COUPLING MICROBIAL ACTIVITY AND NUTRIENT CYCLING IN SOILS OF THE EVERGLADES AGRICULTURAL AREA

By

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To those who have faith and love for me

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TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
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The Everglades Agricultural Area (EAA) is an important agricultural region, but has been implicated in contributing to the deterioration of water quality in the Everglades wetlands primarily through runoff of nutrients. The objective of this research was to investigate impacts of selected agricultural management practices on soil biochemical processes as related to microbial activities, nutrient cycling, and water quality.

Long-term cultivation and nutrient and water management of EAA increased organic matter decomposition rates and altered supply of bioavailable nutrients. Evaluation of the major land uses in the EAA indicated that labile phosphorus (P) was the main soil chemical property affected by agricultural management practices. Phosphorus fertilization resulted in 465% higher concentrations of labile P in sugarcane soils compared to uncultivated soils. The uncultivated sites were the least disturbed, but the most P-limited and therefore had the smallest size of microbial population and lowest efficiency of C and N utilization. Statistical analysis revealed that P availability was the primary factor regulating the soil microbial community structure and function for land uses. Increased P availability promoted significant accumulation of P in microbial

biomass, which indicates that P fertilization is likely to enhance microbial activity and potentially increase organic matter decomposition and soil subsidence. Future management practices should consider the role of labile P on the function of microbial communities and their control of nutrient cycling.

Elemental sulfur (S) application to EAA soils is used as one of management practices to decrease pH and therefore increase the bioavailability of nutrients to crops. Sulfur application at rates up to 448 kg ha^{-1} did not significantly reduce soil pH due to the high buffering capacity against acidification. However, the highest S rate did increase P concentrations in the Fe-Al bound P fraction by 55% compared to unamended soils at 2 months. The stimulatory effects were limited and did not last beyond 2 months. Higher S application rates may be needed to overcome the soil buffering capacity and increase nutrient availability. However, the risk of P export as runoff should be well recognized since the Ca-bound P pool, which comprised of 32% of the total soil P, is not stable under acidic conditions.

Similar to labile P, water-extractable potassium (K) and acetic acid extractable Zinc (Zn) increased at 2 months after S application at the highest S rates by 71% and 134% respectively, but the stimulatory effects did not extend beyond 2 months. Correspondingly, S application at current recommended rates did not increase sugar yield. The failure of S application to enhance nutrient availability throughout the growing season indicates the limited benefit of applying elemental S to reduce pH and increase nutrient availability to sugarcane. Alternatives, such as different P and micronutrient fertilizer application methods, timings, and sources, may be better for increasing nutrient availability for these changing soils.

Sulfur did promote short-term changes in soil microbial activities. The activities of phosphatase and glucosidase in soils receiving 448 kg S ha^{-1} were 115% and 560% higher, respectively, than unamended soils at 2 months. Yet, microbial respiration and potential N and P mineralization rates were not affected by S amendment, suggesting that S application under the current recommendations would not enhance soil subsidence and regeneration rates of N and P. However, mineralized S increased with increasing S application rates and the effects continued throughout the growing season. Averaged across time, mineralizable S was 2, 6, 9, and 27 mg kg^{-1} d^{-1} for the increasing S application rates, indicating that S fertilization may pose an environmental hazard to the nearby aquatic ecosystem through potential export of SO_4. Therefore, using higher S application rates to produce favorable responses in terms of nutrient availability and sugarcane yield should be well evaluated in terms of both effects on sugarcane yield and adverse environmental effects.

Agricultural management practices have significantly altered nutrient distribution and regeneration in EAA soils, especially the P cycling. Phosphorus fertilization is to stimulate microbial activities and soil oxidation rates. Current land use as sugarcane cropping continues to promote soil subsidence. Elemental S application under current recommendation rates introduced temporary and limited effects on increasing nutrient availability to sugarcane and posed minimal impacts on microbial activity and function. However, S application at a large scale may increase the risk of SO_4 export from the fields and stimulate nutrient regeneration rates and microbial activity.

CHAPTER 1
INTRODUCTION

Soil supports diverse microbial communities that play important roles in ecosystem level processes such as decomposition of organic matter and nutrient cycling (Wright and Reddy, 2001). In natural systems, the soil microbial community composition and activity are related to the efficiency of nutrient cycling and ecosystem function. However, the richness, abundance, and activity are vulnerable to influence by biogeochemical properties such as pH, moisture, organic matter content, and nutrients (Carreira et al., 2000; Allison et al., 2007; Castillo and Wright, 2008; Morra et al., 2009). Alterations in the physical and chemical nature of soils may lead to shifts in microbial community composition and changes in microbial function.

Decomposition of organic matter is the major nutrient source in soils and is mediated by heterotrophic microorganisms. Microorganisms play a critical role in regulating nutrient cycling, as soil microbial biomass is considered both a source and sink for nutrients (Sicardi et al., 2004; Zhang et al., 2006). Plants exclusively utilize inorganic nutrients and therefore their growth depends on decomposition of organic matter, particularly in natural and agricultural ecosystems having low nutrient inputs (Yao et al., 2000; Chen et al., 2003). Microbial activity, such as extracellular enzyme activities and respiratory rates, reflects the potential of nutrient immobilization and mineralization, and hence are widely utilized to indicate changes in soil quality,

organic matter status, and nutrient cycling processes (Stevenson et al., 2004; Monkiedje et al., 2006; Nogueira et al., 2006).

Nutrient limitation is a key factor commonly constraining agricultural production and soil microbial populations. Agricultural management practices, such as organic amendments and fertilizer use, are commonly applied to enhance crop yields. These practices result in alterations of nutrient distribution in the soil profile and shifts in microbial community structure and function. For instance, tillage enhances organic matter turnover by increasing the presence of oxygen throughout the soil profile and consequently enhances microbial respiration (Gesch et al., 2007). Fertilization overcomes potential nutrient limitations and stimulates microbial activity and crop growth (Castillo and Wright, 2008a). Organic amendments often improve soil characteristics and enhance microbial activity (Tejada et al., 2006). However, excessive nutrients from fertilization or decomposition can be immobilized in soil organic matter, or lost as leaching or runoff from fields, which may cause serious environmental problems to the adjacent ecosystems. Thus, nutrient management in agricultural fields is especially important for critical or sensitive ecosystems, such as those in south Florida near the Everglades.

Everglades Agricultural Area

The Everglades Agricultural Area (EAA) is located south of Lake Okeechobee and north of the Water Conservation Areas (WCAs) in south Florida (Fig. 1-1). It consists of an area of approximately 283,300 ha which was drained in the early

1900s for agricultural production (Chen et al., 2006). Currently, the land use is predominantly sugarcane with a smaller portion dedicated to rice, vegetables, and turf sod production. The soils are primarily Histosols with an organic matter content as high as 80-90% (Snyder et al., 2005). Due to the draining and establishment of the EAA, soil subsidence has increasingly become a critical problem. Subsidence resulted from the oxidation of organic matter upon initiation of drainage in the early 1900s (Gesch et al., 2007). Along with subsidence, excessive N and P are released during organic matter mineralization which has altered nutrient cycling and soil processes (Morris and Gilbert, 2005). The current long-term estimate of soil subsidence is approximately 1.5 cm yr^{-1} (Morris and Gilbert, 2005). At this rate of loss, soils may become too shallow for many agricultural use and sugarcane production in the near future (Anderson and Flaig, 1995; Morris and Gilbert, 2005).

Long-term cultivation of these soils, specifically tillage, has resulted in the incorporation of bedrock $CaCO_3$ into surface soil and has gradually increased the pH from the historic 5.0 to 5.5 to approximately 7.0 to 7.5 today (Snyder, 2005). As a result, P and micronutrient availability to crops has decreased and necessitated new fertilizer management practices. Elemental S is occasionally applied for the purpose of reducing pH and therefore improving the availability of micronutrients and P to sugarcane (Schueneman, 2001). The microbial oxidation of elemental S to SO_4 produces acidity which reacts with the soil and reduces pH, which in turn releases P and micronutrients from adsorption sites into soil solution. However, the buffering

capacity of these calcareous Histosols is strong and can counteract the acidifying effects of elemental S oxidation, thus effects of amendments may only be temporary and may need to be repeated each growing season (Beverly and Anderson, 1986).

Sugarcane is the dominant crop grown in the EAA, and requires approximately 30 kg P ha^{-1} $year^{-1}$ and extensive tillage for pre-plant preparation and weed control (Rice et al. 2006). Elements that are of nutritional concern for sugarcane production include N, P, K, Mg, B, Cu, Fe, Mn, Si, and Zn (Rice et al., 2006). To prevent nutrient deficiency and maximize sugar production, fertilizer recommendations were introduced by the University of Florida's Institute of Food and Agricultural Science (IFAS) (Anderson, 1985; Rice et al., 2006). Long-term P application has resulted in P accumulation in the soil profile as well as export into Everglades wetlands through canal systems, which was a major factor contributing to the deterioration of water quality and alterations of the Everglades wetland ecosystem (Childers et al. 2003). Studies have demonstrated that most of applied P fertilizers enter the Fe-Al bound and Ca-Mg bound P fractions, which together account for 60% of the total P in EAA soils (Wright, 2009). Phosphorus retained in Fe-Al and Ca-Mg bound pools of these calcareous soils are considered relatively stable under current drained conditions (Wright, 2009). Several factors are capable of influencing P stability and mobility in the soil profile including pH, microbial activity, and soil amendments (Arai et al. 2005; Jaggi et al. 2005). Application of elemental S at a rate of 500 µg g^{-1} significantly enhanced available P concentrations in alkaline soils having a pH of 10.2 (Jaggi et

al., 2005). Jaggi et al. suggested that increased P concentrations resulted from lowered pH and replacement of SO_4 with PO_4 from soil adsorption sites. However, such stimulatory effects were not observed for other studies of calcareous soils with a pH of 8.1 (Hassan and Olson, 1966).

The ecosystem of south Florida has been greatly modified as a result of agriculture and urbanization. Driven factors include deterioration of water quality and alterations in ecosystem-level processes (Chimney and Goforth, 2006), thus improvements in water quality is a major goal of Everglades rehabilitation projects (Gabriel, 2008). Reducing nutrient exports from the EAA to the remnant Everglades is an ongoing strategy for improving water quality (Chimney and Goforth, 2006; Gabriel, 2008). Therefore, a better understanding of nutrient dynamics and land management within the EAA is critical.

Land Use Change

Acknowledging the fact that subsidence clouds the future of agriculture in the EAA, strategies have been implemented to increase sustainability of these soils (Grigg et al., 2002; Morris et al., 2004). Major land use changes are considered inevitable in the near future, likely in the order of decades (Anderson and Rosendahl, 1998; Snyder, 2005). An emerging interest is to convert current land uses back to prior uses as wetland prairie. Under flooded conditions, degradation of organic matter and the subsidence rate can be reduced or eliminated (Grigg et al., 2002). Snyder (2005) proposed possible land uses in the EAA over the next 50 years

including growing pasture grasses and utilizing forestry in areas with shallow organic soils. Additionally, in consideration of the continuous growing population and urbanization, more agricultural lands are expected to change to home development sites with turfgrass coverage (Anderson and Rosendahl, 1998). Clearly, many land use options can be explored to serve as alternatives to traditional agriculture. However, far less is known about the influence of land use changes on the EAA ecosystem with regard to nutrient cycling and microbial community dynamics.

Sulfur in the Everglades

Sulfur is required by all biological materials as an essential macronutrient (Wang et al., 2006) and it is present in soils in various forms, each of which play important biological and chemical roles. Sulfate is the most abundant form of inorganic S found in most soils and the main form available to plants, although reduced forms, such as elemental S, thiosulfate, and sulfide, can be found under anaerobic conditions (Zhou et al., 2005). However, the majority of soil S is in organic form, which consists of two components, ester S and C-bound S, serving as the main source for inorganic S (Solomon et al., 2001). Mineralization of organic S compounds is mediated by heterotrophic microbial activity and closely associated with C and N mineralization (Gharmakher et al., 2009). McGill and Cole (1981) proposed a conceptual model for cycling of organic C, N, S and P through soil organic matter, in which S mineralization involves biological and biochemical processes. In biological processes, SO_4 is released as a by-product of C oxidation,

while SO_4 can also be a direct product of enzymatic hydrolysis (McGill and Cole, 1981).

Potential S sources to the EAA include soil amendments, fungicides, and fertilizers (Orem, 2007). Since the 1920s, S has been applied to soil as $CuSO_4$ to enhance crop yields via increasing the availability of Cu to these micronutrient poor soils (Allison et al., 1927). In later years, elemental S was recommended to lower soil pH when it exceeded 6.6 for the purpose of improving the availability of micronutrients needed for sugarcane growth (Anderson, 1985). The IFAS recommended rate is 560 kg S ha^{-1}, but Schueneman (2001) indicated that actual S application rates in the EAA are lower than the IFAS recommendations. Everglades Agricultural Area growers tend to use micronutrient sprays to alleviate nutrient deficiency caused by elevated pH since it is more cost effective, so S application may not be considered necessary at this stage. However, due to the increasing soil pH and decreasing soil depth to bedrock since 1985, revision of this recommendation may be required. There is a need to determine the level of S application producing favorable responses in terms of nutrient availability and sugarcane yield.

There is widespread S contamination of surface water and sediments in the northern Everglades (Bates et al., 1998; Bates et al., 2002). Potential sources contributing to the S enrichment include agricultural S, groundwater, rainwater, seawater aerosol, S flux from sediments, and influx of water from Lake Okeechobee

(Bates et al., 2002). However, it has been suggested that S from fertilizers and soil amendments are the primary contributors to SO_4 enrichment of the Everglades (Bates et al., 2002; Orem, 2007).

The environmental and ecological significance of S in Everglades wetlands is principally the stimulation of MeHg formation, which is catalyzed by SO_4-reducing bacteria under anaerobic conditions (Bates et al., 2002). The MeHg is a neurotoxin that is bioaccumulative and found in high concentrations in fish and other wildlife in the Everglades (Orem, 2007). During the process of SO_4 reduction, P bound to the organic matter can be released. As such, the presence of high SO_4 concentrations can lead to internal eutrophication (Gilmour et al., 2007). Two primary mechanisms are possibly responsible for the internal eutrophication. First, production of sulfide results in the release of nutrients, particularly NH_4-N and PO_4. Second, NH_4-N and PO_4 are mobilized through the generation of alkalinity (Gilmour et al., 2007; Gabriel et al., 2008). Furthermore, SO_4 reduction produces sulfide, which can be toxic to the aquatic plants and animals (Orem, 2004).

In consideration of the adverse impacts that S poses to the Everglades wetlands, reducing potential S exports from the EAA is beneficial for protecting water quality and ecosystem health (Gabriel at al., 2008). Explicit quantification of S budgets and transformations within EAA soils is limited, so there is a need to study the seasonal dynamics of S as related to S application practices and the risk of S exportation.

Objectives and Hypothesis

The present study was designed to experimentally determine the influence of select land uses (sugarcane, forest, and uncultivated) and management on the microbial activity and nutrient cycling in EAA soils (Fig. 1-2).

The specific objectives and hypotheses were to:

- Investigate impacts of land uses (sugarcane, forest, and uncultivated) on microbial activities and cycling of C, N, and P (Chapter 2).

  Hypothesis: Long-term land management (P fertilization) has altered C, N and P cycling as well as microbial activity and community composition

- Identify linkages between microbial activities and soil biogeochemical properties using multivariate methods (Chapter 3).

  Hypothesis: Differences in microbial function under various land-use types can be explained by variations in soil biogeochemical properties

- Determine effects of S amendment on P cycling and related processes during the sugarcane growing season (Chapter 4).

  Hypothesis: Sulfur application will reduce soil pH and consequently influence soil P forms and availability

- Evaluate the microbial eco-physiological response to S amendment (Chapter 5).

  Hypothesis: Sulfur application will increase nutrient availability and stimulate microbial activity

- Determine S addition effects on nutrient availability to sugarcane (Chapter 6).

  Hypothesis: Micronutrient availability to sugarcane will be enhanced due to S application

- Quantify S forms and transformations in soils amended with elemental S (Chapter 7).

  Hypothesis: Sulfur application will modify S forms and transformations in EAA soils

The completion of this study is expected to provide information to better manage nutrient and lands within the EAA while minimizing adverse environmental impacts. More specifically, completion of this project would provide information to a) predict effects of future land use changes on nutrient cycling; b) reduce nutrient export from fields; c) improve water quality; d) satisfy nutrient requirements for crops; e) optimize fertilizer use efficiency; and f) contribute to more efficient and effective management of south Florida ecosystems.

