patterns returned, a much deeper marsh community would have developed and this change seems to be suggested by the isotopic evidence.

Table 6.3. Results of ^{14}C AMS dating. Values in parentheses represent standard error of analytical dating procedure.

Station	Depth	Sample Description	^{14}C Age, YBP	Calibrated Age, AD/BC
B4	-35	Seed	125 (±20)	>1800 AD
B4	-65	Woody husk	345 (±20)	1550 AD (±100)
B4	-85	Panicum root	440 (±20)	1440 AD (±50)
B4	-115	Stem fragment	1905 (±20)	95 AD (±20)
B4	-125	Pine needle	2930 (±20)	1150 BC (±125)
B4	-145	Seed husk	2790 (±20)	950 BC (±60)
C1	-35	Grass blade	120 (±20)	>1800 AD
C1	-55	Seed pod case	525 (±25)	1415 AD (±10)
C1	-75	Grass blade	1070 (±20)	985 AD (±60)
C1	-85	Twig	2560 (±15)	780 BC (±10)
C1	-95	Nuphar rhizome	2695 (±20)	825 BC (±25)
C1	-125	Nuphar rhizome	3025 (±20)	1290 BC (±30)
C1	-135	Wood fragment	2920 (±20)	1150 BC (±100)
C1	-145	Wood fragment	3365 (±20)	1650 BC (±30)

The important implication for this study is that the slow biogeochemical processes that lead to immobilization of organic phosphorus were probably punctuated by extreme climatic events. These events would have had important consequences for the flora and fauna of the marsh, the organic remains of these organisms, and thus the peat deposit itself. These stochastic, geologically sudden changes are superimposed on the peat record and need to be considered when determining such processes as average rate of marsh accretion and long-term burial rates of primary nutrients.

7. Conclusions

Results from this study provide information on the biogeochemical transformations that organic matter undergoes after it is deposited on the soil surface.

Peat cores taken to a depth of approximately 1.5-m covered a soil age range that dated to approximately 3000 years before present.

There is ample evidence that substantial changes occur both in organic matter composition, and organic P. The fluorescence and spectrophotometric procedures indicated a progression to more humified material, but not necessarily a concomitant change in molecular weight of extractable organic matter. Nuclear magnetic resonance analysis of carbon shows a decline in plant carbohydrates with respect to depth and an increase in aromatic compounds. Nuclear magnetic resonance of phosphorus showed stability of diester-P compounds, and an absence of inositol hexaphosphate. This contrasts sharply with upland soil ^{32}P-NMR work, where diester-P is has been shown to quickly degrade, and inositol phosphates are the dominant P-storage compound. Organic forms of phosphorus increased with respect to depth.

Lignin fractionation showed that the lignin content of soil fibric material increases with age, while cellulose and hemicellulose decline. Stable isotopic composition seem to indicate stable plant community with a community change approximately 1000 years ago, perhaps due to an extended drought.

Results from this study suggest that P does not behave as a conservative compound. It is subject to the same biogeochemical processes that dramatically alter soil organic matter. These processes act to transform the original floral and faunal material into inorganic and organic compounds that are progressively less and less similar to the original material. These processes lead to loss of labile forms of P, and an increasing proportion of stable organic phosphorus compounds. Comparative studies that contrast surficial peat soils to their deeper counterparts need to consider the effects that soil forming processes have had on soil properties, particularly in the case of organic soils.

8. References

Baldock, J.A., J.M. Oades, P.N. Nelson, T.M. Skene, A. Golchin, and P. Clarke. 1997.Assessing the extent of decomposition of natural organic materials using solis-state 13C NMR spectroscopy. Australian J. Soil Research. 35:1061-1083.

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. Paleolimnology. 26:241-247.

Buck, D. G., M. Brenner, D.A. Hodell, J.H. Curtis. 2005. A late Holocene record of climate variability from hypersaline Lago Enriquillo, Dominican Republic. Oral presentation at 2005 American Society of Limnology and Oceanography meetings, Salt Lake City, Utah.

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

Cade-Menun, B.J., C.W. Liu, R. Nunlist, and J. G. McColl. 2002. Soil and litter 31PNMR spectroscopy: extractants, metals and phosphorus relaxation times. J. Env. Qual. 31:457-465.

Chen, Y., N. Senesi, and M. Schnitzer. 1977. Information provided on humic substances by E4/E6 ratios. Soil Sci. Soc. Am. J. 41:352-358.

Cox, L., R. Celis, M.C. Hermosin, J. Cornejo, A. Zsolnay, and K. Zeller. 2000. Effect of organic amendments on herbicide sorption as related to the nature of dissolved organic matter. Environ. Sci. Technol. 34: 4600-4605.

Davis, S.M. 1991. Growth, decomposition, and nutrient retention of Cladium jamaicense Crantz and Typha domingensis Pers. in the Florida Everglades. Aquat. Bot. 40: 203-224.

DeBusk, W.F. and K.R. Reddy. 1998. Turnover of detrital organic carbon in a nutrient impacted Everglades Marsh. Soil Sci. Soc. Am. J. 62: 1460-1498.

Doran, G. 2002. Windover : multidisciplinary investigations of an early Archaic Florida cemetery. University Press of Florida, Gainesville FL.

Florida Department of Environmental Protection. 2004. Integrated Water Quality Assessment for Florida: 2004 305(b) Report and 303(d) List Update.

Kadlec, R.H. and R.L Knight. 1996. Treatment Wetlands. Lewis Publishers. Chelsea, MI.

Lowe, E.F. and L.W. Keenan. 1997. Managing phosphorus-based cultural eutrophication in wetlands: a conceptual approach. Ecol. Eng. 9:109-118.

Ohno, Tsutomu. 2002. Fluorescence inner filtering correction for determining the humification index of dissolved organic matter. Environ. Sci. Technol. 36: 742-746.

Olila O. G., K.R. Reddy and L. Keenan. 1995. Labile and non-labile pools of phosphorus in surface waters and soils in the Upper St. Johns River Basin. Final report to the St. Johns River Water Management District. Contract No. 94D179.

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.

Sjögersten, Sofie, B.L. Turner, Nathalie Mahieu, L.M. Condron and P. A. Wookey. 2003. Global Change and Biol. 9: 759-772.

Stevenson, F.J. 1994. Humus Chemistry: Genesis, Composition, Reactions. 2nd Edition. John Wiley & Sons.

Steward, K.K. and W.H. Ornes. 1975. The autoecology of sawgrass in the Florida Everglades. Ecology 56: 162-171.

Stuiver, M., and Reimer, P. J., 1993, Extended 14C database and revised CALIB radiocarbon calibration program, Radiocarbon 35:215-230.

Turner, B.L. 2004. Optimizing phosphorus characterization in animal manures by solution Phosphorus-31 Nuclear Magnetic Resonance spectroscopy. J. Environ. Qual. 33: 757-766.