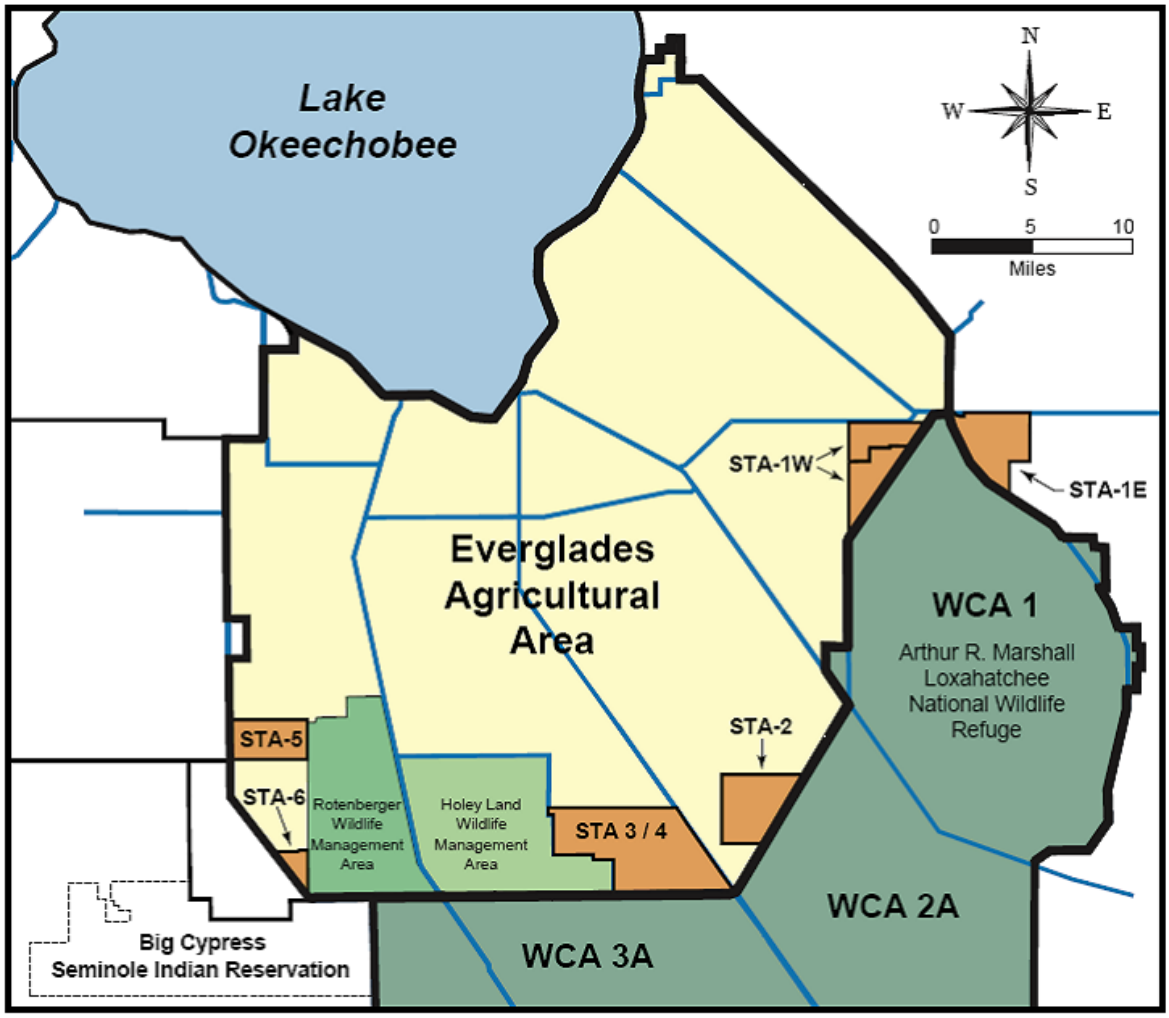

Fig. 1-1. Map of the Everglades Agricultural Area in south Florida. Source: South Florida Water Management District.

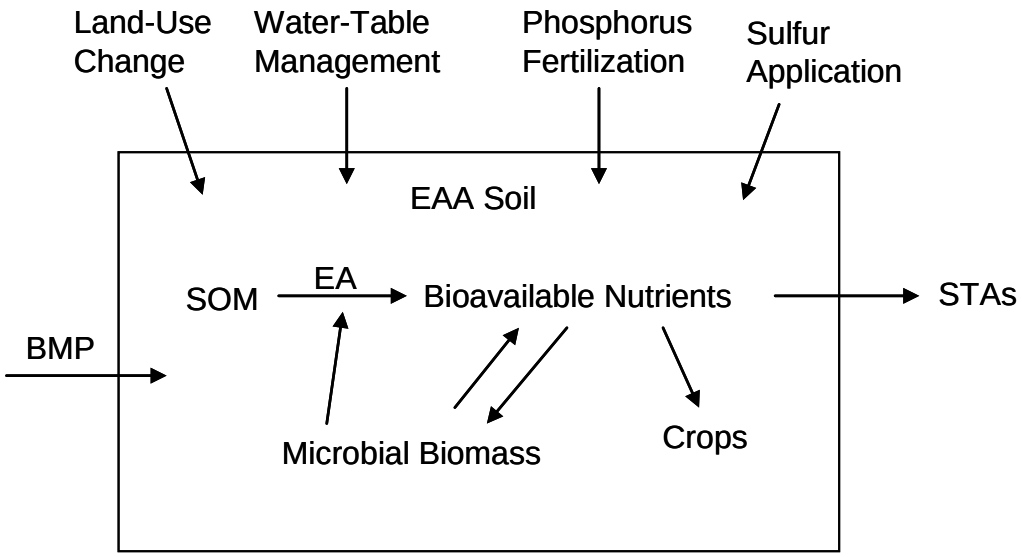

Fig. 1-2. Conceptual scheme showing selected land managements that potentially affect biogeochemical processes in EAA soils. BMP, best management practices; SOM, soil organic matter; EA, enzymatic activity; STAs, storm-water treatment areas.

CHAPTER 2
LAND USE EFFECTS ON SOIL NUTRIENT CYCLING AND MICROBIAL COMMUNITY DYNAMICS IN THE EVERGLADES AGRICULTURAL AREA, FLORIDA

Introduction

Soil supports diverse microbial communities that play important roles in ecosystem level processes such as decomposition of organic matter and nutrient cycling (Wright and Reddy, 2001a). In natural systems, soil microbial community composition and activity are related to the efficiency of nutrient cycling and ecosystem function (Yao et al., 2000). However, the richness, abundance, and activity of the microbial community is vulnerable to influence by soil physical and chemical properties such as pH, moisture, organic matter content, and nutrient availability. Alterations in the physical and chemical nature of the soil may lead to shifts in microbial community composition and changes in microbial function. Agricultural practices such as fertilization and tillage influence soil chemical properties and nutrient dynamics throughout the soil profile (Gesch et al., 2007; Wright et al., 2007). Therefore, changes in land uses with different intensity or history of agricultural practices consequently results in distinctive changes in microbial community composition and function (Grayston et al., 2004). Land use changes can also disrupt carbon (C) and nitrogen (N) dynamics and organic matter storage in soils across a range of habitats (Garcia-Oliva et al, 2006; Monkiedje et al., 2006), which are commonly viewed as major factors causing shifts in microbial community composition (Schimel and Bennett, 2004; Cookson et al., 2007).

The Everglades Agricultural Area (EAA) is located south of Lake Okeechobee and north of the Water Conservation Areas (WCAs) in south Florida. It consists of an area of approximately 283,300 ha which was artificially drained in the early 1900s for agricultural production (Chen et al., 2006). Currently, the land use is primarily

sugarcane with a smaller portion dedicated to vegetables. The soils in the EAA are primarily Histosols with organic matter contents as high as 80-90% (Snyder et al., 1978). Due to the draining and establishment of the EAA, soil subsidence has become a critical problem, which resulted from the oxidation of organic matter (Gesch et al., 2007). Along with subsidence, excessive N and phosphorus (P) were released from organic matter mineralization, which altered nutrient cycling and soil processes (Morris and Gilbert, 2005). The current long-term estimate of soil subsidence is approximately 1.5 cm yr^{-1} (Morris and Gilbert, 2005). At this rate of loss, soils will become too shallow for agricultural use and sugarcane production in the future (Anderson and Flaig, 1995; Morris and Gilbert, 2005). Acknowledging the fact that the subsidence clouds the future of agriculture in EAA, strategies have been implemented to increase sustainability of agriculture (Grigg et al., 2002; Morris et al., 2004). However, land use changes in EAA are considered inevitable in the near future, likely on the order of decades (Anderson and Rosendahl, 1998; Snyder, 2005).

An emerging interest is to convert current land uses back to prior uses as prairie. Under flooded conditions, degradation of organic matter and the subsidence rate can be reduced or eliminated (Grigg et al., 2002). Snyder (2005) further proposed the possible land uses in the EAA over the next 50 years, which included growing pasture grasses and planting cypress trees in areas with shallow organic soils. Additionally, in consideration of the continuous growing population and urbanization, more agricultural lands are expected to change to home development sites with turfgrass coverage (Anderson and Rosendahl, 1998). Obviously, many land use options can be explored to serve as alternatives to traditional agriculture. However, far less is known about the

influence of land use change on EAA ecosystems with regard to nutrient cycling and microbial community dynamics. The objectives of the present study were to determine and characterize the impacts of land use on the soil microbial community composition and activity and to investigate the effects of land use on nutrient cycling.

Materials and Methods

Site Description

The study sites are located in the northern EAA at the Everglades Research and Education Center near Belle Glade, FL. The long-term average annual rainfall is 133 cm and temperature is 24°C. All soils are Dania muck (euic, hyperthermic, shallow Lithic Medisaprists) with a depth to the bedrock of approximately 45 cm. These organic soils developed under seasonal flooding and low nutrient status and supported vegetation adapted to these conditions, primarily sawgrass (*Cladium jamaicense* Crantz). Due to conversion to agricultural use by drainage, the dominant vegetation shifted to annual crops of vegetable and sugarcane (*Saccharum* sp.) in the early 1900s. Four land uses were selected for this study to mirror possible land uses in the future: soils under forest for 19 years, fields under sugarcane production for approximately 50 years, turfgrass lawns for 60 years, and fields under perennial pasture for approximately 100 years. Four field sites were randomly sampled for each land use. The forest soils were previously cropped to sugarcane but planted to bald cypress (*Taxodium distichum*) and pond cypress (*Taxodium ascendens*) in 1988. These fields did not receive any fertilization after land use change, but were extensively tilled prior to seedling establishment, and no further management has been applied. The sugarcane fields were managed for vegetable production from the early 1900s to the 1950s, but mainly

for sugarcane since the 1950s. Fertilization was applied at a rate of 40 kg P ha^{-1} yr^{-1} (Gilbert and Rice, 2006) prior to planting. Sugarcane is planted from August through January and harvested from October through April. Tillage operations included several disking (to 15 cm depth) after crop harvest, subsoil chiseling (to 30 cm depth) to improve drainage, and frequent in-season tine cultivations (to 4 cm depth) for weed control (Morris et al., 2004). The turf fields were vegetated by St. Augustinegrass [*Stenotaphrum secundatum* (Walt) Kuntze] turf since the mid 1940s. The uncultivated field was primarily occupied by paragrass [*Panicum purpurascens* (L.) Raddi] and bermudagrass [*Cynodon dactylon* (L.) Pers]. Turf and uncultivated fields were periodically mowed with residues returned to soil, but received no fertilization and tillage since establishment.

Soil Sampling and Physical-Chemical Analysis

Surface soil (0-15 cm) samples were collected from 4 replicate fields of each land use on March 2007. The soils were homogenized after the removal of large plant particles and stored at 4°C until use. Moisture content was measured as the mass loss after drying at 70°C for 5 days. Soil organic matter content was estimated by the loss-on-ignition method after ashing at 550°C for 4 hours (Anderson, 1976). Total organic C was then calculated from the organic content by using a factor of 0.51 (Anderson, 1976). Total C, total N, and total P were determined using the oven dried (70°C) and ground soil. Total C and N were measured with a Carlo-Erba NA 1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ), while total P was evaluated after ashing (Bremner, 1996) using the ascorbic acid-molybdenum blue method (Kuo, 1996) with an AQ2+ discrete analyzer (Seal Analytical Inc., Mequon, WI).

Microbial Biomass

Microbial biomass C (MBC), N (MBN) and P (MBP) were measured by the fumigation-extraction method (White and Reddy, 2001). The amount of K_2SO_4-extracted C was determined with a total organic carbon analyzer TOC-5050A (Shimadzu, Norcross, GA). The microbial biomass C was calculated from the difference in extractable C between fumigated and unfumigated samples using a conversion factor of 0.37. After digestion, the K_2SO_4-extracts were measured for total Kjeldahl N using an AQ2+ discrete analyzer (Seal Analytical Inc., Mequon, WI). The microbial biomass N was calculated from the difference in total Kjeldahl N between fumigated and unfumigated samples using K_{EC} = 0.54. The total P content of the $NaHCO_3$ extracts used for microbial biomass P analysis was measured for P as previously described (Kuo, 1996). The microbial biomass P was determined as the difference in total P of $NaHCO_3$ extracts from fumigated and unfumigated samples. Labile inorganic P ($NaHCO_3$-Pi) was measured for unfumigated soil extracts and analyzed for P as previously described (Kuo, 1996).

Potentially Mineralizable N and P

Potentially mineralizable N (PMN) was determined according to methods of White and Reddy (2000) based on a 10-day incubation followed by extraction with 2M KCl. Extracts were analyzed for NH_4-N (White and Reddy, 2000). Potentially mineralizable P (PMP) was measured using the method of Corstanje et al. (2007) with slight modifications. 0.5 g dry soil were placed in 30-ml serum bottles and mixed with 5 ml of water. The bottles were then capped incubated in the dark at 40°C for 10 days. At 10 d, 20 ml of 1M HCl was injected and soil shaken for 3 hours. Extracts were filtered through

0.45 µm membrane filters. Another set of equivalent weight samples, without incubation, were directly extracted with 25 ml of 1M HCl and extracts were analyzed for total P. The potentially mineralizable P was determined as the difference in total P of extracts between incubated and non-incubated soil.

Enzyme Activity Assay

Approximately 1 g moist soil was placed in polypropylene centrifuge tubes, mixed with 30 ml of water, and shaken for 25 minutes. Homogenized samples were further diluted 5 times for enzyme assays. Enzyme assays were conducted in three or four replicates with controls to offset non-enzymatic production. For cellobiohydrolase assay, the substrate used was 2 mM p-nitrophenol-cellobioside (ACROS Organics, Geel, Belgium). 0.75 ml of diluted samples and 0.75 ml of substrates were mixed in 2-ml micro centrifuge tubes and incubated at 20°C for 20 hours with gentle shaking. At the end of the incubation, the mixtures were centrifuged at 10,000 rpm for 1 minute, 0.75 ml supernatants were transferred to new tubes, followed by the addition of 0.075 ml of 1 N NaOH to stop the reaction and develop the color. The mixtures were then analyzed for absorbance with a spectrophotometer UV-160 (Shimadzu, Norcross, GA) at 420 nm. The enzymatic activity was expressed as mg p-nitrophenol released per gram dried soil per hour. Leucine aminopeptidase assay was conducted in 96-well microtiter plates (Prenger and Reddy, 2004). 200 µl of samples was incubated with 50 µl substrates, 5 mM L-Leucine 7-Amino-4-methylcoumarin (Biosynth, Naperville, IL), at 20°C for 8 hours. The florescence readings were collected at 1 hour intervals using a fluorescence plate reader, Bio-TEK FL600 (Bio-TEK Instruments Inc., Winooski, VT), at a setting of 365 nm excitation and 450 nm emission. Enzyme activity was determined by calculating

the mean florescent reading changes over time with a standard curve and expressed as mg 7-amino-4-methylcoumarin released per gram dried soil per hour. Alkaline phosphatase and sulfatase assays were conducted according to the methods of Wright and Reddy (2001a).

Community-Level Physiological Profile by BIOLOG Assay

Community-Level physiological profiles (CLPPs) were determined by direct incubation of fresh soil extracts in BIOLOG Eco-Plates (31 substrates) (BIOLOG Inc.). Approximately 1 g moist soil samples was mixed with 20 ml of water and gently shaken for 20 minutes. The homogenized samples were then diluted 400 times and soil particles allowed to settle for 15 minutes at 4°C. 150 μl of the supernatants was subsequently dispensed into each well of Eco-Plates and incubated at 20°C for 7 days. Optical densities were measured every 6 or 12 hours using Bio-TEK FL600 (Bio-TEK Instruments Inc., Winooski, VT) at 590 nm. Absorbance values of each well with C sources were blanked against control wells before analysis. Negative values were considered as 0. Community metabolic diversity (CMD) was calculated by summing up the numbers of positive responses in each plate. Positive responses were defined as any absorbance values greater than 0.25 (Garland, 1997). Average well color development (AWCD) was determined as described by Garland (1996, 1997). To overcome possible interference by inoculum density on color development, absorbance values for various C sources were standardized by dividing the blanked value of each well by the AWCD of the plate and were subsequently used for principal component analysis.

Statistical Analysis

Significant differences among land uses for all the variables were determined by one-way ANOVA and Tukey's test at $\alpha = 0.05$. Multivariate analysis on the BIOLOG profiles was carried out by principal component analysis (Preston-Mafham et al., 2002; Campbell et al., 2003). All computations were conducted on computing software JMP V4 (SAS Institute Inc., NC).

Results

Soil Physical and Chemical Properties

Soil organic matter content was highest in uncultivated soil and decreased in the order of forest, sugarcane and turf soil, respectively (Table 2-1). Total C and N were also highest in uncultivated, averaging 461 g C $kg^{-1}$and 32 g N kg^{-1}, respectively. However, in contrast to total C and N, total P was lowest in uncultivated soils, averaging 0.78 g P kg^{-1}. Extractable C was significantly higher in sugarcane (2.34 g C kg^{-1}) than other land uses (Table 2-1). However, no difference in extractable C was found among forest, turf and uncultivated soil. Extractable NH_4-N in forest and sugarcane soils was lower than turf and uncultivated soils (Table 2-1). Labile inorganic P was higher in sugarcane soil (96 mg P kg^{-1}) than forest (43 mg P kg^{-1}) and uncultivated (17 mg P kg^{-1}). The highest inorganic P was found in turf soil (173 mg P kg^{-1}); however, it was not statistically different from other land uses. Potentially mineralizable N was four times lower in sugarcane (2.66 mg N kg^{-1} d^{-1}) than other land uses (Table 2-2). Potentially mineralizable P varied widely in the turf soil, averaging 33.1 mg P kg^{-1} d^{-1}, which was about 8 times higher than other land uses (Table 2-2), but the difference was not significant.

Microbial Biomass

Soil microbial biomass C, ranging from 9.3 to 13.5 g C kg^{-1}, was lower in uncultivated soil than forest and sugarcane (Table 2-2). The microbial biomass C to organic matter ratio in the turf soil (23374 mg kg^{-1}) was two times higher than uncultivated soil, which was also higher than the forest (15183 mg kg^{-1}) and sugarcane (16857 mg kg^{-1}) soils. As well, the microbial biomass C to organic matter ratio was lower in uncultivated soil than forest and sugarcane. Additionally, microbial biomass N was lowest in uncultivated soil (0.12 g N kg^{-1}) and highest in turf soil (0.32 g N kg^{-1}). Soil microbial biomass P was not significantly different among land uses.

Extracellular Enzyme Activity

Cellobiohydrolase activity was highly varied in uncultivated soil, averaging 271 mg g^{-1} h^{-1}, which was not significantly different from other land uses (Table 2-2). However, activities in forest, sugarcane and turf soils varied considerably, averaging 140, 51, and 191 mg g^{-1} h^{-1}, respectively. Uncultivated soil had higher alkaline phosphatase (1.21 mg g^{-1} h^{-1}) than forest (0.70 mg g^{-1} h^{-1}) and sugarcane (0.94 mg g^{-1} h^{-1}). Sulfatase activities were higher in uncultivated soil (0.42 mg g^{-1} h^{-1}) than sugarcane soil (0.24 mg g^{-1} h^{-1}), but were not different from turf soil (0.44 mg g^{-1} h^{-1}) and forest soil (0.32 mg g^{-1} h^{-1}).

Microbial Community Composition and Function

We used the CMD and AWCD to describe the average numbers of substrates potentially utilized and respiration rates from the C-sources by microbial communities. Both CMD (Fig. 2-1) and AWCD (data not shown) followed a sigmoidal curve over time. On day 1, no color development was observed; nevertheless on day 3.25 both CMD and AWCD of forest and turf reached their mid-point. Since the actual rates of color development changed with time, the comparison among land uses in relative rates was

made in the interval of day 1 to day 3.25. The CMD of the microbial communities was higher in forest and turf than sugarcane and uncultivated (Fig. 2-1). On day 3.25, more than 55% of the wells were positive in plates inoculated with turf or forest soil, yet only 15% of wells were found positive in plates from sugarcane soils, and 6% of wells from uncultivated soils showed positive responses (Fig. 2-1). The rates of color development (AWCD) followed the same pattern as CMD, being highest in turf and forest and lower for sugarcane and uncultivated (data not shown). Principal component analysis on the utilization patterns of all of the 31 substrates on day 3.25 revealed clear differentiation among land uses in the composition of active members of the soil microbial community (Fig. 2-2). In particular, ordination axis 1 demonstrated obvious separation between turf and either the uncultivated or sugarcane. Furthermore, ordination axis 1 separated the soil microbial community in forest from that of uncultivated soil. No apparent separations were observed on axis 2. However, microbial community composition in forest soils was distinct from sugarcane soils on ordination axis 3.

Discussion

Nutrient Distribution and Cycling

To investigate land use effects on nutrient distribution and cycling, samples from the 0-15 cm depth were utilized since surface soils are most susceptible to changes in chemical and physical properties (Garcia-Oliva et al., 2006). Long term cultivation and fertilization in the EAA greatly altered nutrient distribution and increased the organic matter turnover rates (Table 2-1). Uncultivated soils had higher organic matter content than other land uses, and were the only land use never subjected to tillage, while various tillage practices were often applied to sugarcane fields to maintain drainage and

for weed control (Morris et al., 2004). It has been reported that tillage practices alter the below ground ecosystem and expose subsurface organic matter to the aerobic environment, which increases organic matter decomposition rates and may lead to soil subsidence (Reicosky and Lindstrom, 1993; Gesch et al., 2007). Thus, tillage operations in sugarcane likely contributed to its lower organic matter content than other land uses. Lohila et al. (2003) proposed that tillage-induced soil C loss was likely greater for Histosols than mineral soils. Since the dominant soil of EAA is Histosols and the subsidence problem is considered a result of aerobic oxidation of organic C (Shih et al., 1998), it is reasonable to postulate that frequent tillage may worsen the subsidence problems in EAA (Gesch et al., 2007).

Labile inorganic P is a readily available fraction of P that remains soluble until either absorbed or precipitated to Fe, Al, Ca, and Mg (Anderson et al., 1994). Therefore, it is mobile with drainage, runoff or shallow groundwater. Sugarcane soil had about 6 times more labile inorganic P than uncultivated soil and 2 times more than forest due to its intensive fertilization history. However, we did not find any significant difference in labile organic P and PMP among soils of those land uses. In addition, labile inorganic P of the sugarcane soil accounted for 10% of its total P stock, which was higher than other land uses, especially uncultivated soil (2%). In contrast, uncultivated soil had 10% of its total P as labile organic P, which was significantly greater than other land uses. Thus, intensive fertilization and management increased soil P retention in inorganic forms rather than organic and conversely, land uses with minimal cultivation had a greater P sequestration in organic fractions (Graham et al., 2005). Furthermore, practices under sugarcane cropping releases more dissolved inorganic P to drainage water than both

uncultivated and forest, which poses a greater threat to the downstream ecosystems. The Florida Everglades is highly sensitive to small increases in P concentrations (Noe et al., 2001). Phosphorus in the drainage water from the EAA is considered to be the key contributor to the eutrophication of Lake Okeechobee and the Everglades (Childers et al., 2003). Our results support the idea that reduced management practices and P fertilization intensity potentially decrease the P content in the EAA drainage water (Izuno et al., 1991), thus land uses that minimize soil disturbance decrease potential for eutrophication of downgradient aquatic systems.

The C: N: P molar ratios of forest, sugarcane, turf and uncultivated soil were 939:54:1, 1178:67:1, 399:23:1, and 1528:93:1, respectively, suggesting that the uncultivated soil was the most P-limited land use, followed by the sugarcane and forest. There were no obvious differences in C: N ratios among land uses. However, the difference in both the C: P and N: P ratios were significant, indicating that agricultural practices greatly influenced the P sequestration in the soil. Alkaline phosphatase activity plays an important role in the P-limited ecosystem with respect to the regeneration of P from organic forms (Wright and Reddy, 2001a). Hence, it was not surprising to find that the uncultivated soil exhibited the highest alkaline phosphatase activity, followed by the sugarcane and forest soils. No significant correlation between alkaline phosphatase activity and soil P parameters implied other environmental factors likely contributed to this observation.

Microbial Community Dynamics

Microbial communities are in close contact with soil microenvironments, and therefore are easily subjected to change following alteration of soil properties (Corstanje

et al., 2007). Hence, changes in land use may cause a shift in the composition of active fractions of microbial communities, which can be explained by the changes to indices of microbial activity such as respiratory capacities and extracellular enzymatic activities (Wright and Reddy, 2001b; Corstanje et al., 2007).

Significant correlations between microbial biomass C and either organic matter or dissolved organic C has been frequently observed (Yao et al., 2000; Cookson et al., 2007). However, in the present study we did not find any of such correlation. The microbial biomass C to organic matter ratio is thought to be indicative of the organic matter quality and availability (Monkiedje et al., 2006). Our results showed that the ratio was highest in turf and lowest in uncultivated soil (Table 2-2), which suggested that turf had the highest chemical diversity in organic matter sources and efficiency of C utilization and conversely, the uncultivated soil had the lowest. This statement was further supported by the findings that microbial biomass C to organic matter ratio was significantly correlated with AWCD (R^2 = 0.81) and CMD (R^2 = 0.65). Uncultivated soil has lower overall plant coverage than turf, forest, and sugarcane (Shih et al., 1982), thus it is reasonable to expect lower chemical diversity of organic matter sources. Analysis of PMN to microbial biomass N ratio also revealed that uncultivated soil had a significantly higher ratio than other land uses, indicating that its microbial communities had the lowest efficiency in utilizing N resources. Regarding the fact that uncultivated soil had lower microbial biomass C and N, it was possible that uncultivated soil had the lowest abundance of microbial populations, which may explain its low efficiency in C and N utilization. No statistical difference was found in PMP to total P ratio and to microbial biomass P ratio, suggesting less effects of land use on the overall potential P

turnover rates and utilization efficiency. In consideration of its lower abundance of microbial populations and poorer efficiency of C and N utilization, uncultivated soil may experience lower rates of organic matter degradation, which was also evidenced by its higher organic matter content (Table 2-1). Shih et al. (1982) reported that oxidation rates were higher in uncultivated than sugarcane and forest due to its higher soil temperature, mainly resulting from lower vegetative cover and greater exposed area. Uncultivated fields were periodically mowed with residues returned back to soil, and soil was periodically covered with layers of plant residues, which provided inputs of organic matter and prevented the temperature increases. Thus, results of temperature effects on soil oxidation may be confounded by differences in sampling time and sites. Interestingly, we found significant correlations between microbial biomass C to organic matter ratio and total P (R^2 = 0.70), labile inorganic P (R^2 = 0.72), total labile P (R^2 = 0.73), and microbial biomass P (R^2 = 0.58) implying that P plays the critical role in determining the efficiency of C utilization and degradation of organic matter by soil microorganisms. It is well known that nutrient availability, such as P, greatly influences soil microbial activity and function (Wright and Reddy, 2001a, b; Corstanje et al., 2007). As demonstrated previously, uncultivated soil was the most P limited system, followed by the sugarcane and forest soils. Probably, P deficiency deeply confined the microbial population, limited the microbial activity, and subsequently inhibited the efficiency of C utilization and reduced oxidation rates of organic matter for uncultivated soil.

Extracellular enzymes are excreted by the microorganisms to the soil for sequestering nutrients. The activities of those enzymes are critical for the degradation of soil organic matter and plant detritus (Wright and Reddy, 2001a) and regulated by the

availability of the substrates and other environmental factors (White and Reddy, 1999; Corstanje et al., 2007). To characterize the effects of land uses on microbial activity, we conducted the enzymatic assays of cellobiohydrolase in the C cycle, leucine aminopeptidase in the N cycle, alkaline phosphatase in the P cycle and arylsulfatase in the S cycle. Most of the enzymes were sensitive to the changes in land use, except leucine aminopeptidase, indicating possible differences in the composition of active fractions of soil microbial populations and biochemical processes among different land uses (Monkiedje et al., 2006). Application of land management and plant coverage greatly affected the distribution and availability of substrates and the quantity and quality of organic matter sources, which primarily contributed to differences in enzyme activities. Significantly negative correlations were found between the leucine aminopeptidase activities and moisture content (R^2 = -0.55), loss-on-ignition (R^2 = -0.80), labile organic P (R^2 = -0.70), and microbial biomass C (R^2 = -0.58), respectively. It was possible that leucine aminopeptidase was sensitive to factors other than the quality and quantity of organic matter sources.

Sole C-source utilization patterns are commonly used to evaluate changes in microbial community composition and functional diversity and have been successfully applied to ranges of soil habitats undergoing changes in land use (Garland, 1996; Campbell et al., 2003). BIOLOG EcoPlates were employed to investigate the utilization patterns of 31 different substrates by soil microbes. Our results indicated clearly that the microbial community composition and functional diversity differed across land uses. The CMD was highest in turf and forest soils and lowest in uncultivated soil (Fig. 2-1). Since the difference in the CMD profile reflects the variation in substrate diversity and

availability (Grayston et al., 2004), the C sources in turf and forest soils appeared more divisive than those of the sugarcane and uncultivated fields, which further affirmed our aforementioned statements on the results of microbial biomass C to organic matter ratios. Average well color development describes the average respiration of the C sources by the microbial community (Garland, 1997). Results demonstrated that microbial communities of turf and forest soils were more functionally adapted to use those C resources than sugarcane and uncultivated soil, which was consistent with our previous postulation that turf had the highest efficiency of C utilization and uncultivated soil the lowest. Results also indicated that microbial communities of turf and forest had higher capacities to acclimatize to alterations in land use (Preston-Mafham et al., 2002). Principal component analysis on the color development profiles revealed no similarity in the microbial community composition between any two of those four land uses, except that of the forest and turf (Fig. 2-2). It has been proposed recently that both dissolved organic C and dissolved organic N indeed greatly regulate the composition of the active fractions of the soil microbial community (Schimel and Bennett, 2004; Cookson et al., 2007). In the present study, we did not find any such effect. On the contrary, we found remarkable correlations between AWCD and total P (R^2 = 0.70), labile inorganic P (R^2 = 0.64), total labile P (R^2 = 0.62), soil C to P ratio (R^2 = -0.92), and soil N to P ratio (R^2 = -0.92), respectively. Therefore, concentrations of labile inorganic P in soil profiles play a critical role in regulating microbial community composition. Moreover, labile inorganic P is of importance in determining the C utilization efficiency and organic matter decomposition by microbes, which was evidenced by the fact that it was highly negatively correlated with organic matter content (Table 2-3). Apparently, good

agreement between results of microbial biomass C to organic matter ratio and BIOLOG profiles analysis firmly supported the statement that long-term intensive P fertilization in EAA might stimulate microbial communities with higher efficiency of C utilization, which in turn will enhance the organic matter decomposition rates, and, consequently, result in increasing soil subsidence and nutrient regeneration.

Indicators of Land Use Changes

Indicators have been widely recommended to specify early effects of land use change or nutrient enrichment (Corstanje et al., 2007; Monkiedje et al., 2006). Soil organic matter is one of the key indicators for its role as nutrient sources and impacts on soil physical structure. Organic matter content of soils in the EAA approximates 80-90%. Hence, alteration of the organic matter content in the short term is probably not sensitive enough to indicate changes in land use. Instead, our results show that microbial biomass C to organic matter ratio was highly distinct across land uses, which indicates substrate availability to soil microorganisms, and can be used as an indicator of prospective alterations in organic matter status along with land use changes in the EAA. Soil enzyme activities are generally the most sensitive indicators of changes of belowground microbial communities (Sicardi et al., 2004). In our study, cellobiohydrolase activity exhibited the most variation among land uses, suggesting that this enzyme can be considered as a sensitive indicator of land use changes.

Conclusions

Long term cultivation and fertilization in the EAA greatly altered soil nutrient distribution and increased organic matter decomposition rates. Sugarcane cropping sequestered more P in inorganic fractions and may pose threats to the downstream Everglades ecosystems. Uncultivated soils retained more P in organic fractions.

Uncultivated soil was the most P-limited system and thus had the lowest efficiency of C and N utilization. Soil microbial community structure and metabolic diversity significantly changed after variable long-term land management. Turf and forest soils had the highest diversity of C sources and utilization rates of C resources, while the uncultivated soil had the lowest. Soils of forest and turf were close to each other in terms of microbial community composition, but were significantly different from sugarcane or uncultivated. Labile inorganic P played important roles in regulating organic matter decomposition and microbial community composition and function. Our results also support the notion that changes in microbial activity represent a shift in microbial community composition. Microbial biomass C to organic matter ratio and cellobiohydrolase activity was sensitive indicators of alterations in land uses. Turf soils potentially had a higher rate of soil subsidence and uncultivated soils had the lowest. Land use change from sugarcane cropping to turf grass in EAA is likely to enhance soil subsidence. Nonetheless, land use change from sugarcane cropping to uncultivated tends to slow down the oxidation rates of organic matter and subsequently may minimize soil subsidence.

Table 2-1. Properties of forest, sugarcane, turf and uncultivated soils (0-15 cm).