Turner, B.L., M.J. Paphazy, P.M. Haygarth, and I.D. McKelvie. 2002. Inositol phosphates in the environment. Phil. Trans. R. Soc. Lond. 357:449-469.

Turner, B.L., N. Mahieu, and L.M. Condron. 2003. ^{31}P MNR spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. Soil Sci. Soc. Am. J. 67: 497-510.

Turner, B.L., N. Mahieu, and L.M. Condron. 2003. The phosphorus composition of temperate pasture soils determined by NaOH-EDTA extraction and solution ^{31}P MNR spectroscopy. Organic Geochemistry. 34: 1199-1210.

United States Environmental Protection Agency. 1998. 303d List of Impaired Waters.

Urban, N.H., S.M. Davis, N.G. Aumen. 1993. Fluctuations in sawgrass and cattail densities in Everglades Water Conservation Area 2A under varying nutrient, hydrologic and fire regimes. Aquat. Bot. 46: 203-223.

Vollenweider, R.A. 1975. Input-output models with special reference to the phosphorus loading concept in limnology*).Schweiz. Z. Hydrol. 37:53-84.

Walker, W.W., Jr. 1995. Design basis for the Everglades stormwater treatment areas. Water Res. Bull. 31:671-685.

9. Figures

10. Appendices

Appendix B. Physico-chemical properties of BCMCA soil samples retrieved on March 16, 2004.

Station	Rep	Types	Depth	UF ID	Water	BD	LOI	Total C	Total N
			cm		%	g /cm3	%	g/kg	g/kg
B4	A	Soil	0-10	133	93	0.056	94	481	33
B4	A	Soil	10-20	134	94	0.071	95	489	32
B4	A	Soil	20-30	135	93	0.081	97	511	35
B4	A	Soil	30-40	136	91	0.104	96	507	32
B4	A	Soil	40-50	137	90	0.110	95	507	32
B4	A	Soil	50-60	138	90	0.108	98	514	29
B4	A	Soil	60-70	139	90	0.103	94	509	29
B4	A	Soil	70-80	140	88	0.131	93	523	28
B4	A	Soil	80-90	141	89	0.119	95	532	27
B4	A	Soil	90-100	142	91	0.106	95	535	28
B4	A	Soil	100-110	143	90	0.109	94	545	28
B4	A	Soil	110-120	144	90	0.102	96	544	26
B4	A	Soil	120-130	145	92	0.089	98	548	29
B4	B	Soil	0-10	146	93	0.054	96	492	31

Appendix B. Physico-chemical properties of BCMCA soil samples retrieved on March 16, 2004.

Station	Rep	Types	Depth	UF ID	Water	BD	LOI	Total C	Total N
			cm		%	g /cm3	%	g/kg	g/kg
B4	B	Soil	10-20	147	93	0.068	94	488	29
B4	B	Soil	20-30	148	93	0.072	96	511	33
B4	B	Soil	30-40	149	94	0.069	98	563	37
B4	B	Soil	40-50	150	92	0.082	96	424	26
B4	B	Soil	50-60	151	92	0.091	96	524	27
B4	B	Soil	60-70	152	92	0.092	96	500	27
B4	B	Soil	70-80	153	91	0.097	94	515	28
B4	B	Soil	80-90	154	90	0.118	94	509	27
B4	B	Soil	90-100	155	86	0.156	91	536	25
B4	B	Soil	100-110	156	88	0.134	92	517	26
B4	B	Soil	110-120	157	90	0.107	94	550	29
B4	B	Soil	120-130	158	90	0.105	94	544	25
B4	B	Soil	130-140	159	91	0.101	94	532	22
B4	B	Soil	140-150	160	92	0.092	96	547	25
B4	C	Soil	0-10	161	92	0.064	94	481	30
B4	C	Soil	10-20	162	92	0.086	95	501	31
B4	C	Soil	20-30	163	93	0.072	96	522	31
B4	C	Soil	30-40	164	92	0.095	96	516	35
B4	C	Soil	40-50	165	91	0.101	95	514	31
B4	C	Soil	50-60	166	91	0.105	95	510	28

B4	C	Soil	60-70	167	91	0.104	93	511	27
B4	C	Soil	70-80	168	90	0.110	93	506	27
B4	C	Soil	80-90	169	87	0.151	92	537	27
B4	C	Soil	90-100	170	90	0.115	93	508	28
B4	C	Soil	100-110	171	91	0.093	94	538	28
B4	C	Soil	110-120	172	90	0.104	95	532	23
B4	C	Soil	120-127	173	89	0.131	95	551	21
C1	A	Soil	0-10	174	90	0.084	92	471	27
C1	A	Soil	10-20	175	88	0.121	90	470	34
C1	A	Soil	20-30	176	91	0.086	93	490	30
C1	A	Soil	30-40	177	93	0.081	94	511	32
C1	A	Soil	40-50	178	92	0.084	96	526	31
C1	A	Soil	50-60	179	90	0.115	92	516	27
C1	A	Soil	60-70	180	89	0.118	91	537	24
C1	A	Soil	70-80	181	91	0.095	93	539	27
C1	A	Soil	80-90	182	92	0.093	93	539	25
C1	A	Soil	90-100	183	93	0.070	95	555	27
C1	A	Soil	100-110	184	94	0.065	96	534	26
C1	A	Soil	110-120	185	94	0.062	95	526	30
C1	A	Soil	120-130	186	91	0.097	83	460	27
C1	A	Soil	130-140	187	92	0.090	81	424	25
C1	A	Soil	140-150	188	87	0.158	54	298	15
C1	B	Soil	0-10	189	88	0.111	87	438	30

Appendix B. Physico-chemical properties of BCMCA soil samples retrieved on March 16, 2004.

Station	Rep	Types	Depth	UF ID	Water	BD	LOI	Total C	Total N
			cm		%	g /cm3	%	g/kg	g/kg
C1	B	Soil	10-20	190	90	0.101	93	484	35
C1	B	Soil	20-30	191	90	0.097	94	509	33
C1	B	Soil	30-40	192	91	0.100	96	524	33
C1	B	Soil	40-50	193	90	0.108	95	521	31
C1	B	Soil	50-60	194	88	0.135	91	538	25
C1	B	Soil	60-70	195	91	0.107	92	521	28
C1	B	Soil	70-80	196	92	0.082	93	539	27
C1	B	Soil	80-90	197	93	0.079	95	544	27
C1	B	Soil	90-100	198	93	0.076	97	539	28
C1	B	Soil	100-105	199	93	0.105	95	526	30
C1	C	Soil	0-10	200	90	0.093	94	461	25
C1	C	Soil	10-20	201	90	0.091	91	461	33
C1	C	Soil	20-30	202	91	0.098	93	488	34
C1	C	Soil	30-40	203	91	0.095	93	498	32
C1	C	Soil	40-50	204	91	0.106	96	520	33
C1	C	Soil	50-60	205	89	0.124	93	519	28
C1	C	Soil	60-70	206	89	0.121	93	487	22
C1	C	Soil	70-80	207	92	0.091	94	526	28
C1	C	Soil	80-90	208	93	0.080	94	517	25
C1	C	Soil	90-100	209	94	0.066	95	546	27