	Forest	Sugarcane	Turf	Uncultivated
Moisture (%)	49 a	54 a	46 ab	37 b
LOI (%)	83 ab	81 a	57 a	85 b
TC (g kg^{-1})	449 a	445 a	319 a	461 b
TN (g kg^{-1})	30 a	29 a	21 ab	32 b
TP (g kg^{-1})	1.25 a	0.98 b	2.71 ab	0.78 c
TOC (g kg^{-1})	424 ab	411 a	293 a	433 b
DOC (g kg^{-1})	1.13 a	2.34 b	1.14 a	1.14 a
DON (mg kg^{-1})	141 a	197 b	148 ab	132 a
NH_4-N (mg kg^{-1})	12 a	10 a	21 b	42 c
LIP (mg kg^{-1})	43 a	96 b	173 abc	17 c
TLP (mg kg^{-1})	122 a	165 b	236 ab	97 a

Notes: LOI, Loss-On-Ignition; TOC, Total Organic C; DOC, Dissolved Organic C; DON, Dissolved Organic N; NH_4-N, Extractable NH_4^+; LIP, Labile Inorganic P ($NaHCO_3$-extractable P); TLP, Total Labile P; Different letters following numbers indicate significant differences among land uses ($p < 0.05$).

Table 2-2. Microbial biomass, enzymatic activities, potentially mineralizable N (PMN) and potentially mineralizable P (PMP) of forest, sugarcane, turf and uncultivated soils (0-15 cm).

	Forest	Sugarcane	Turf	Uncultivated
MBC (g kg^{-1})	12.7 a	13.5 a	13.3 ab	9.3 b
MBN (g kg^{-1})	0.24 a	0.16 a	0.32 ab	0.12 b
MBP (mg kg^{-1})	52 a	48 a	99 a	41 a
MBC/OM (mg kg^{-1})	15183 a	16857 a	23374 b	10918 c
PMN (mg kg^{-1} d^{-1})	10.14 a	2.66 b	10.42 a	12.79 a
PMP (mg kg^{-1} d^{-1})	4.75 a	4.18 a	33.10 a	3.92 a
CBHase (mg g^{-1} h^{-1})	140 a	51 b	191 c	271 abc
LAPase (mg g^{-1} h^{-1})	1.42 a	1.96 a	3.64 a	2.58 abc
APase (mg g^{-1} h^{-1})	0.70 a	0.94 a	1.29 ab	1.21 b
Sulfatase (mg g^{-1} h^{-1})	0.32 ab	0.24 a	0.44 ab	0.42 b

Notes: MBC, Microbial Biomass C; MBN, Microbial Biomass N; MBP, Microbial Biomass P; OM, Organic matter content; CBHase, Cellobiohydrolase; LAPase, Leucine Aminopeptidase; APase, Alkaline Phosphatase; Different letters following numbers indicate significant differences among land uses ($p < 0.05$).

Table 2-3. Significant correlations of soil and microbial properties at $p < 0.05$, $n = 16$.

	OM	Total P	LIP	MBC/OM	MBC	MBN	MBP	CMD	AWCD
OM	1								
Total P	-0.61	1							
LIP	-0.67	0.92	1						
MBC/OM	-0.72	0.70	0.72	1					
MBC	NS	NS	NS	0.48	1				
MBN	NS	NS	NS	NS	0.76	1			
MBP	NS	0.56	NS	0.58	0.62	0.62	1		
CMD	-0.57	0.56	NS	0.65	NS	NS	NS	1	
AWCD	-0.78	0.70	0.64	0.81	NS	NS	NS	0.88	1

Notes: OM, Organic Matter Content; LIP, Labile Inorganic P; MBC/OM, Microbial Biomass C to Organic Matter Content ratio; MBC, Microbial Biomass C; MBN, Microbial Biomass N; MBP, Microbial Biomass P; CMD, Community Metabolic Diversity; AWCD, Average Well Color Development; NS, not significant at $p < 0.05$.

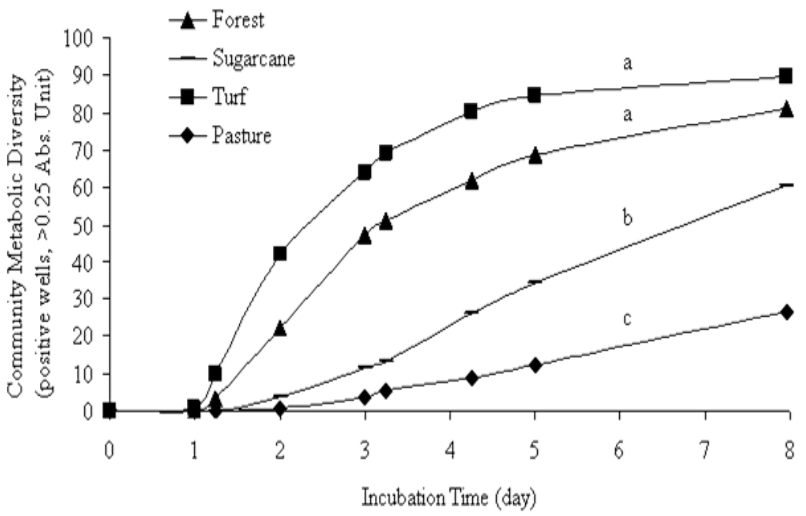

Fig. 2-1. Microbial community metabolic diversities of forest, sugarcane, turf and uncultivated soils. Different letters indicate significant differences between land uses ($p < 0.05$).

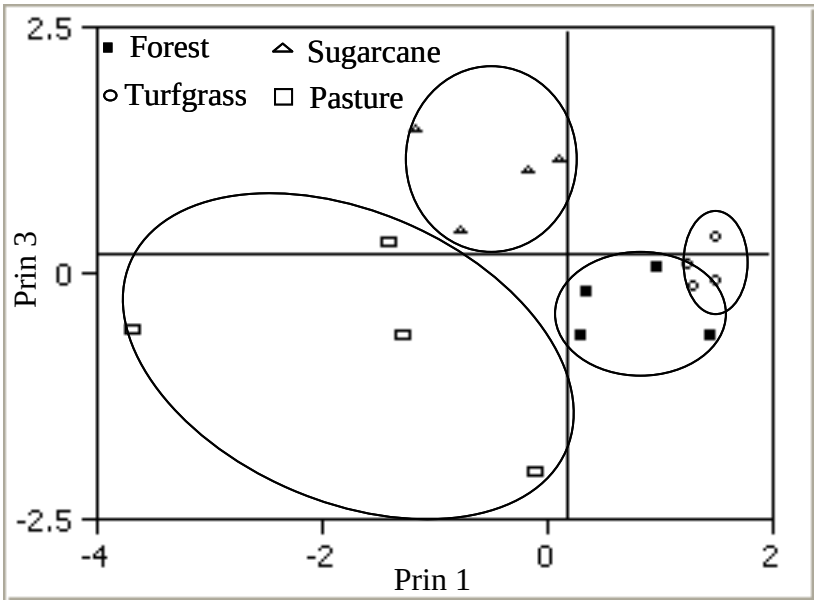

Fig. 2-2. Principal component analysis of community-level physiological profiles from forest, sugarcane, turf and uncultivated soils.

CHAPTER 3
MULTIVARIATE ANALYSIS OF CHEMICAL AND MICROBIAL PROPERTIES IN HISTOSOLS AS INFLUENCED BY LAND-USE TYPES

Introduction

Univariate data analysis is essential in many experiments, but it is considered appropriate when just one variable is measured for several samples (Sena et al., 2002). To better understand whole soil ecosystem processes, various properties are systematically collected and multivariate analytical methods are employed, which allows analysis of multiple variables simultaneously while interpreting results with better summarized information (Sena et al., 2002). Although considered underutilized (Sena et al., 2000), multivariate methods have been well recognized and commonly applied in soil research. For instance, principal component analysis (PCA) has been performed to investigate management impacts on soil quality (Wander and Bollero, 1999) and microbial community structure and function (Grayston et al., 2004; Bossio et al., 2005; Allison et al., 2007; Cookson et al., 2007). Recently, application of canonical correlation analysis (CCA) and discriminant analysis (DA) has also been reported for soils research (Zhang et al., 2006; Banning and Murphy, 2008; Sanchez-Moreno et al., 2008).

Canonical correlation analysis is a method used to assess the dependent relationships between two data sets. The method is designed to find linear combinations of variables in one data set that account for the most variation in a linear combination of variables for the other data set (Lattin et al., 2003). In this way, much of the relationship between two data sets is detected and visualized. A potential

application for CCA in soil research is the identification of the relationship between soil chemical properties and microbial community structure and function. Soil supports diverse microbial populations and, in natural systems, microbial activity is essentially related to the efficiency of nutrient cycling and organic matter turnover (Wright and Reddy, 2001). Yet, the richness, abundance, and activity of microbial communities are influenced by chemical properties such as organic matter content and nutrient availability (Rutigliano et al., 2004; Ye et al., 2009). Alterations in the chemical conditions of soils may lead to shifts in microbial community composition and changes in microbial function, which is frequently observed upon change in land uses (Nogueira et al., 2006; Cookson et al., 2007; Castillo and Wright, 2008a).

The Everglades Agricultural Area (EAA) in south Florida was drained in the early 1900s and converted from wetlands to sugarcane and vegetable cropping. Soils of the EAA are primarily Histosols with high organic matter content, and contain high N yet low P and micronutrient concentrations that require supplemental fertilization (Snyder, 2005; Ye et al., 2009). These soils have undergone subsidence since they were drained at rates currently at 1.5 cm/yr (Shih et al., 1998). This has decreased the soil depth to bedrock to the point where major changes in soil chemical properties, such as pH, are becoming problematic and increasingly prohibitive for agricultural use. Sugarcane is the major land use in the EAA and requires approximately 30 kg P ha^{-1} yr^{-1} and extensive tillage (Rice et al., 2006). Its long-term cultivation typically changes soil chemical properties and microbial community structure and function (Grayston et al., 2004;

Cookson et al., 2007; Castillo and Wright, 2008a, 2008b). Due to economic factors, soil subsidence, and water management concerns, current land use patterns indicate a shift from sugarcane cropping back to the historic seasonally-flooded wetland prairie ecosystem, tree islands, or pastures (Snyder, 2005).

The purpose of this study was to evaluate land use effects on soil biochemical processes using multivariate analytical methods. Land uses under sugarcane cropping and cypress were compared with uncultivated land. Application of multivariate methods was performed to determine (1) whether land uses distinguished by integrated soil chemical properties and microbial parameters, (2) which variables contributed most for those differentiations, (3) and whether discriminations in microbial parameters were dependent on soil chemical properties.

Materials and Methods

Site Description

The study sites are located in the northern EAA near Belle Glade, FL (26° 39' N, 80° 38' W). All soils are Dania muck (euic, hyperthermic, shallow Lithic Medisaprists) with depths to the bedrock of approximately 45 cm. Three land uses with different management history were selected for this study: soils under forest for 21 years, soils under sugarcane production for approximately 50 years, and reference soils that have never been cultivated since drainage. The forest soils were previously cropped to sugarcane but planted to bald cypress (*Taxodium distichum*) and pond cypress (*Taxodium ascendens*) in 1988. These did not receive any fertilization after land use

change, but were tilled prior to seedling establishment with no further management applied. The sugarcane soils were managed for vegetable production from the early 1900s to the 1950s, but mainly for sugarcane since the 1960s. Phosphorus fertilization is commonly applied at 30 kg ha^{-1} yr^{-1} prior to planting (Rice et al., 2006). Tillage operations included several diskings (to 15 cm depth) after crop harvest, subsoil chiseling (to 30 cm depth) to improve drainage, and frequent in-season tine cultivations (to 4 cm depth) for weed control (Morris et al., 2004). The uncultivated soils were primarily occupied by paragrass [*Panicum purpurascens* (L.) Raddi] and bermudagrass [*Cynodon dactylon* (L.) Pers] and mowed periodically with residues returned to soil, and received no fertilization and tillage.

Soil Chemical Properties

Surface soil (0-15 cm) samples were collected from four replicate sites of each land use in March 2007. The soils were homogenized after the removal of visible plant particles and stored at 4°C. Soil organic matter content was measured by the loss-on-ignition method after ashing at 550°C for 4 hr (Wright et al., 2008). Total C, total N, and total P were determined using the oven dried (70°C) soil. Total C and N were measured with a Carlo-Erba NA 1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ) while total P was measured after ashing (Bremner, 1996) using the ascorbic acid-molybdenum blue method (Kuo, 1996) with an AQ2+ discrete analyzer (Seal Analytical Inc., Mequon, WI). Labile inorganic N (LIN) was determined by extraction with 0.5 *M* K_2SO_4 followed by colorimetric analysis of NH_4 (Castillo and Wright, 2008b). Labile

organic N (LON) was calculated from the difference between total N of the K_2SO_4 extracts and LIN (Castillo and Wright, 2008b). Labile inorganic P (LIP) was analyzed as described previously after $NaHCO_3$ extraction (Kuo, 1996). The $NaHCO_3$ extracts were measured for total P after Kjeldahl digestion (Castillo and Wright, 2008b), with labile organic P (LOP) being difference between total extractable P and LIP.

Soil Microbial Parameters

Microbial biomass

Microbial biomass C (MBC), biomass N (MBN) and biomass P (MBP) were measured by the fumigation-extraction method (White and Reddy, 2001). The amount of K_2SO_4-extracted C was determined with a Shimadzu TOC-5050A total organic carbon analyzer. The MBC was calculated as the difference in extractable C between fumigated and unfumigated samples using a conversion factor of 0.37. Dissolved organic C (DOC) was referred to as the total C contained in the unfumigated K_2SO_4 extract. After digestion, the K_2SO_4 extracts were measured for total Kjeldahl N using an AQ2+ discrete analyzer (Seal Analytical Inc., Mequon, WI). The MBN was calculated as the difference in total N between fumigated and unfumigated samples using k=0.54. The MBP was determined as the difference in total P of $NaHCO_3$ extracts between fumigated and unfumigated soil. The P content of the $NaHCO_3$ extracts was measured as previously described (Kuo, 1996).

Potentially mineralizable N and P

Potentially mineralizable N (PMN) was determined according to methods of White and Reddy (2000) based on a 10-d incubation followed by extraction with 2*M* KCl and analysis of NH_4. Potentially mineralizable P (PMP) was measured using the method of Corstanje et al. (2007) with slight modifications. Soil (0.5 g) was placed in 30-ml serum bottles and mixed with 5 ml double distilled water. The bottles were then capped and incubated in the dark at 40°C for 10 d. At 10 d, 20 mL of 1 *M* HCl was injected and samples were shaken for 3 hr. Extracts were then filtered through 0.45 µm membrane filters. Another set of equivalent weight samples, without incubation, were directly extracted with 25 mL of 1 *M* HCl and extracts analyzed for P. The PMP was determined as the difference in P concentration between extracts from incubated and non-incubated soil.

Enzyme activity assay

Four enzyme activities were measured in this study, including glucosidase in the C cycle, leucine aminopeptidase in the N cycle, alkaline phosphatase in the P cycle and arylsulfatase in the S cycle. Approximately 1 g moist soil was placed in polypropylene centrifuge tubes, mixed with 30 mL of water, and shaken for 25 min. Homogenized samples were further diluted 5 times for enzyme assays, which were conducted in triplicate with controls to assess non-enzymatic production. Leucine aminopeptidase assay was conducted in 96-well microtiter plates (Prenger and Reddy, 2004) with 200 µL samples incubated with 50 µL of 5 mM L-leucine 7-amino-4-methylcoumarin

(Biosynth, Naperville, IL) at 20°C for 8 hr. The florescence readings were collected at 1 hr intervals using a Bio-TEK FL600 fluorescence plate reader (Bio-TEK Instruments Inc., Winooski, VT) at a setting of 365 nm excitation and 450 nm emission. Enzyme activity was determined by calculating the mean fluorescent reading changes over time with a standard curve and expressed as mg 7-amino-4-methylcoumarin released per g soil per hr. Alkaline phosphatase and sulfatase assays were conducted according to Wright and Reddy, 2001.

Community-level physiological profile by BIOLOG assay

Community-level physiological profiles (CLPPs) were determined by direct incubation of fresh soil extracts in BIOLOG Eco-Plates (31 substrates) (BIOLOG Inc.). Approximately 1 g moist soil was mixed with 20 mL of water and gently shaken for 20 min. The homogenized samples were then diluted 400 times and soil particles allowed settling for 15 min at 4°C. 150 µL of the supernatant was subsequently dispensed into each well of Eco-Plates and incubated at 20°C for 7 d. Optical densities were measured every 6 or 12 hr using Bio-TEK FL600 (Bio-TEK Instruments Inc., Winooski, VT) at 590 nm. Absorbance values of each well with C sources were blanked against control wells before analysis. Negative values were considered as 0. Average well color development (AWCD) was determined as described by Garland (1996; 1997). To overcome possible interference by inoculum density on color development, absorbance values for various C sources were standardized by dividing the blanked value of each well by the AWCD of the plate and were subsequently used for PCA.