C1	C	Soil	100-110	210	95	0.060	94	525	26
C1	C	Soil	110-120	211	94	0.071	80	513	28
C1	C	Soil	120-130	212	88	0.138	69	366	21
C1	C	Soil	130-140	213	90	0.107	69	379	20
C1	C	Soil	140-150	214	81	0.240	42	227	13
B4	A	Detritus		215			98	475	16
B4	B	Detritus		216			98	472	15
B4	C	Detritus		217			99	475	11
B4	A	Plant		218			99	477	8
B4	B	Plant		219			99	474	7
B4	C	Plant		220			100	481	10
C1	A	Detritus		221			95	496	25
C1	B	Detritus		222			97	494	26
C1	C	Detritus		223			95	487	25
C1	A	Plant		224			96	456	12
C1	B	Plant		225			95	466	15
C1	C	Plant		226			95	454	14

Appendix C. Results of phosphorus fractionation of BCMCA soil samples retrieved on March 16, 2004.

Station	Rep	Types	Depth	UF ID	HClPi	FAP	HAP	ResidueP	TP
			cm		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
B4	A	Soil	0-10	133	73	169	150	160	403
B4	A	Soil	10-20	134	28	117	103	28	276
B4	A	Soil	20-30	135	26	69	79	87	261
B4	A	Soil	30-40	136	11	57	62	50	180
B4	A	Soil	40-50	137	9	34	57	48	148
B4	A	Soil	50-60	138	7	27	52	34	120
B4	A	Soil	60-70	139	6	35	49	39	129
B4	A	Soil	70-80	140	5	31	51	37	124
B4	A	Soil	80-90	141	4	20	33	14	71
B4	A	Soil	90-100	142	3	23	13	31	58
B4	A	Soil	100-110	143	3	17	17	17	53
B4	A	Soil	110-120	144	2	16	13	22	54
B4	A	Soil	120-130	145	2	18	10	20	51
B4	B	Soil	0-10	146	60	158	134	56	408
B4	B	Soil	10-20	147	43	111	85	32	271
B4	B	Soil	20-30	148	33	88	71	45	238
B4	B	Soil	30-40	149	26	62	62	46	196
B4	B	Soil	40-50	150	16	53	70	42	181

B4	B	Soil	50-60	151	9	41	59	45	154
B4	B	Soil	60-70	152	7	26	47	42	122
B4	B	Soil	70-80	153	6	33	44	53	135
B4	B	Soil	80-90	154	6	39	51	53	148
B4	B	Soil	90-100	155	5	27	44	37	112
B4	B	Soil	100-110	156	4	19	25	26	73
B4	B	Soil	110-120	157	3	17	15	29	64
B4	B	Soil	120-130	158	2	17	13	27	59
B4	B	Soil	130-140	159	2	18	10	27	57
B4	B	Soil	140-150	160	5	15	18	27	64
B4	C	Soil	0-10	161	95	168	100	110	472
B4	C	Soil	10-20	162	50	71	82	120	323
B4	C	Soil	20-30	163	30	70	75	93	269
B4	C	Soil	30-40	164	18	57	67	84	225
B4	C	Soil	40-50	165	9	33	53	67	161
B4	C	Soil	50-60	166	7	29	47	60	143
B4	C	Soil	60-70	167	6	24	52	65	147
B4	C	Soil	70-80	168	6	32	60	41	140
B4	C	Soil	80-90	169	5	19	61	27	112
B4	C	Soil	90-100	170	4	16	27	30	77
B4	C	Soil	100-110	171	3	16	22	22	62
B4	C	Soil	110-120	172	2	13	17	22	53
B4	C	Soil	120-127	173	2	18	9	29	58
C1	A	Soil	0-10	174	50	353	192	225	821

Appendix C. Results of phosphorus fractionation of BCMCA soil samples retrieved on March 16, 2004.

Station	Rep	Types	Depth	UF ID	HClPi	FAP	HAP	ResidueP	TP
			cm		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
C1	A	Soil	10-20	175	53	155	149	115	473
C1	A	Soil	20-30	176	42	55	93	52	242
C1	A	Soil	30-40	177	30	31	74	55	189
C1	A	Soil	40-50	178	19	26	60	28	133
C1	A	Soil	50-60	179	11	24	60	35	130
C1	A	Soil	60-70	180	6	13	34	19	73
C1	A	Soil	70-80	181	4	14	27	7	53
C1	A	Soil	80-90	182	3	10	23	52	89
C1	A	Soil	90-100	183	2	11	16	16	45
C1	A	Soil	100-110	184	2	13	15	16	46
C1	A	Soil	110-120	185	2	13	16	23	54
C1	A	Soil	120-130	186	2	17	10	20	49
C1	A	Soil	130-140	187	2	23	22	24	72
C1	A	Soil	140-150	188	3	18	22	13	56
C1	B	Soil	0-10	189	45	236	180	125	587
C1	B	Soil	10-20	190	52	118	152	83	404
C1	B	Soil	20-30	191	39	69	105	33	246
C1	B	Soil	30-40	192	21	26	68	49	164

C1	B	Soil	40-50	193	15	31	55	33	134
C1	B	Soil	50-60	194	8	23	47	15	93
C1	B	Soil	60-70	195	7	18	28	25	78
C1	B	Soil	70-80	196	4	13	21	26	64
C1	B	Soil	80-90	197	3	13	12	27	55
C1	B	Soil	90-100	198	3	14	13	27	57
C1	B	Soil	100-105	199	2	13	20	25	61
C1	C	Soil	0-10	200	53	312	231	297	893
C1	C	Soil	10-20	201	59	187	165	173	584
C1	C	Soil	20-30	202	43	72	77	89	280
C1	C	Soil	30-40	203	27	49	69	67	212
C1	C	Soil	40-50	204	14	24	58	50	146
C1	C	Soil	50-60	205	11	30	54	96	191
C1	C	Soil	60-70	206	8	18	33	33	92
C1	C	Soil	70-80	207	6	22	14	22	65
C1	C	Soil	80-90	208	4	7	28	23	62
C1	C	Soil	90-100	209	3	13	20	24	59
C1	C	Soil	100-110	210	3	32	-2	24	56
C1	C	Soil	110-120	211	3	16	11	47	76
C1	C	Soil	120-130	212	3	15	10	24	52
C1	C	Soil	130-140	213	4	17	15	23	58
C1	C	Soil	140-150	214	11	24	15	20	70
B4	A	Detritus		215	58	152	55	70	336
B4	B	Detritus		216	82	166	66	76	390

Appendix C. Results of phosphorus fractionation of BCMCA soil samples retrieved on March 16, 2004.