Data Analysis

To classify soils by integrated chemical properties, data were standardized to zero mean and unit variance and subject to cluster analysis (CA) according to K-means partitioning method. The importance of a given variable X in defining the differences among clusters was justified by the ratio of between-cluster sum of squares of X to the total sum of squares of X (R^2) and the ratio of between-cluster sum of squares of X to within-cluster sum of squares of X [$R^2/(1-R^2)$]. High ratios indicate high importance. Microbial data were analyzed with DA to determine differences among land uses, and stepwise variable selection was conducted to identify the important variables in discriminating land uses. Due to the existence of missing data, MBP and PMP were not eligible for DA and hence excluded from the analysis. Principal components analysis was used to reduce the numbers of chemical and microbial variables by extracting the most important principal components separately. Canonical correlation analysis was then carried out to investigate the dependent relationship between extracted chemical and microbial principal components. The significant level was set at $\alpha = 0.05$. Application of PCA and DA was conducted with JMP 7 (SAS Institute Inc., NC), while CA and CCA were performed with SAS 9.1 (SAS Institute Inc., NC).

Results

Land-Use Effects on Integrated Soil Chemical Properties

Soil chemical properties are listed in Table 3-1. The K-means procedure suggested a three-group clustering as the best clustering scheme for these land uses

(Fig. 3-1). The dendrogram clearly displayed that cluster 1 contained only soils from cypress, cluster 2 included soils solely under sugarcane production, and cluster 3 exclusively was comprised of uncultivated soils. The cluster of sugarcane soil was closer to cypress than to uncultivated soil. Analysis of the variances revealed that LIP, LIN, DOC, and total P were the most important parameters defining the differences among clusters (Table 3-2). Additionally, the plot of cluster means across all chemical variables demonstrated that sugarcane, cypress, and uncultivated soils were highly distinguished by total P and LIP (Fig. 3-2).

Variable reduction was made by PCA while retaining as much as possible the original variances. The analysis extracted two principal components, C-Prin1 and C-Prin2, representing 54% and 19% of the original variances, respectively. Score plots showed that sugarcane, cypress, and uncultivated soils were distributed separately along ordination axis Prin1, while on axis Prin2 cypress was separated from sugarcane and uncultivated (Fig. 3-3). Variables with significant loadings on Prin1 were total C (R^2 = 0.80), total N (R^2 = 0.84), DOC (R^2 = -0.81), LIN (R^2 = 0.81), LON (R^2 = -0.75), and LIP (R^2 = -0.95), while total P had high negative loadings on Prin2 (R^2 = -0.74) (Fig. 3-4).

Land-Use Effects on Integrated Microbial Properties

Considering the high numbers of variables (31) for BIOLOG data, PCA was first conducted to created new variables representing the carbon utilization patterns. Two variables were extracted (CLPP1 and CLPP2), and each explained 20% of the total

variance in the BIOLOG data set. The new variables were then combined with other microbial parameters and used for DA (Table 3-3). Canonical plots showed that cypress, sugarcane, and uncultivated soils were significantly different on the first discriminant function (Canonical 1), and the second discriminant function (Canonical 2) demonstrated the differences between uncultivated soils and those under sugarcane and cypress (Fig. 3-5). To identify variables that significantly contributed to the discriminations, stepwise variable selection was conducted. The CLPP2 was the first significant variable selected into the model with a partial R^2 of 0.78, and was followed by MBC (R^2 = 0.79), MBN (R^2 = 0.60) and PMN (R^2 = 0.84) (Table 3-4).

Application of PCA extracted two principal components, M-Prin1 and M-Prin2, from the original microbial data, which together explained 68% of the total variance (Fig. 3-3). Score plots indicated that uncultivated soils were distinct from sugarcane and cypress soils on the ordination axis M-Prin1, while sugarcane and cypress were separated on axis M-Prin2. Glucosidase exhibited the highest loading (R^2 = 0.81) on M-Prin1 followed by sulfatase (R^2 = 0.80), phosphatase (R^2 = 0.77), MBC (R^2 = -0.77), PMN (R^2 = 0.70), leucine aminopeptidase (R^2 = 0.67) and MBN (R^2 = -0.65), while CLPP1 exhibited the highest loading on M-Prin2 (R^2 = 0.81) (Fig. 3-4).

Dependent Relationship between Chemical Properties and Microbial Parameters

Canonical correlation analysis was performed with chemical (C-Prin1, 2) and microbial (M-Prin1, 2) principal components, and two pairs of canonical variates (CVs) were extracted. Canonical correlation between the first chemical canonical variate (C-

CV1) and the first microbial canonical variate (M-CV1) was significant (R = 0.91), with a goodness of fit of p = 0.0006. Approximately 83% of the variance in M-CV1 was explained by C-CV1. The importance of a given principal component for obtaining the maximum correlation between C-CVs and M-CVs was expressed as a standardized canonical coefficient. The coefficient of M-Prin1 for M-CV1 was 0.99 and of M-Prin2 was -0.17. Therefore, M-Prin1 gave a greater contribution increasing the M-CV1, while M-Prin2 contributed less in an opposite way. The coefficients of C-Prin1 and C-Prin2 for C-CV1 were 0.81 and 0.58, respectively. The second pair of canonical variates explained 43% of the variance shared by M-CV2 and C-CV2 (R = 0.65, p = 0.03). The canonical coefficients of M-Prin1 and M-Prin2 for M-CV2 were 0.17 and 0.99, respectively, while those of C-Prin1 and C-Prin2 for C-CV2 were 0.58 and -0.81, respectively. Further redundancy analysis revealed that 42% of total variance in M-Prin1 and M-Prin2 was explained by C-CV1 and another 21% by C-CV2.

Discussion

Land-Use Effects on Soil Chemical Properties

Cluster analysis is a method that involves classifying objects into groups so that objects within each group are relatively similar, while objects in different groups are relatively dissimilar (Lattin et al., 2003). This analytical method has been used to identify discriminations of management effects on soil properties (Sena et al., 2002; Gila et al., 2008; Micó et al., 2008). In the present study, a K-means partitioning method was utilized to describe the heterogeneity of EAA soils under different land uses by

integrated analysis using chemical parameters. Soils were clustered into three groups in corresponding to the three land uses (Fig. 3-1), indicating that cypress, sugarcane, and uncultivated soils were highly different in the sense of soil chemistry. Apparently, long-term cultivation of the EAA soils resulted in such discrimination. It has been reported that agricultural management poses remarkable impacts on the distribution of C (Wu et al., 2004; Zhang et al., 2006), N (Cookson et al., 2007) and P (Castillo and Wright, 2008b; Wright, 2009). Soils of the EAA are primarily organic and contain high N and low P and micronutrient concentrations. Therefore, sugarcane production requires supplemental fertilization and as well as extensive tillage (Rice et al., 2006). Fertilization is likely to promote nutrient accumulation, especially P, in the soil profile (Wright, 2009), while tillage disrupt organic matter and leads to nutrient stratification (Gesch et al., 2007). Analysis of cluster means across all variables further revealed that cypress, sugarcane, and uncultivated soils were highly distinguished by total P and LIP (Table 3-1, Fig. 3-2), indicating a significant difference in P availability between land uses. It was reasonable since sugarcane soils received long-term P fertilization of approximately 30 kg ha^{-1} yr^{-1}, and those of uncultivated did not, whereas the cypress soils received P application up until their establishment. This may also explain why LIP (R^2 = 0.91; $R^2/(1-R^2)$ = 10.4) was the most important variable in defining clusters (Table 3-2), and why the cluster of cypress was closer to sugarcane rather than to uncultivated soil (Fig. 3-1).

Land-Use Effects on Microbial Parameters

Discriminant analysis utilizes the information from a set of variables to achieve the clearest possible discrimination between or among groups (Lattin et al., 2003). In the present study, DA was applied to differentiate soils under multiple land-uses as well as to determine whether the difference was actually significant. Analysis of discriminant functions clearly showed that sugarcane, cypress, and uncultivated soils had distinctive microbial characteristics (Fig. 3-5). Similar results have also been observed for other ecosystems, in which nutrient availability caused shifts in microbial community structure and function (Allison et al., 2007; Cookson et al., 2007; Matsushita et al., 2007). The stepwise variable selection procedure initially suggested that phosphatase (R^2 = 0.63), sulfatase (R^2 = 0.51), glucosidase (R^2 = 0.62), PMN (R^2 = 0.67), MBC (R^2 = 0.68), MBN (R^2 = 0.57), and CLPP2 (R^2 = 0.78) were all significant and eligible to enter the discriminant model. However, only CLPP2, MBC, MBN, and PMN were finally included, which indicated their great significance to the discrimination (Table 3-4). In other words, cypress, sugarcane, and uncultivated soils were highly distinguished by those four variables. Considering the fact that CLPP2, MBC, and MBN characterized the microbial community composition and population size, it may be true that microbial community structure, rather than function, was more sensitive to agricultural management. This concept was also supported by other studies (Bossio et al., 2005; Zhang et al., 2006).

Variable Reduction

Principal component analysis is a useful method for dimension reduction (Lattin et al., 2003), as it allows for re-expression of data with fewer variables while capturing as much of the available variance as possible. The objective of variable reduction is to make data analysis more manageable and straightforward. Application of PCA in the present study successfully reduced nine chemical variables to two principal components and was able to capture 73% of the original variance, while nine microbial parameters were reduced to two principal components explaining 68% of the total variance. The reduction increased the sample size to variable ratio and made subsequent CCA analysis and interpretation easier. In addition, score plots showed that cypress, sugarcane, and uncultivated soils were dispersed differently along ordination axes (Fig. 3-3), indicating their significant difference in both soil chemistry and microbial community structure and function, which further supports the aforementioned results of CA and DA. Principal component loadings determine the amount of variance of a given variable accounted by principal components (Lattin et al., 2003). Our results demonstrated that LIP had the highest loadings on C-Prin1 (R^2 = -0.95), while total P was the only variable that had significant loadings on C-Prin2 (R^2 = -0.74), which is in agreement with the previous statement that LIP and total P significantly contributed to the discrimination in soil chemistry (Figs. 3-2 and 3-4).

Dependent Relationship between Chemical Properties and Microbial Parameters

Microbial communities are in close contact with soil microenvironments, and therefore are easily subjected to change following alteration of soil chemical properties (Corstanje et al., 2007). Thus, land use patterns are likely to affect the microbial community structure and function, which can be described by the changes in microbial parameters such as respiratory capacities, microbial biomass and extracellular enzymatic activities (Wright and Reddy, 2001; Castillo and Wright, 2008a). In the present study, multivariate data analysis demonstrated the contrasting soil chemistry and distinctive microbial community composition and function across soils under different land uses (Figs. 3-1 and 3-5). It was likely that discriminations in microbial parameters were directly connected to variations in soil chemical properties, and CCA suggested that the dependent relationship was indeed significant. Changes in soil chemical properties resulted in alterations in microbial community composition and function. Nutrient availability often plays a critical role in regulating the microbial community structure and function (Cookson et al., 2007; Corstanje et al., 2007; Wright and Reddy, 2008). Cluster analysis suggested that LIP and total P were the most important variables discriminating the integrated chemistry of cypress, sugarcane, and uncultivated soils (Table 3-2, Fig. 3-2), which was further supported by the results of PCA (Fig. 3-4). In consideration of the fact that Everglades soils are historically P limited (Wright and Reddy, 2008), it was likely that P availability was the major factor

that controlled the microbial communities of these land uses, which indeed are P limited (Table 3-1).

Soil chemical properties and the microbial community are two key components in ecosystem function (Finlay et al., 1997). Net results of chemical-microbial interactions define the way ecosystems function. Factors capable of interrupting or modifying the interactions are likely to cause significant ecological impacts. Our results suggested that agricultural cultivation has drastically altered soil chemical properties within the EAA, especially P availability. Intensive application of P fertilizers is likely to stimulate microbial communities, which in turn would enhance organic matter decomposition rates and contribute to greater rates of soil subsidence. Thus, future P fertilization should be evaluated well for its potential long-term impact on microbial activity, which in turn may affect the sustainability of different land uses of these subsiding soils.

Conclusions

Long-term cultivation in the EAA significantly altered nutrient distribution and availability for different land uses, especially for P cycling. Correspondingly, soil microbial community composition and function was modified, and applications of CA and DA successfully described the alterations. Canonical correlation analysis clearly demonstrated a significant dependence relationship between chemical properties and microbial community composition and function. Phosphorus availability was one of the major factors regulating the soil microbial activity and function for the land uses. Intensive application of P fertilizer is likely to stimulate microbial community and

subsequently increase soil oxidation and subsidence. Future land use changes in the EAA should consider effects of P on the functioning of microbial communities and their control of nutrient cycling.

Table 3-1. Chemical properties of cypress, sugarcane, and uncultivated soils (0-15 cm) with standard error values, n = 4.

	Unit	Cypress	Sugarcane	Uncultivated
Loss-On-Ignition	%	83 (3)	81 (2)	85 (1)
Total C	g kg^{-1}	449 (3)	445 (2)	461 (2)
Total N	g kg^{-1}	30 (0.5)	29 (0.3)	32 (0.3)
Total P	g kg^{-1}	1.3 (0.07)	1.0 (0.01)	0.8 (0.01)
Dissolved organic C	g kg^{-1}	1.1 (0.1)	2.3 (0.1)	1.1 (0.2)
Labile organic N	mg kg^{-1}	141 (11)	197 (8)	132 (16)
Labile inorganic N	mg kg^{-1}	12 (1)	10 (1)	42 (5)
Labile organic P	mg kg^{-1}	79 (5)	69 (4)	80 (4)
Labile inorganic P	mg kg^{-1}	43 (9)	96 (4)	17 (3)

Table 3-2. The ratio of between-cluster sum of square to total sum of square (R^2) and the ratio of between-cluster sum of square to within-cluster sum of square ($R^2/(1-R^2)$) for chemical variables in defining differences among clusters, n = 12.

	R^2	$R^2/(1-R^2)$
Loss-On-Ignition	0.21	0.26
Total C	0.72	2.60
Total N	0.63	1.72
Total P	0.86	6.10
Dissolved organic C	0.86	6.30
Labile organic N	0.65	1.83
Labile inorganic N	0.87	6.49
Labile organic P	0.30	0.43
Labile inorganic P	0.91	10.43

Table 3-3. Microbial parameters in cypress, sugarcane, and uncultivated soils (0-15 cm) with standard error values, n = 4.

	Unit	Cypress	Sugarcane	Uncultivated
Microbial biomass C	g kg^{-1}	13 (1.0)	14 (0.5)	9 (0.6)
Microbial biomass N	g kg^{-1}	0.2 (0.04)	0.2 (0.01)	0.1 (0.01)
Potentially mineralizable N	mg kg^{-1} d^{-1}	10 (2.5)	3 (0.3)	13 (1.6)
Leucine aminopeptidase	mg kg^{-1} d^{-1}	1.4 (0.4)	2.0 (0.2)	2.6 (0.7)
Phosphatase	mg g^{-1} h^{-1}	0.7 (0.12)	1 (0.08)	1 (0.08)
Sulfatase	mg g^{-1} h^{-1}	0.3 (0.04)	0.2 (0.04)	0.4 (0.05)
Glucosidase	mg g^{-1} h^{-1}	0.2 (0.01)	0.1 (0.02)	0.3 (0.04)
CLPP1	None	1.2 (1.0)	-1.6 (0.4)	0.4 (1.8)
CLPP2	None	2 (0.7)	1 (0.2)	-3 (0.9)

CLPP1 and 2, first and second principal components extracted from datasets of community-level physiology profiles.

Table 3-4. Stepwise discriminant model for differentiating cypress, sugarcane, and uncultivated soils based on microbial parameters, n = 12.

Variable	Partial R^2	*F* value	*P* value
CLPP2	0.78	15.9	0.001
Microbial biomass C	0.79	15.3	0.002
Microbial biomass N	0.60	5.3	0.039
Potentially mineralizable N	0.84	16.0	0.004

CLPP2, second principal component extracted from datasets of community-level physiology profiles.