Station	Rep	Types	Depth	UF ID	HClPi	FAP	HAP	ResidueP	TP
			cm		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
B4	C	Detritus		217	50	109	34	17	210
B4	A	Plant		218	59	83	30	1	173
B4	B	Plant		219	58	80	16	13	166
B4	C	Plant		220	112	130	38	3	283
C1	A	Detritus		221	199	363	215	169	946
C1	B	Detritus		222	206	319	177	205	906
C1	C	Detritus		223	249	380	216	97	942
C1	A	Plant		224	379	279	72	37	766
C1	B	Plant		225	450	404	108	-3	960
C1	C	Plant		226	360	401	88	14	862

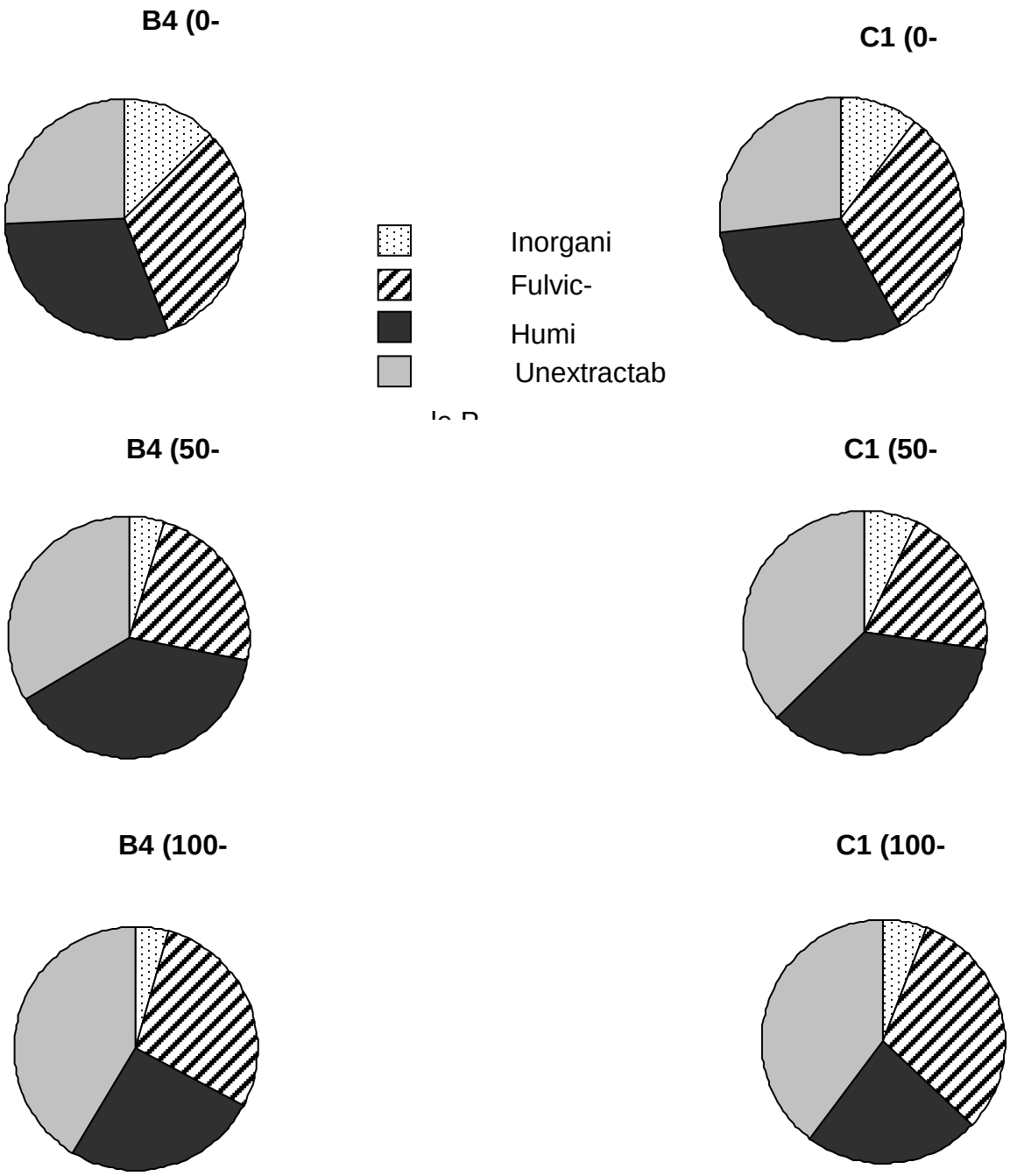
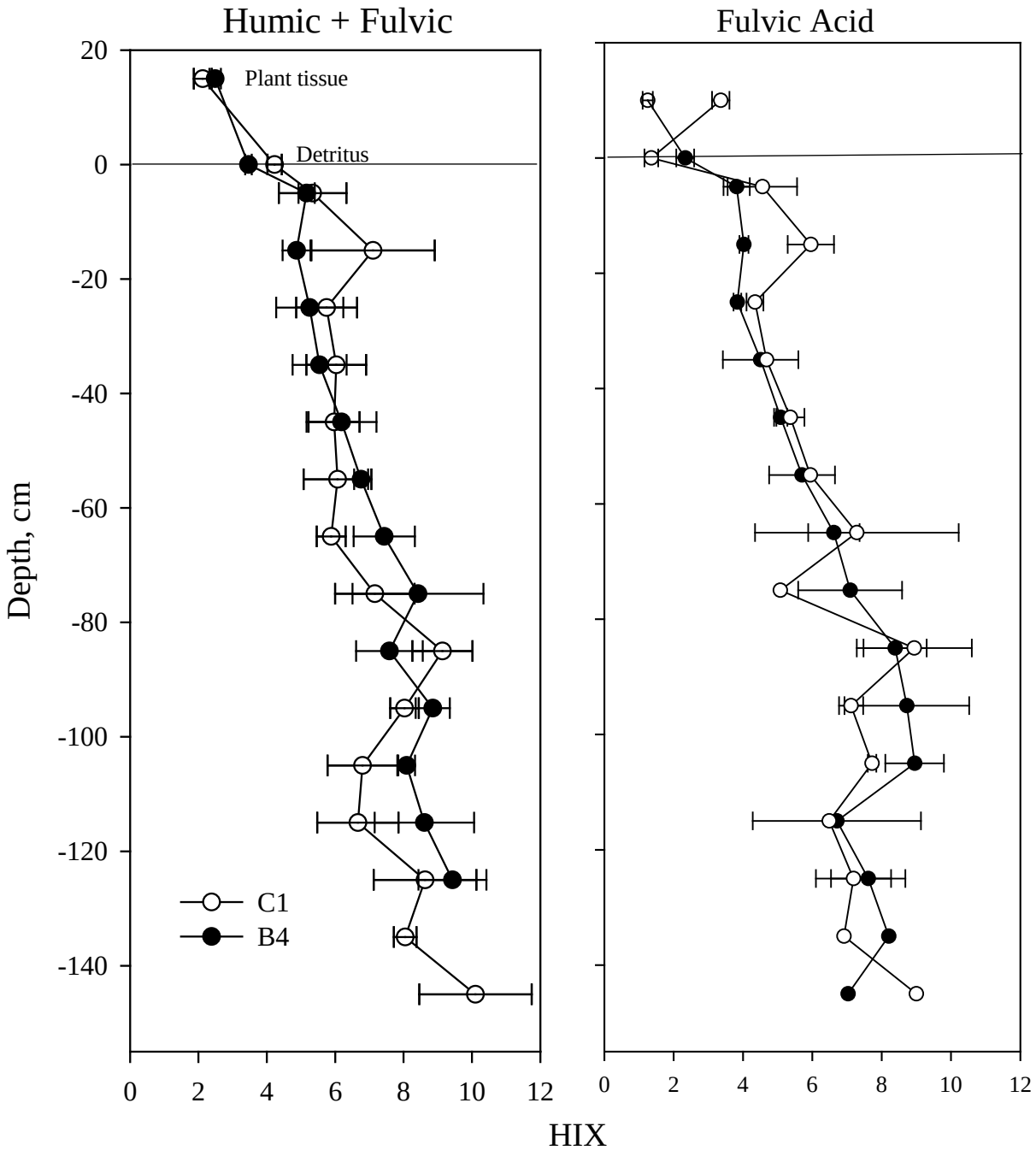