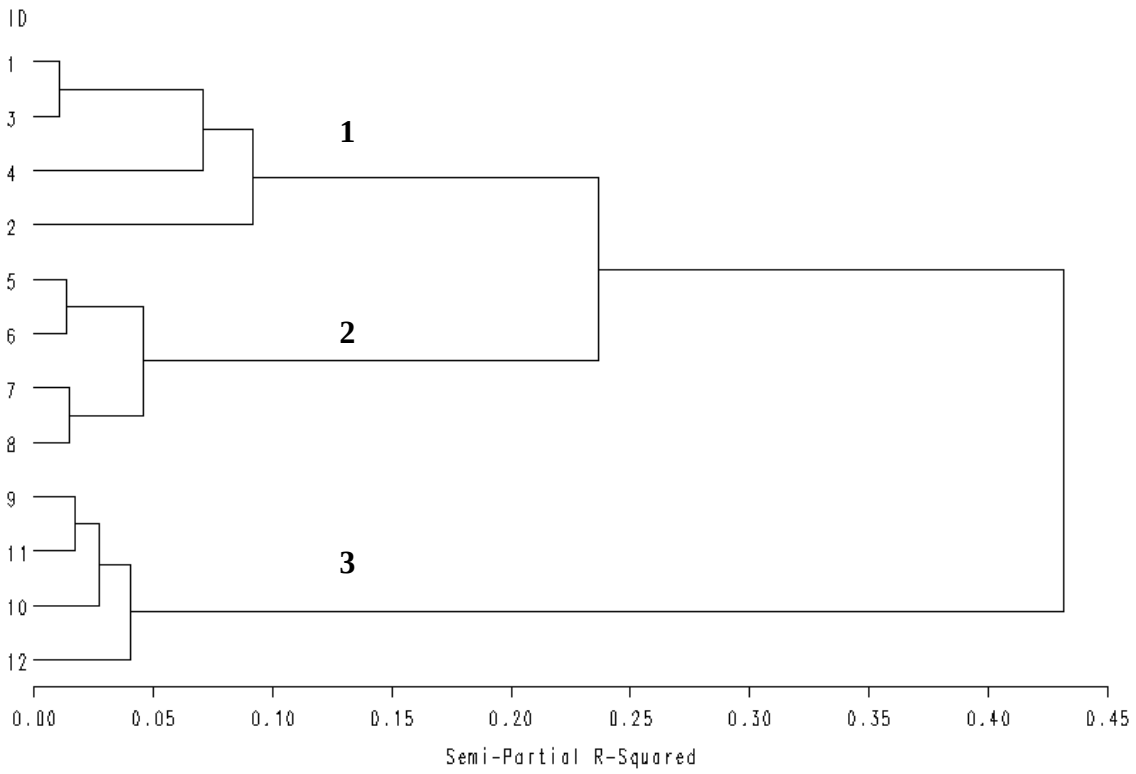

Fig. 3-1. Dendrogram from K-means cluster method applied to soil chemical data.

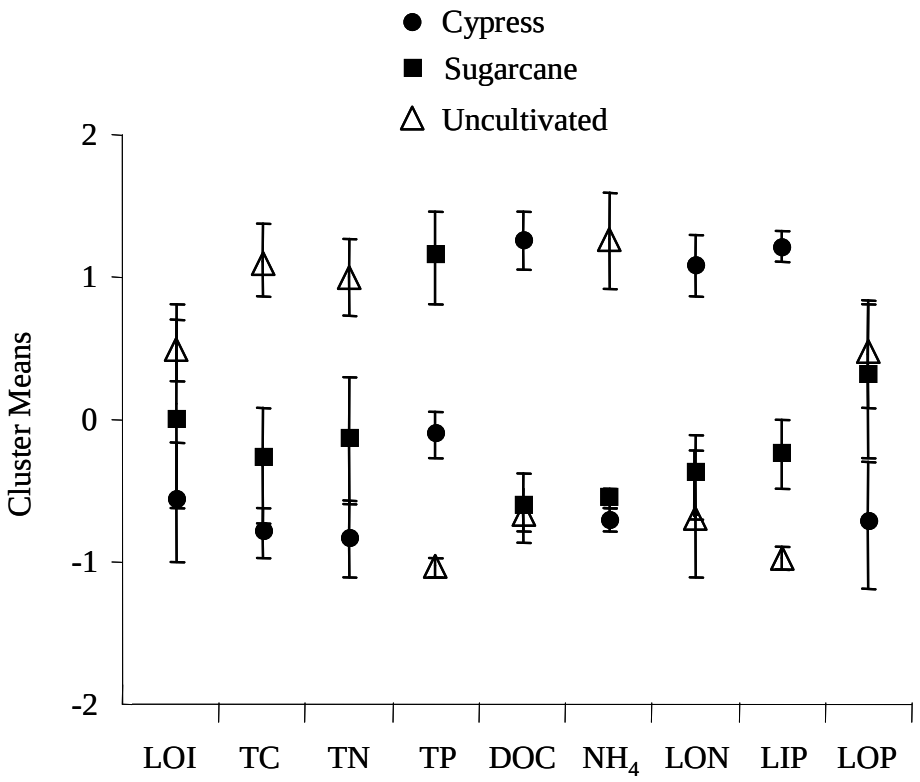

Fig. 3-2. Cluster means across chemical variables. LOI, loss-on-ignition; TC, total C; TN, total N; TP, total P; DOC, dissolved organic C; NH_4, labile inorganic N; LON, labile organic N; LIP, labile inorganic P; LOP, labile organic P.

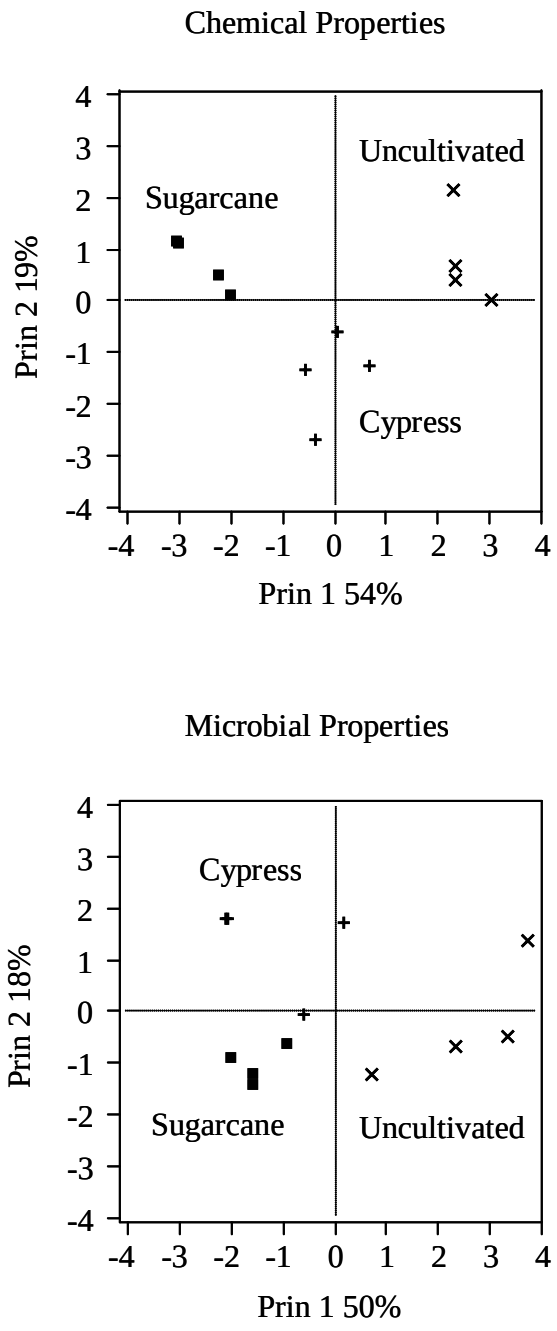

Fig. 3-3. Score plots of principal components analysis on soil chemical properties and microbial parameters.

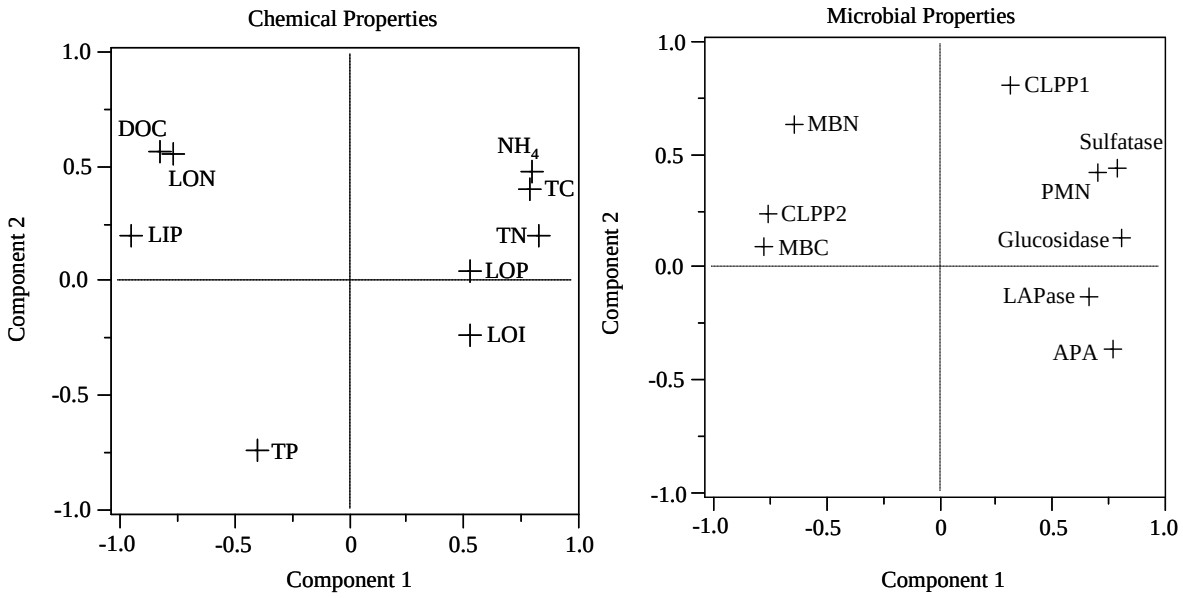

Fig. 3-4. Loading plots of principal components analysis on soil chemical properties and microbial parameters. LOI, loss-on-ignition; TC, total C; TN, total N; TP, total P; DOC, dissolved organic C; NH_4, labile inorganic N; LON, labile organic N; LIP, labile inorganic P; LOP, labile organic P; MBC, microbial biomass C; MBN, microbial biomass N; MBP, microbial biomass P; LAPase, leucine aminopeptidase; APA, alkaline phosphatase; CLPP1, 2, first and second principal components extracted from datasets of community-level physiology profiles.

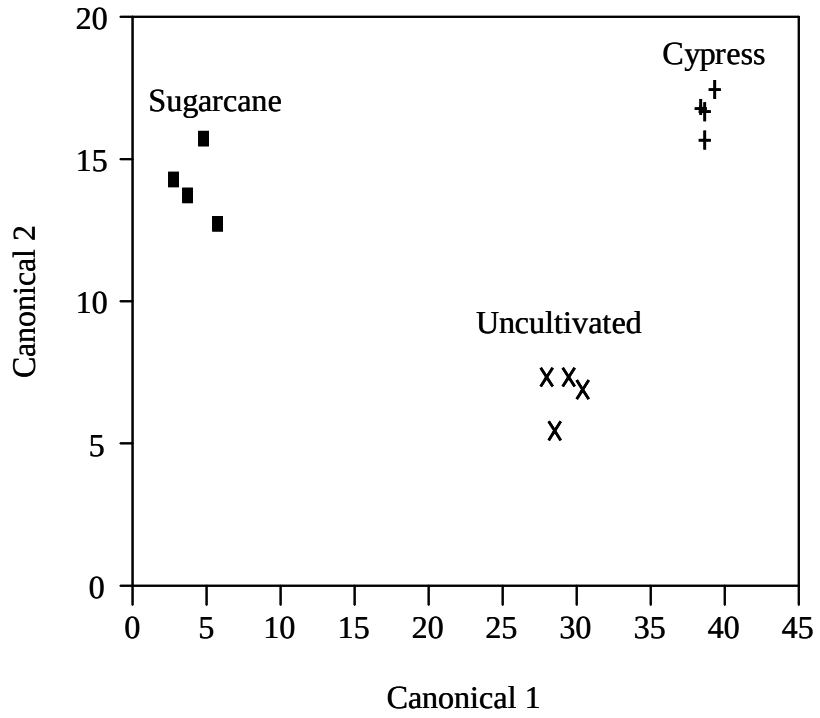

Fig. 3-5. Canonical plots of discriminant analysis on soil microbial parameters.

CHAPTER 4
SULFUR-INDUCED CHANGES IN PHOSPHORUS DISTRIBUTION IN EVERGLADES AGRICULTURAL AREA SOILS

Introduction

The Everglades Agricultural Area (EAA) in south Florida was drained in the early 1900s and converted to sugarcane and vegetable cropping. The EAA primarily consists of Histosols with high organic matter content, approximately 85% by weight, which contain high N yet low P and micronutrient concentrations that require supplemental fertilization (Snyder 2005; Castillo and Wright, 2008). Due to the conversion of land use from seasonally-flooded wetlands to agricultural use, oxidation or subsidence of the drained peatlands has occurred at a rate currently approximating 1.5 cm yr^{-1} (Shih et al., 1998). Consequently, the depth of the soil has declined considerably to the point of causing significant interaction with the underlying bedrock. Cultivation of these drained peatlands, specifically the use of tillage, has resulted in incorporation of bedrock $CaCO_3$ into soil, which has gradually increased the pH since drainage from the historic 5.0-5.5 to approximately 7.0-7.5 today (Snyder, 2005; Gabriel et al., 2008). Subsequently, these soil pH increases have decreased P and micronutrient availability to crops and necessitated new fertilizer management practices. Sugarcane is the dominant crop grown in the EAA, but it requires approximately 30 kg P ha^{-1} $year^{-1}$ and extensive tillage for pre-plant preparation and weed control (Rice et al., 2006). Long-term P application has resulted in P accumulation in soil profile and as well as export into Everglades wetlands through canal systems, which was a major factor contributing to the

deterioration of water quality and alterations of the Everglades wetland ecosystem (Childers et al., 2003).

An understanding of P transformations and distribution in soil is necessary to maximize efficient P use by sugarcane, while minimizing potential export from fields into adjacent wetlands. Fractionation schemes have been developed to determine the P distribution and allocation in different pools related to their degree of recalcitrance (Reddy et al., 1998; Harrell and Wang, 2007). Such schemes assume that different extractants selectively extract discrete P chemical forms sequentially (Adhami et al., 2007). Though the assumption and procedure is operationally defined, the methods provide a convenient way to characterize the availability and mobility of P in soils and to assess their impacts on the environment (Maguire et al., 2000). Soils with high labile P content indicate high P availability to plants and also potential export by leaching or runoff. Inorganic P associated with $CaCO_3$ or bound to Fe and Al oxides are considered relatively stable, but may be susceptible to dissolution and regeneration upon change in environmental conditions (Castillo and Wright, 2008). Phosphorus associated with organic pools is unstable in these drained peatlands due to organic matter oxidation and subsequent P mineralization (Wright, 2009).

Several factors are capable of influencing P stability and mobility in the soil profile including pH, microbial activity, and soil amendments (Arai et al., 2005; Jaggi et al., 2005). Elemental S is occasionally applied in the EAA as soil amendment for the purpose of reducing pH and therefore increasing P availability to crops (Gabriel,

et al., 2008). The microbial oxidation of elemental S to SO_4 produces acidity which reacts with the soil and reduces pH, which in turn releases P bound to Ca and Fe minerals into soil solution. However, the buffering capacity of these calcareous histosols is strong and can counteract the acidifying effects of S oxidation, thus effects of amendments are temporary and may need to be repeated each growing season (Beverly and Anderson, 1986). Problems with large scale S application include a potential pulsed P release from soils that may pose runoff or leaching hazards into proximal aquatic ecosystems (Santoso et al., 1995). Additionally, increased nutrient availability resulting from pH reduction can potentially stimulate the microbial population to decompose organic matter and increase soil oxidation rates.

Everglades wetlands of south Florida are traditionally P limited and sensitive to small increases in P loading (Noe et al., 2001). Reducing P export from the EAA is critical to fulfilling the emerging interests of protecting water quality and restoring south Florida ecosystems. Due to the increases in pH and the decreasing depth to bedrock of soils in the EAA, use of S application to counteract the rising pH may increase in the future. Therefore, a better understanding of how S influences pH and P distribution and availability within various pools during the sugarcane growing season is essential and the objective of this study.

Materials and Methods

Site Description

The experimental site is located in the central EAA on Dania muck (euic, hyperthermic, shallow Lithic Haplosaprist) with a depth to bedrock of approximately 45 cm. The experimental design was a randomized complete block with four S application rates and four field replications. Each field plot measured 9 m x 13 m and consisted of 6 rows of sugarcane (*Saccharum officinarum*). Elemental granular S (90%) was applied at rates of 0, 112, 224, and 448 kg S ha^{-1} to the furrow and covered after planting sugarcane in the furrow. Other fertilization was provided using typical guidelines for this region (Rice et al., 2006). All fertilizers were soil-applied just prior to planting and included 17 kg N ha^{-1} and 37 kg P ha^{-1} as monoammonium phosphate, 228 kg K ha^{-1} as KCl, 8.5 kg Mn ha^{-1}, 4.5 kg Cu ha^{-1}, 5.6 kg Fe ha^{-1}, 2.8 kg Zn ha^{-1}, and 1.1 kg B ha^{-1}. All plots received typical cultural practices including cultivation and herbicide application. Water was applied via seepage irrigation in field ditches approximately 182 m apart. Sugarcane cultivar CP 89-2143 was planted in November 2007 and harvested in February 2009.

Soil Sampling and Analysis

Soil samples were collected before planting and fertilizer application and then in January 2008, May 2008, August 2008, and December 2008, corresponding to approximately 0, 2, 6, 9, and 13 months after planting, respectively. Twelve soil (0-15 cm) cores (2.54 cm diameter) were randomly collected from rows within each field

plot and composited. Soils were homogenized after the removal of visible plant residues and stored at 4 °C.