Figure 2.1. Results of phosphorus fractionation on soils from BCMCA. Numbers indicate the percentage of the total phosphorus in each fraction. Each plot represents the average of five discrete 10-cm soil samples.

Figure 2.2. The humification index (HIX) of NaOH-extracted peat samples from BCMCA. Error bars represent the standard deviation of three soil extracts of the same sample. Panel A reflects the HIX of the whole extract, whereas panel B has had humic materials removed by acidic precipitation, leaving only fulvic compounds.



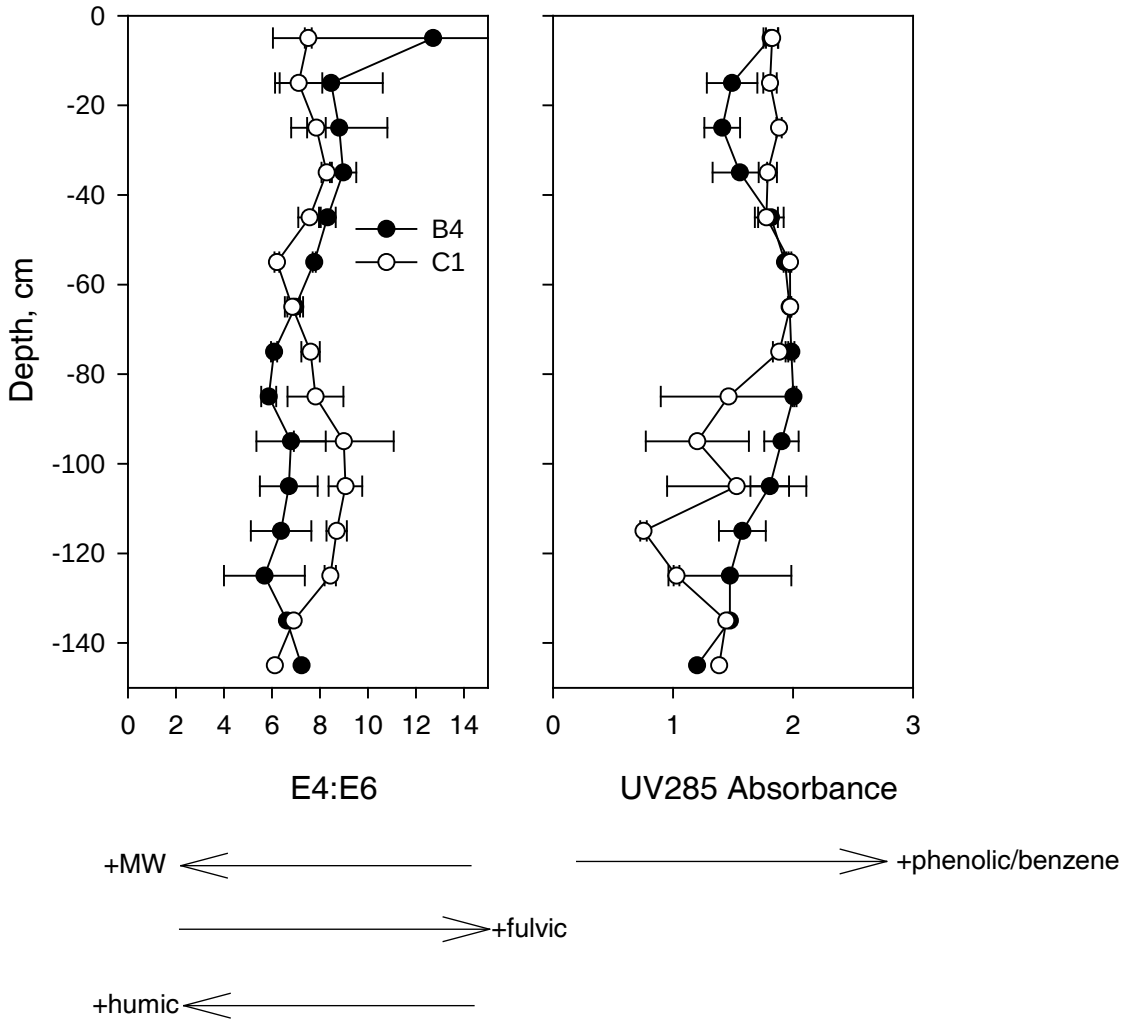


Figure 3.1. Ratio of absorbance at 665 and 465nm (E4:E6) and absorbance at 285nm of NaOH peat extracts at stations C1 and B4 in BCMCA.