Soil pH was measured with a soil to water ratio of 1:3 after equilibration for 30 min. Total organic C was measured by loss-on-ignition at 550°C for 4 hr after conversion to organic C with a coefficient factor of 0.51 (Wright et al., 2008). Total organic N was measured by Kjeldahl digestion followed by NH_4 analysis (Bremner, 1996). Extractable NH_4-N and NO_3-N were determined by extraction with 2 *N* KCl followed by colorimetric analysis (Castillo and Wright, 2008). Acetic acid extractable nutrients were measured according to guidelines for muck soils (Sanchez, 1990) by extracting 4 g soil with 25 mL 0.5 *N* acetic acid for 1 hr, then filtering through Whatman #42 filters. Extracts were analyzed for Ca, Mg, Fe, and Al concentrations by ICP (Perkin-Elmer, Waltham, MA) using EPA method 200.7. Select soil nutrient concentrations and properties before S application are listed on Table 4-1.

Soils underwent a sequential chemical P fractionation procedure (Reddy et al., 1998; Wright, 2009). Approximately 1 g soil was extracted with 25 mL water for 1 hr, passed through 0.45 µm membrane filters, and analyzed for P (labile P). The remaining samples were extracted with 25 mL of 0.1 *N* NaOH for 17 hr, filtered and analyzed for Fe-Al bound P (Fe-Al-P), followed by the extraction of remaining samples with 25 ml of 0.5 *N* HCl for 24 hr and analysis of Ca-bound P (Ca-P). The remaining samples were further digested with 6 *N* HCl for 1 hr at 150°C and analyzed for residual P. Three mL of NaOH extracts was digested with 11 *N* H_2SO_4 for 4 hr at 350°C and analyzed for NaOH-TP. The humic-fulvic acid fraction was

calculated by subtraction of NaOH-P*i* from NaOH-TP. Phosphorus concentrations of extracts were measured using the ascorbic acid-molybdenum blue method (Kuo, 1996) with an AQ2+ discrete analyzer (Seal Analytical Inc., Mequon, WI).

Statistical Analysis

A mixed model was fit using restricted maximum likelihood in the MIXED procedure of SAS (Littell et al., 2006). The fixed effects were S application rate, time and their interaction. Block was a random effect. Degrees of freedom were adjusted using the Kenward-Roger adjustment. An exponential covariance structure was used to model the correlation among observations taken from the same plot over time. Significant differences among individual treatments and time intervals were analyzed with Tukey's test at $\alpha = 0.05$. Pearson correlation and stepwise multiple regression analysis was employed to determine relationships among P fractions and soil properties. All statistical analysis was carried out with SAS 9.1 (SAS Institute).

Results and Discussion

Soil pH

Sulfur application within the range of 0 to 448 kg S ha^{-1} did not significantly reduce soil pH ($p = 0.14$) (Fig. 4-1). In the present study, the background soil pH prior to S application was 6.2, which was not significantly different from soils collected at any time of the growing season. However, soil pH dropped slightly at 2 months after S addition in the highest S application rate and increased thereafter from 6.0 to 6.4 at the end of growing season, which indicated a lagged effect of soil

buffering. Additionally, no interaction effect of S application rate and sampling time (p = 0.58) was found.

The limited effect of acidification may result from the relatively low rates of S application and from the high buffering capacity of this calcareous organic soil (Bloom, 2000; Jaggi et al., 2005; Deubel et al., 2007). Soils with high concentrations of carbonates and bicarbonates are highly buffered against acidification (Bloom, 2000; Rogovska et al., 2007). The buffering effects often take place more slowly than the formation of sulfuric acid, therefore, a re-increase of soil pH is possible (Bloom, 2000; Deubel et al., 2007). A limited reduction in soil pH after S application was observed in other studies of calcareous soils (Hassan and Olson, 1966).

Phosphorus Distribution

Two-way ANOVA demonstrated that the time effect was significant across all P fractions indicating significant seasonal changes in P distribution (Table 4-2). The main effect of S application rate was solely found significant on fractions of labile P and Fe-Al-P. No interaction effect of S application rate and time was observed for any of the P fractions.

Labile P

Labile P is commonly considered the most biological available form of P and consistently represented the smallest fraction of total P throughout the growing season, decreasing from 1.1% to 0.3% from 2 to 13 months (Fig. 4-2). Similar results were also observed in other studies (Maguire et al., 2000; Castillo and Wright, 2008). The concentration of labile P decreased from 15 to 3 mg P kg^{-1} from

2 to 13 months after S application (Fig. 4-3). Phosphorus in labile form is highly mobile, unstable, and prone to loss either by leaching, runoff, or plant uptake, which explains the seasonal decrease observed in this study.

An increase in available P as a result of decreased soil pH has been documented in other studies (Deluca et al., 1989; Codling, 2008). In the present study, labile P was significantly higher in soils receiving 448 kg S ha^{-1} (13 mg P kg^{-1}) when compared to soils receiving 112 (6 mg P kg^{-1}) and 224 kg S ha^{-1} (6 mg P kg^{-1}) (Fig. 4-3). There are two primary mechanisms by which S influences P availability: lowering of soil pH (Gabriel et al., 2008) and replacement of PO_4 with SO_4 and release of PO_4 from association with Fe, Al, and Ca (Jaggi et al., 2005). The significant positive correlations between extractable SO_4 and labile P (Table 4-3) were likely indicative of a partial stimulatory effect of SO_4 on the release of labile P. Statistical analysis also revealed significant correlations of labile P to Fe-Al-P (R^2 = 0.87), Ca-P (R^2 = 0.49), humic-fulvic acid P (R^2 = -0.36), and soil pH (R^2 = -0.33), suggesting possible P replenishment from other pools to the labile fraction (Table 4-3). Yet, Fe-Al-P (R^2 = 0.77) was the major component in explaining the variance in labile P, while Ca-P and humic-fulvic-acid P contributed to a better prediction.

$$\text{Labile P} = 0.51 + 0.35\ (\text{Fe-Al-P}) + 0.01\ (\text{Ca-P}) - 0.07\ (\text{Humic-fulvic-acid P})$$

$$(R^2 = 0.84,\ Cp = 3,\ p < 0.0001)$$

Fe-Al bound P

The Fe-Al fraction contains P associated with amorphous and crystalline Fe and Al oxides (Arai et al., 2005). Therefore, it was not surprising to find that Fe-Al-P

was strongly correlated to Fe and Al content (Table 4-3), a finding that was also observed in other studies (Ryan et al., 1984; Maguire et al., 2000). Phosphorus concentrations in the Fe-Al bound fraction displayed a clear declining pattern during the growing season (Fig. 4-3). Concentrations decreased steadily from the beginning to the end of the season, averaging 62, 48, 41 and 34 mg P kg^{-1}, respectively, for 2, 6, 9, and 13 months after S application. This observed decrease indicates that the Fe-Al bound P was a major source of P for sugarcane. Throughout the season, this fraction had the second lowest contribution to total P, averaging 4% (Fig. 4-2). In acidic soils, the Fe-Al-P is frequently the dominant fraction involved in P retention (Mozaffari and Sims, 1996). However, the calcareous nature of this organic soil is likely to encourage more P sequestration in the Ca-bound rather than the Fe-Al bound fraction. Nonetheless, Fe and Al oxides play important roles in controlling P chemistry in soils with high $CaCO_3$ content (Halajnia et al., 2009). Other researchers also suggested that Fe and Al help to control P loss by leaching and runoff (Arai et al., 2005; Harrell and Wang, 2007).

The pools of Fe-Al-P were not affected by S application rates from 0 to 224 kg S ha^{-1} (Fig. 4-3). However, P concentrations in this fraction were significantly higher in soils amended with 448 kg S ha^{-1}, averaging 59 mg P kg^{-1}, than those receiving lower S rates. The Pearson correlation coefficient between Fe-Al-P and soil pH was significantly negative (R^2 = -0.54) (Table 4-3), indicating that a small decrease in soil pH is likely to encourage P retention in this fraction. Inversely, an increase in soil pH may potentially promote P desorption from fixation sites (Gessa et al., 2005), which

may explain the continuous losses of Fe-Al-P over time along with increasing soil pH (Figs. 4-2 and 4-3). Multiple regression analysis, in addition to correlation analysis, showed that 88% of the variance in Fe-Al-P could be explained by labile P, humic-fulvic acid P, and extractable Fe concentrations.

$$\text{Fe-Al-P} = -25.4 + 2.0\ (\text{labile P}) + 0.2\ (\text{humic-fulvic-acid P}) + 2.2\ (\text{Fe})$$

$$(R^2 = 0.88,\ Cp = 4,\ p < 0.0001)$$

Ca-bound P

Phosphorus stocks in the Ca-bound fraction were much higher than those of labile and Fe-Al fractions, contributing 28-35% of the total P (Fig. 4-4) as a result of high Ca concentrations in the soil. Sugarcane cropping in the EAA requires multiple tillage applications prior to and during the growing season. This consequently results in the incorporation of the bedrock $CaCO_3$ into soil and promotes P retention in Ca-bound fractions (Castillo and Wright, 2008b). Correlation analysis revealed that Ca-P concentration was significantly correlated to soil Ca ($R^2 = 0.56$), Mg ($R^2 = 0.40$), and Mn ($R^2 = 0.47$) concentrations indicating that some portion of P in this soil also exists as Mg-P and Mn-P. Association of P compounds with Mn in highly calcareous soils has recently been reported (Adhami et al., 2007), as such P compounds may originate from hureaulite [$Mn_5H_2(PO_4)_4.4H_2O$] and reddingite [$Mn_3(PO_4)_2.2H_2O$]. The size of the Ca-P fraction declined gradually during the growing season from 454 mg P kg^{-1} to 301 mg P kg^{-1} by 13 months (Fig. 4-5). Previous studies suggested that under cultivated conditions Ca-P, rather than

organic fractions, represents a stable P pool (Zhang and MacKenzie, 1997; Castillo and Wright, 2008).

The Ca-P fraction is considered relatively stable under alkaline condition, but unstable under acidic conditions. In the present study, S application did not impact the size of the Ca-P fraction as a result of the limited reduction in soil pH, which implied that Ca-P in this calcareous soil was not sensitive to slight changes in pH as was the Fe-Al-P and labile P fractions (Hassan and Olson, 1966).

Humic-fulvic acid P

The organic pools are primarily comprised of humic-fulvic acids and the more recalcitrant residual fraction (Turner et al., 2005). Sulfur application did not influence P concentrations in this fraction at any sampling time or alter its proportion to total P. Phosphorus concentrations in the humic-fulvic acid fraction fluctuated during the growing season (Fig. 4-5). The percentage of humic-fulvic acid P to total P followed the same pattern, varying from 12% to 17% (Fig. 4-4). Phosphorus distribution in this fraction was negatively correlated to labile P, Ca-P and residual P fractions (Table 4-3).

Residual P

Residual P was the most abundant P fraction for all sampling times, accounting for 47-51% of the total P (Fig. 4-4). This fraction is considered unavailable to crops since the P is in organic form that must be decomposed before becoming available. However, due to subsidence, P contained in this residual fraction will eventually be made available to crops. Sulfur application did not alter P concentrations in this

fraction or the contribution of this fraction to total P. Phosphorus concentrations in this fraction fluctuated during the growing season in a contrasting pattern to the humic-fulvic acid fraction, averaging 565, 501, 598 and 547 mg P kg^{-1}, respectively, for 2, 6, 9 and 13 months after S application (Fig. 4-5).

Phosphorus Distribution and Availability in S-Amended Soils

Phosphorus concentrations in the top 15 cm of soil averaged 1244 mg P kg^{-1} initially and decreased significantly to an average of 1066 mg P kg^{-1} at the end of the sugarcane growing season. Soils are capable of releasing P constantly into solution over a long period of time (Arai et al., 2005) due to release of mineral-bound P and decomposition of organically-bound P. However, P release varies with soil properties (Zhou and Li, 2001). Active P fractions, such as labile P, Fe-Al-P, and Ca-P, comprise of the primary pools of desorbable P in weekly acidic and calcareous soils (Maguire et al., 2000). In the present study, the size of the inorganic P pools decreased by 13 months and consequently contributed to the decline in total P concentrations and the percentage of inorganic P to total P (Fig. 4-6). The P concentrations in this soil were a net result of the long-term balance between inputs (fertilization, rainfall and soil oxidation) and export (leaching, runoff, and sugarcane uptake). At the current rate of soil oxidation, approximately 60-90 kg P ha^{-1} is generated annually (Wright, 2009), which is greater than typical P fertilization rates to sugarcane (Rice et al., 2006) and P removed as harvested biomass (23 kg P ha^{-1}) (Coale et al., 1993). The significant reduction in total P indicates subsidence is a major source of P in runoff from EAA fields, which contributes to deterioration of

water quality and modifications to proximal aquatic ecosystems (Childers et al., 2003). Therefore, land management that minimizes soil subsidence is likely to reduce the potential for P export. The majority of P in this soil was retained in organic forms (Figs. 4-2 and 4-4), indicating the susceptibility of this soil to oxidation under drained conditions typical of sugarcane production in this region (Castillo and Wright, 2008; Wright, 2009).

Sulfur application at 448 kg S ha^{-1} promoted P accumulation in labile P and Fe-Al-P fractions (Fig. 4-3) suggesting increased P availability to crops and as well as potential increased risk of P export (Codling, 2008). However, no significant effects of S addition were found on total P and total inorganic and organic P concentrations. It was likely that the effects of S amendment on labile P and Fe-Al-P were confounded by the non-influential effects of sulfur on Ca-P, and considering the fact that Ca-P was the dominant inorganic fraction. A declining trend in pH for soils receiving 112 to 448 kg S ha^{-1} corresponded to a declining trend in the size of Ca-P pool (Figs. 4-4 and 4-5). It is reasonable to postulate that application rates beyond 448 kg S ha^{-1} would continue to reduce soil pH and reach a point causing significant releases of P from the Ca-bound fraction (Gessa et al., 2005), which may be a cause of concern since Ca-P comprised 32% of total P and more than 80% of total inorganic P in this soil (Fig. 4-4). Nonetheless, using the current recommended S application guidelines and rates, the risk of P export from the Ca-bound P would be minimal. However, due to the increasing pH problem for EAA soils, there may be a need for greater S application rates in the future, which may overcome the soil's

buffering capacity and in fact release large amounts of P from the Ca-bound pool and therefore pose an environmental hazard to nearby aquatic ecosystems.

Conclusions

Organic P was the major form of P in this soil, averaging 63% of total P, while the Ca-P fraction dominated the inorganic pools, contributing 32% of total P. Total P concentrations in the surface soil decreased significantly at the end of growing season as a result of considerable reduction in inorganic P, especially labile P and Fe-Al-P, which comprised of the majority of available P for crops. Under current sugarcane production, organic P in this soil is susceptible to oxidation and a potential source for P loss. Application of S at rates up to 448 kg S ha^{-1} introduced limited effects on reduction in soil pH, yet a small decrease in soil pH promoted P accumulation in labile and Fe-Al bound fractions, which increased P availability and as well as the risk of P export from these two fractions. The pool of Ca-P was relatively stable under current S application guideline and rates. Higher S rates than currently recommended may overcome the soil's buffering capacity and consequently release large amounts of P from the Ca-bound pool and pose an environmental hazard.

Table 4-1. Chemical properties of the Dania soil in the Everglades Agricultural Area before fertilizer application. Numbers in parenthesis is

Soil Property	Unit	Concentration
Total organic C	g kg^{-1}	416 (3)
Total N	g kg^{-1}	38 (0.6)
Total P	mg kg^{-1}	850 (9)
Extractable NO_3-N	mg kg^{-1}	290 (39)
Extractable NH_4-N	mg kg^{-1}	16 (3)
Extractable Ca	mg kg^{-1}	720 (49)
Extractable Mg	mg kg^{-1}	105 (5)
Extractable Fe	mg kg^{-1}	13 (1)
Extractable Al	mg kg^{-1}	1.1 (0.2)

Table 4-2. Two-way ANOVA on different pools of P in soils amended with variable S application rates.

Variable	*P* Value		
	Treatment	Time	Interaction
Labile P*i*	0.014	0.004	0.234
Fe-Al bound P	0.004	<0.0001	0.069
Ca-bound P	0.272	0.022	0.858
Humic-fulvic acid P	0.350	0.514	0.397
Residual P	0.145	<0.0001	0.275
Total P	0.766	0.011	0.868

Table 4-3. Pearson correlations coefficients for P fractions and soil properties at α=0.05, n=64.