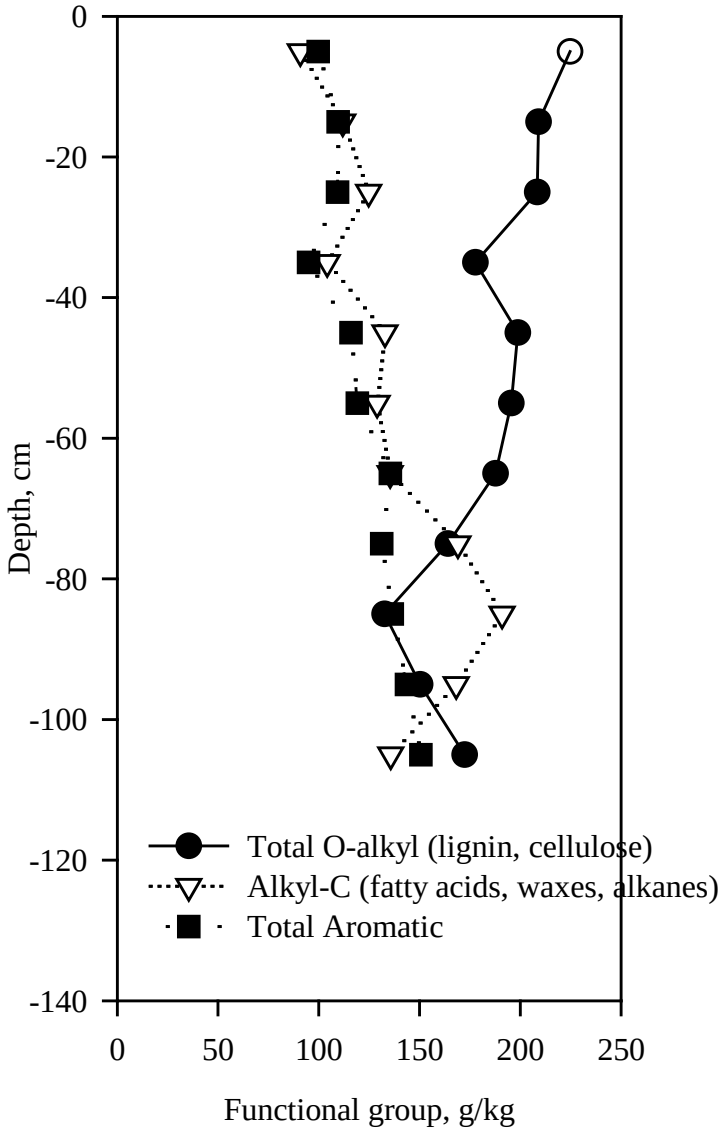


Figure 4.1. Results of solid-state ^{13}C-NMR spectroscopy of dried, ground peat samples collected from station B4 on February 11, 2003.

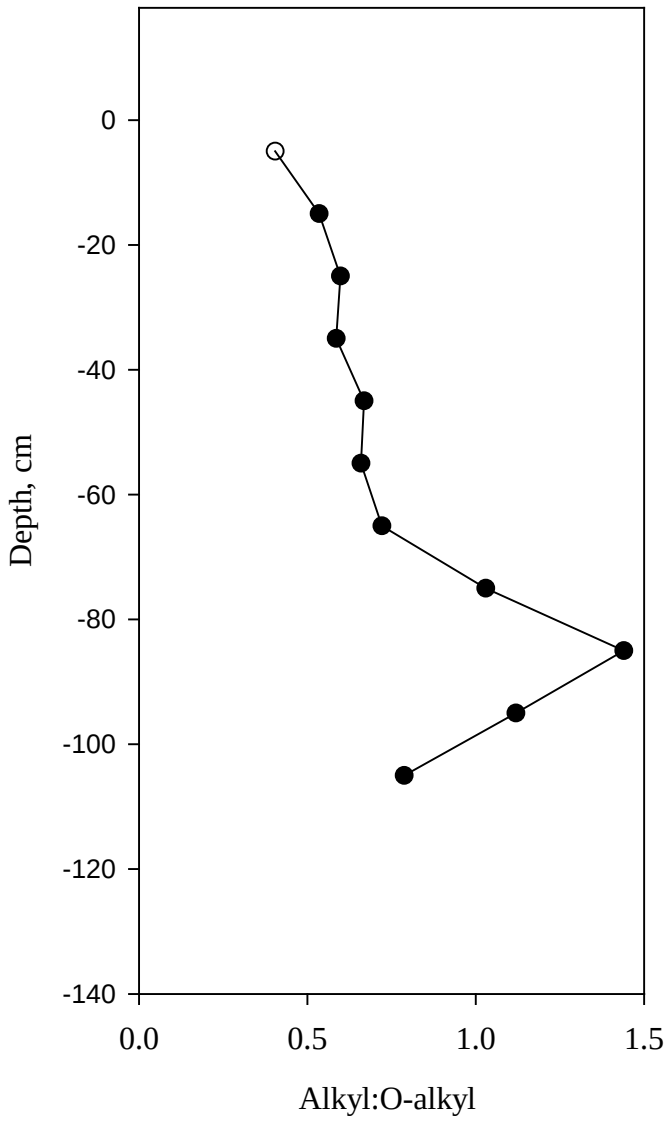


Figure 4.2. The alkyl: o-alkyl ratio of dried, ground peat samples collected from station B4 on February 11, 2003.

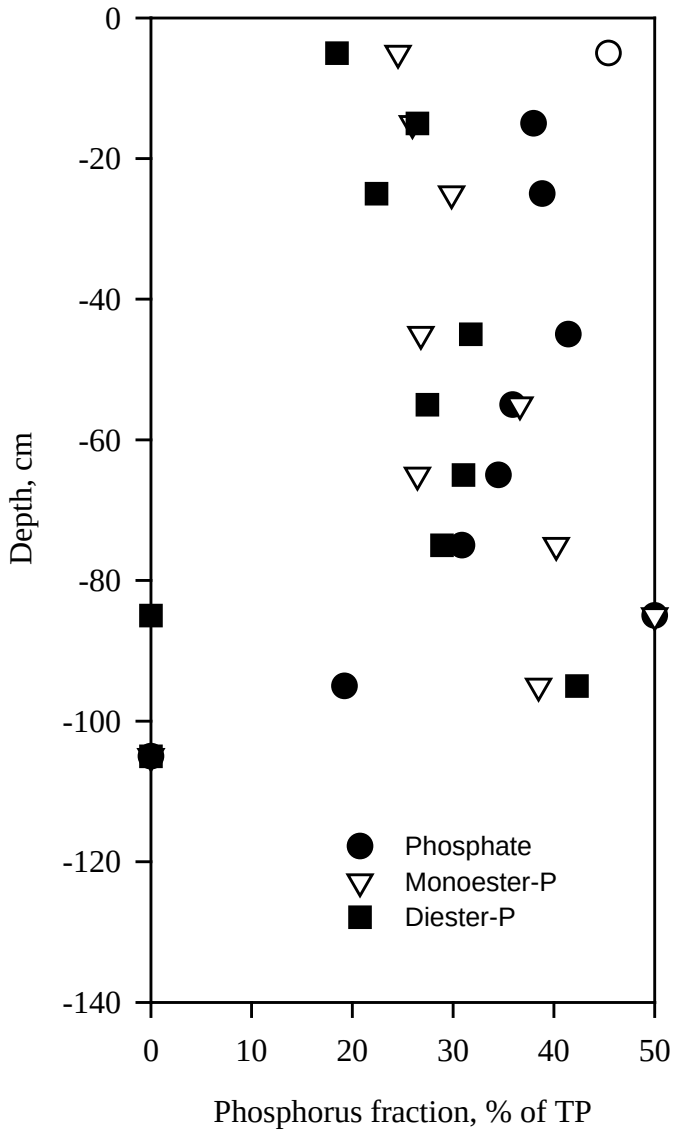


Figure 5.1. Results of solution ^{31}P-NMR spectroscopy of NaOH-extracted samples collected from station B4 on February 11, 2003. Note approximately equal concentration of mono and diester P.

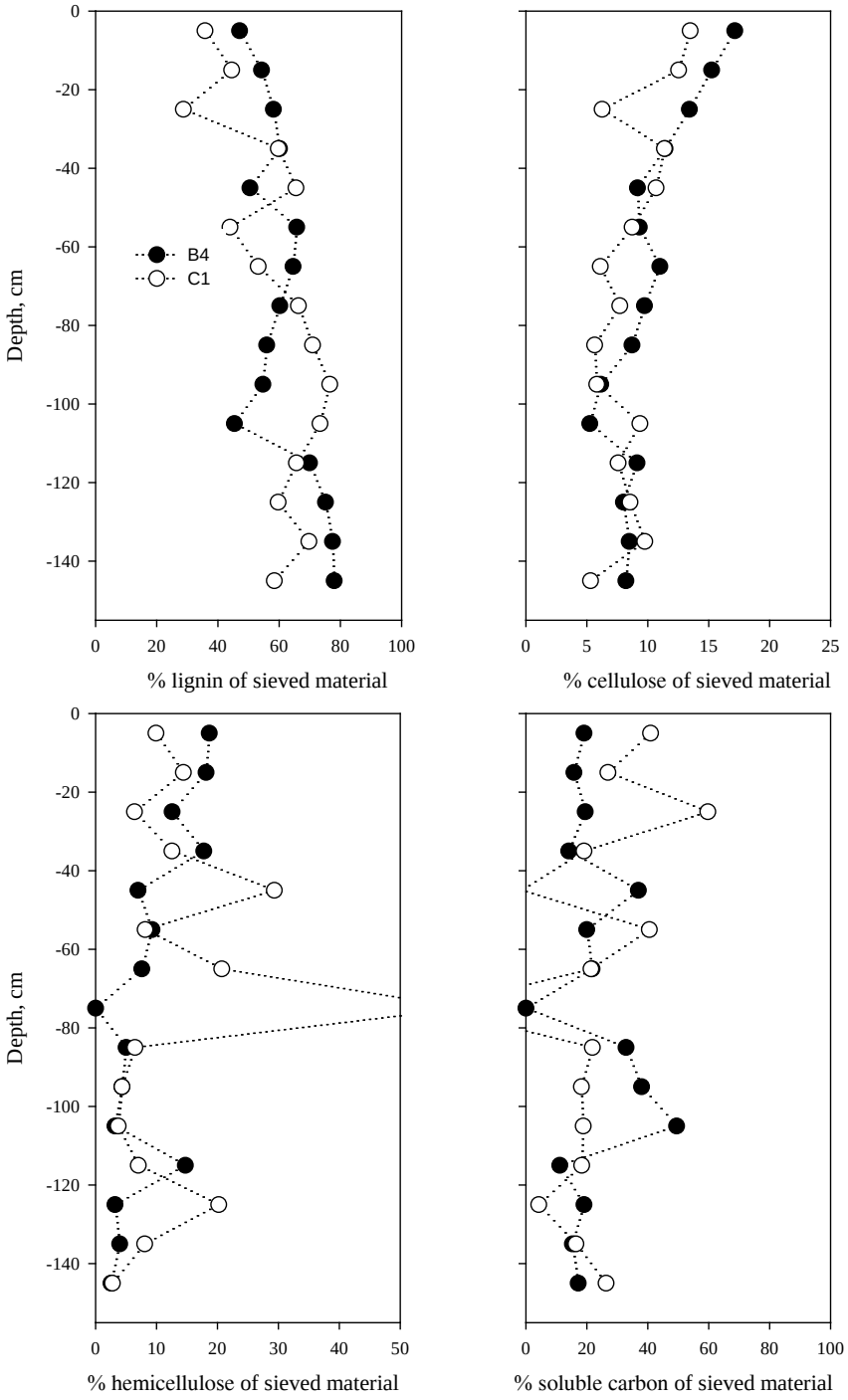


Figure 6.1. Results of lignin fractionation of plant fiber. Fiber was separated from bulk peat samples that were collected from BCMCA stations B4 and C1. Note differing horizontal scales.

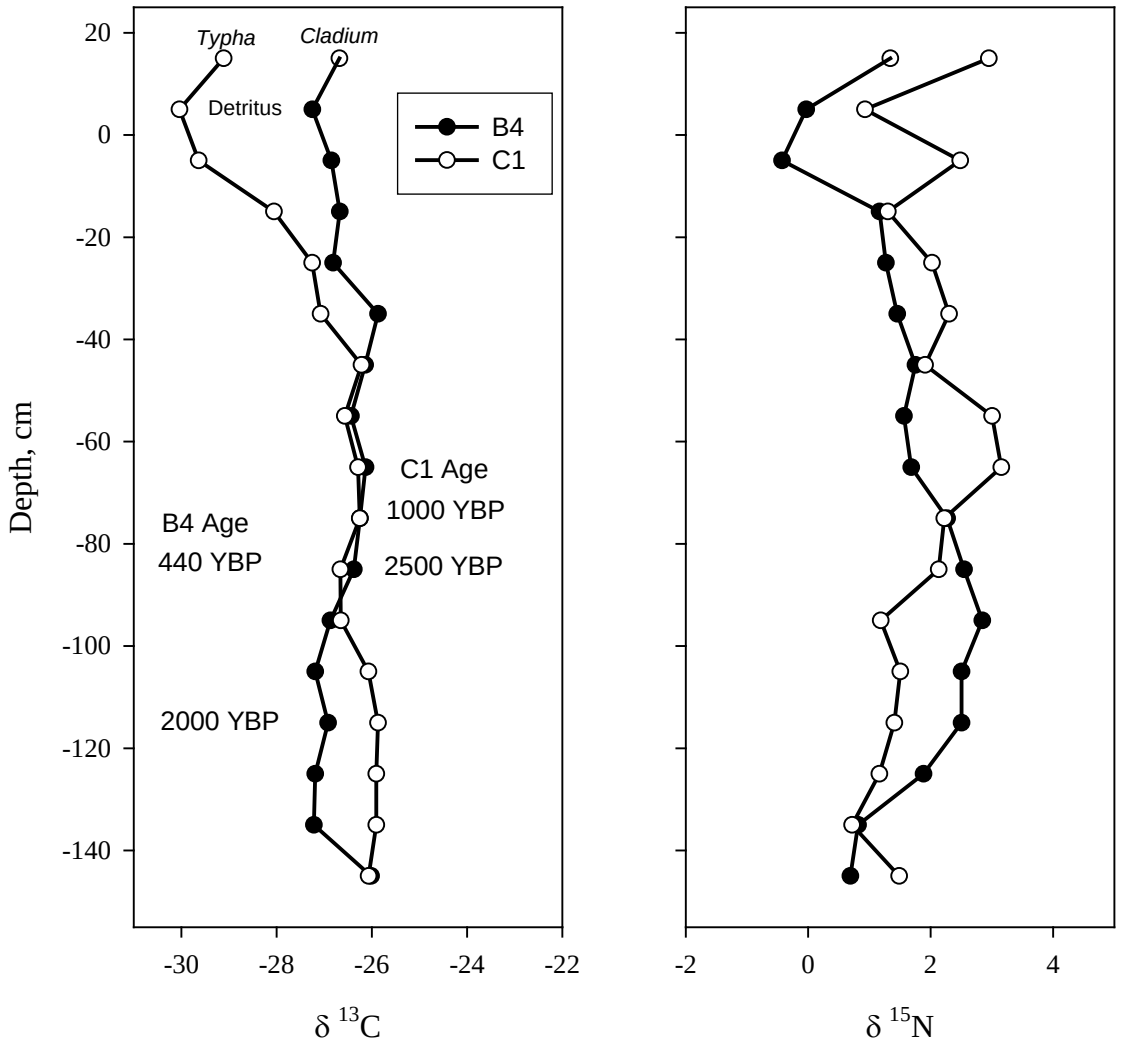


Figure 6.2. Stable isotopic composition of plant fiber that was separated from bulk peat samples collected from BCMCA stations B4 and C1. Carbon-14 dates posted on left plot to illustrate approximate soil age (YBP = years before present).

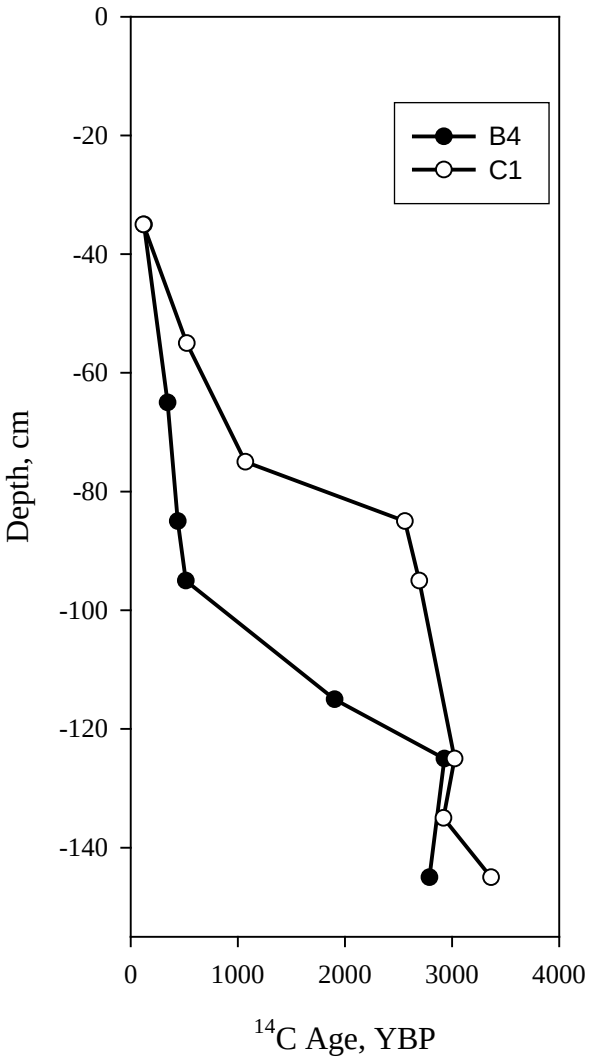
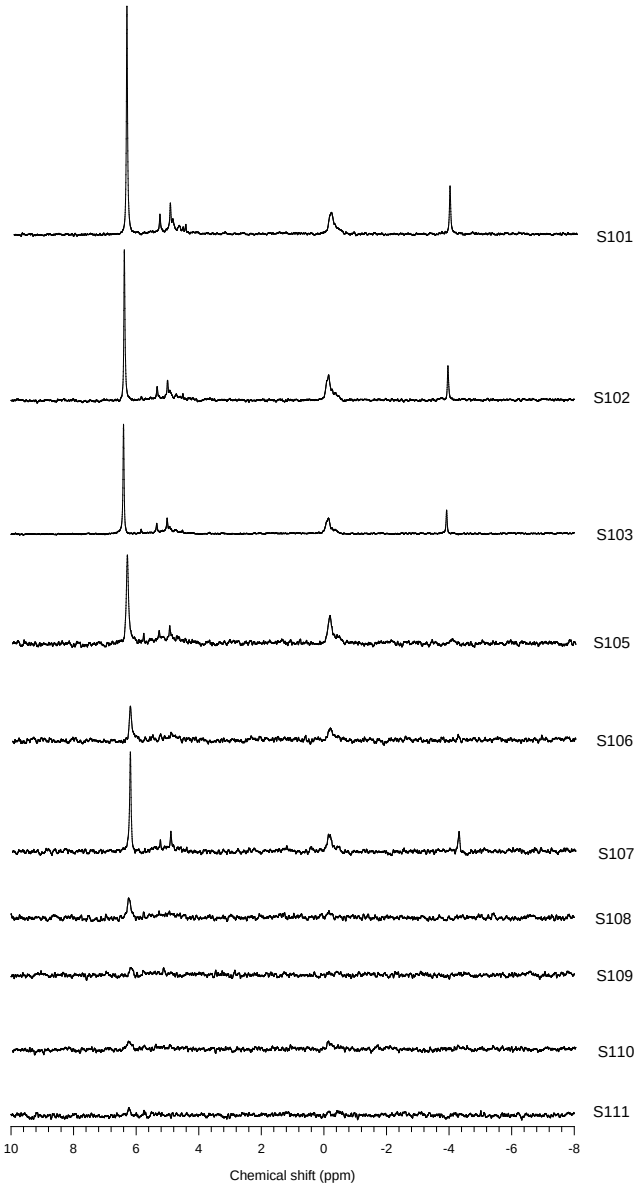


Figure 6.3. Uncalibrated ^{14}C age of plant macrofossils removed from selected depths at stations B4 and C1 in BCMCA (YBP = years before present).

Appendix A.



Chemical P-NMR shifts of extracted soil organic matter for a soil sample from BCMCA. The spectra are vertically stacked at 10-cm intervals, i.e., sample S101 is station B4, 0-10 cm depth interval, whereas sample S111 is the 90-100 cm depth interval.