	Labile P*i*	Fe-Al-P	Ca-P	Humic-fulvic acid P	Residual P	Ca	Mg	Al	Fe	Mn	pH
Labile Pi	1										
Fe-Al-P	0.87	1									
Ca-P	0.49	0.34	1								
Humic-fulvic acid P	-0.36	NS	-0.39	1							
Residual P	NS	NS	0.60	-0.58	1						
Ca	0.26	NS	0.56	-0.53	0.65	1					
Mg	0.40	0.37	0.40	-0.44	0.25	0.66	1				
Al	0.62	0.68	NS	-0.29	NS	NS	0.46	1			
Fe	0.36	0.54	NS	-0.27	NS	NS	0.78	0.57	1		
Mn	0.38	0.36	0.47	-0.49	0.26	0.64	0.88	0.38	0.78	1	
pH	-0.33	-0.54	NS	NS	0.52	0.30	-0.3	-0.51	-0.50	NS	1

Ca, Mg, Al, Fe, and Mn = acetic acid-extractable concentrations; NS = not significant.

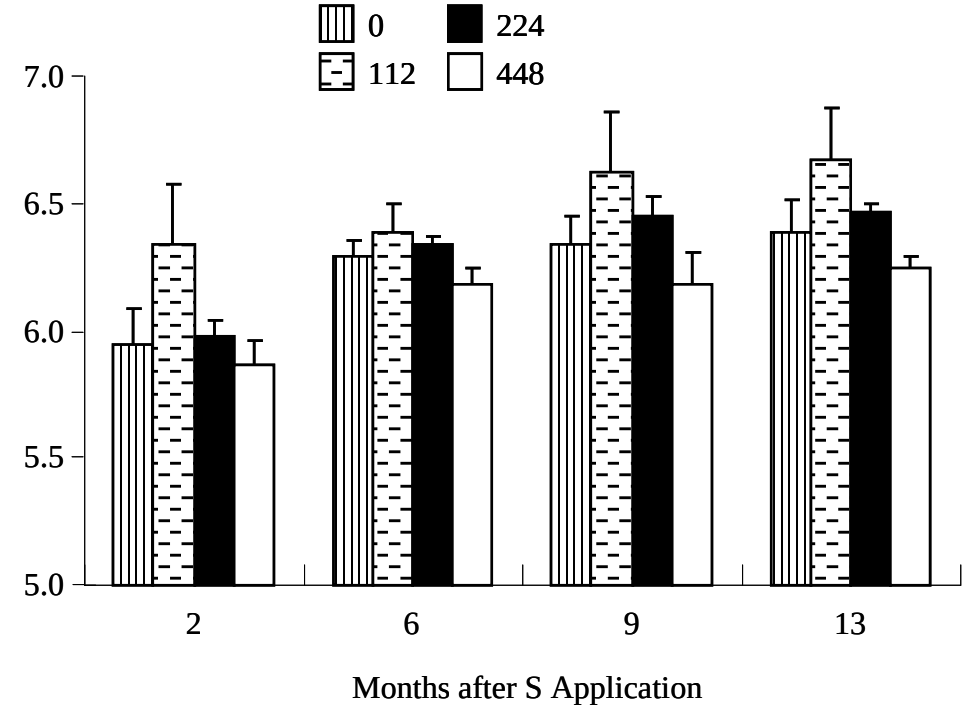

Fig. 4-1. Soil pH changes in response to different S application rates (0, 112, 224, and 448 kg S ha^{-1}) throughout the sugarcane growing season. Error bars represent the standard error of the mean.

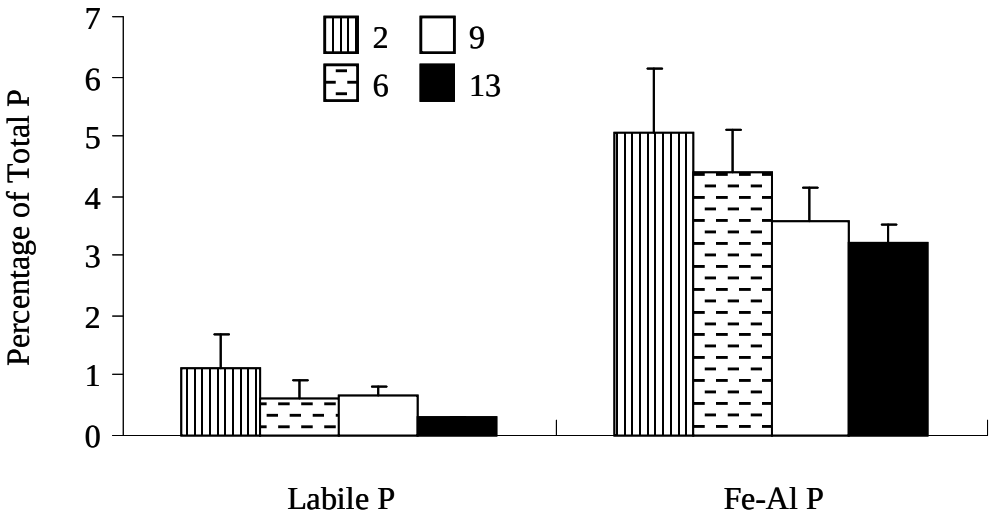

Fig. 4-2. The percentage of labile P and Fe-Al bound P of soil total P throughout the sugarcane growing season. Error bars represent the standard error of the mean.

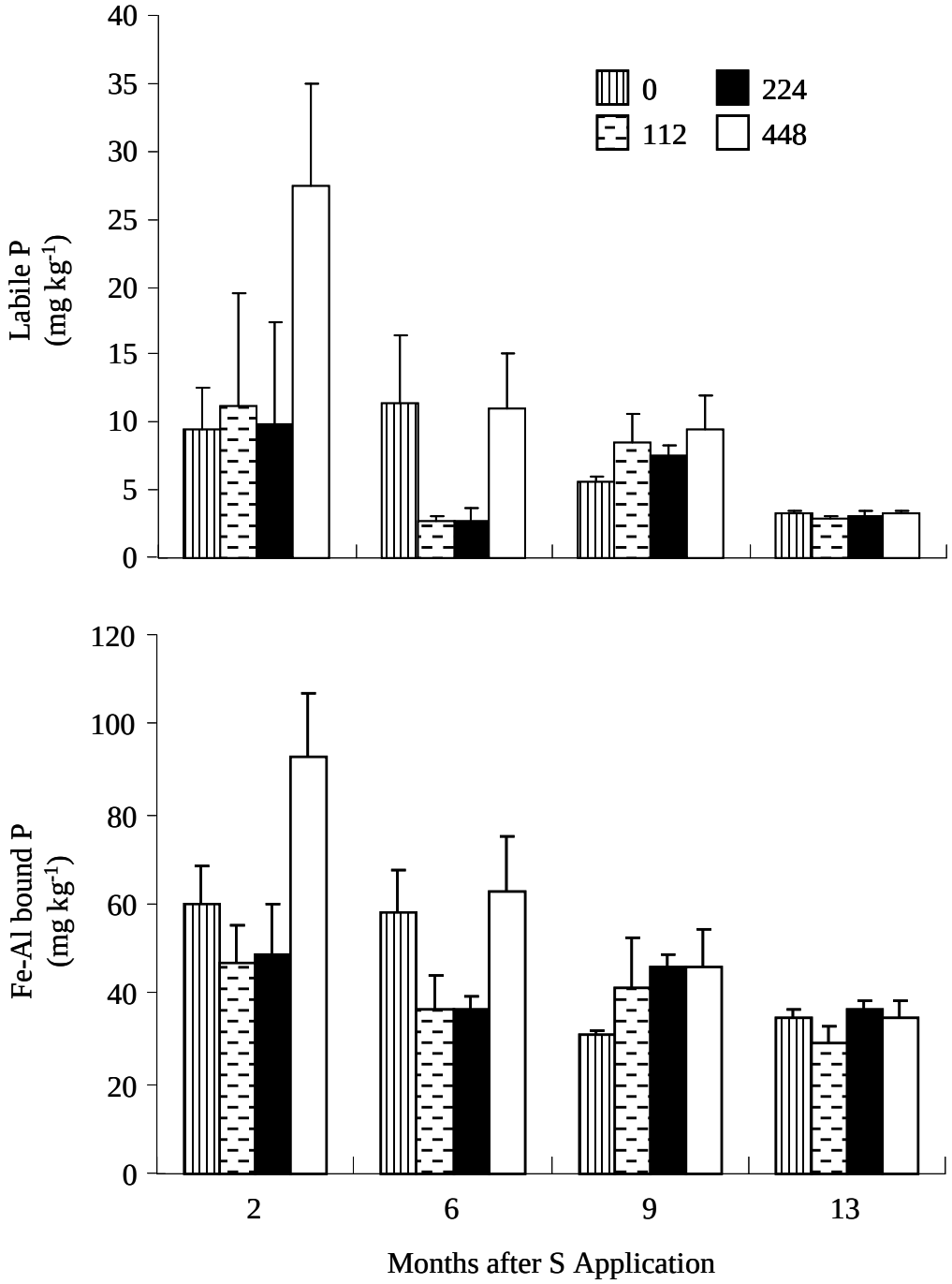

Fig. 4-3. Concentrations of labile P and Fe-Al bound P in soils 2, 6, 9, and 13 months after S application for different rates (0, 112, 224, and 448 S kg ha^{-1}). Error bars represent the standard error of the mean.

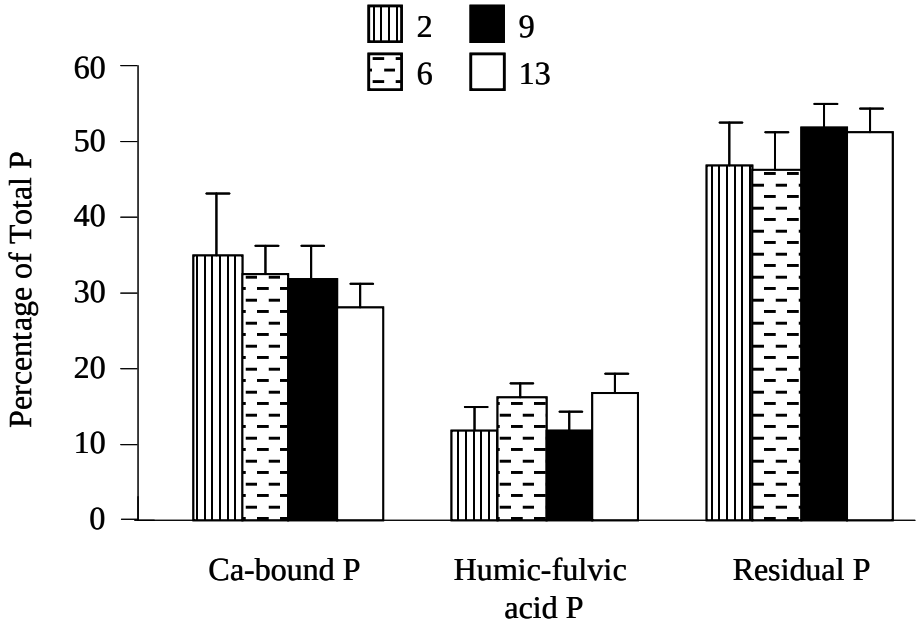

Fig. 4-4. The percentage of three P fractions to total P. Due to lack of significant effects of S, data for S application rates were averaged for presentation. Error bars represent the standard error of the mean.

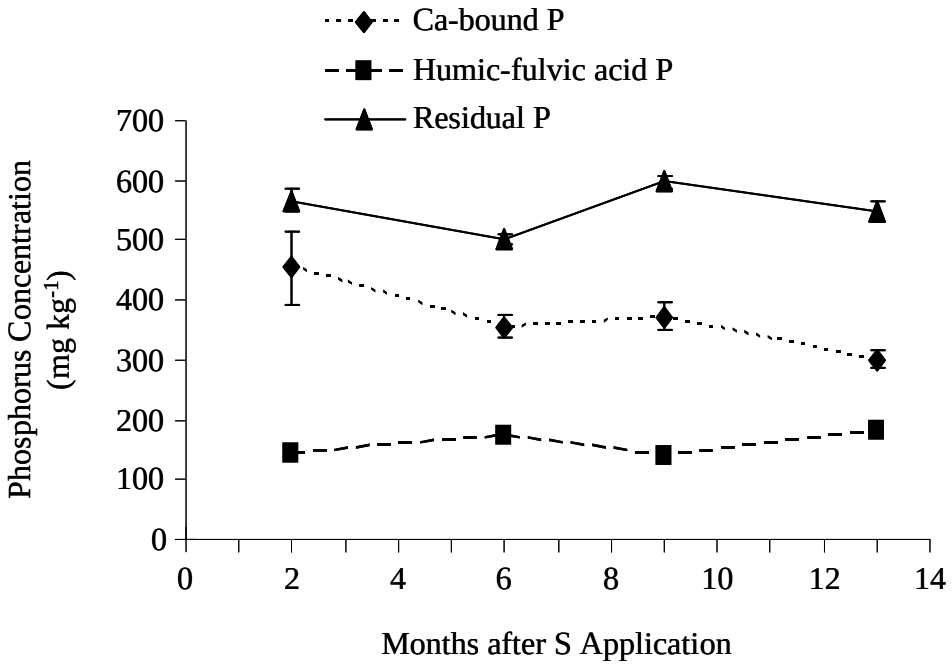

Fig. 4-5. Concentrations of P in Ca-bound, humic-fulvic acid, and residual fractions of soils at various times after S application. Due to lack of significant effects of S, data for S application rates were averaged for presentation. Error bars represent the standard error of the mean.

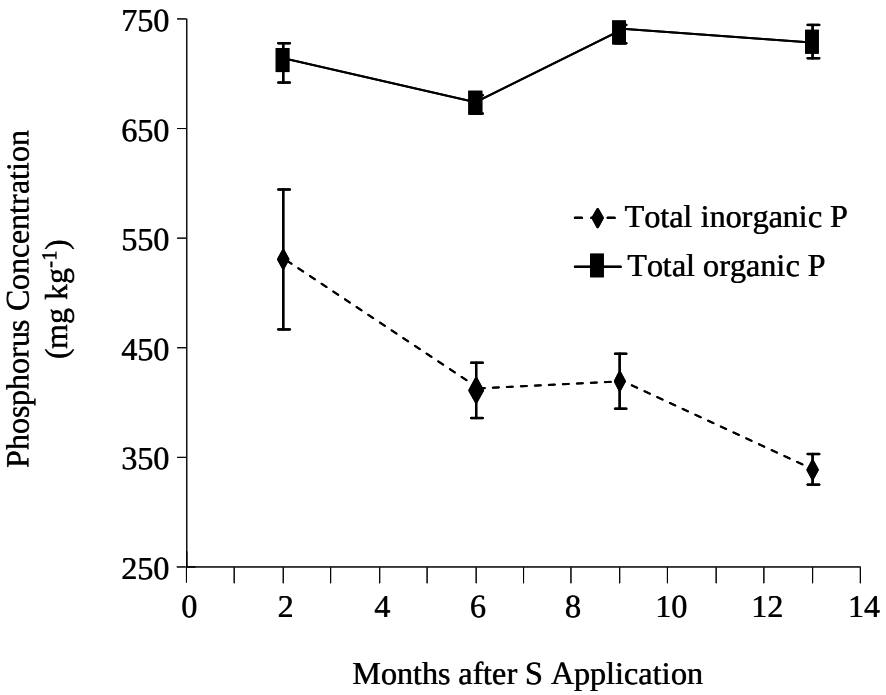

Fig. 4-6. Distribution of P among inorganic and organic pools after S application. Error bars represent the standard error of the mean.

CHAPTER 5
MICROBIAL ECO-PHYSIOLOGICAL RESPONSE OF A CALCAREOUS HISTOSOL TO SULFUR AMENDMENT

Introduction

Microbial communities play important roles in organic matter degradation and nutrient regeneration in soils. Likewise, soil physical and chemical properties and environmental factors greatly influence microbial activities and community composition (Allison et al., 2007). Microbial functional activities, such as extracellular enzyme activities (Allison et al., 2007), microbial respiration (Castillo and Wright, 2008; Iovieno et al., 2009), and nutrient mineralization rates (Corstanje et al., 2007; Wright et al., 2009) have been widely used as indicators to assess soil disturbance. Monitoring the microbial eco-physiological response to soil disturbance provides insights in understanding their effects and extent on nutrient cycling and organic matter turnover (Corstanje et al., 2007).

The EAA in south Florida was historically a seasonally-flooded prairie ecosystem but was converted to agricultural use by drainage in the early 1900s. Long-term drainage has resulted in oxidation of these Histosols resulting in a decreased depth to bedrock. The current estimate of soil loss is 1.5 cm yr^{-1} and many soils are less than 51 cm in depth, such as those classified as the Dania series (Shih et al., 1998; Snyder, 2005). Land use conversion and soil oxidation has contributed to nutrient export from agricultural fields into adjacent wetlands, resulting in SO_4 and P enrichment. Sulfate export from the EAA into Everglades wetlands has been implicated in causing stimulation of Hg methylation (Gabriel et al., 2008).

Long-term cultivation of these drained peatlands, specifically the use of tillage, coupled with soil oxidation, has also resulted in incorporation of bedrock $CaCO_3$ into surface soil and has gradually increased the pH from the historic 5.0 to 5.5 to approximately 7.0 to 7.5 today (Snyder, 2005; Gabriel et al., 2008). As a result, P and micronutrient availability to crops have decreased and necessitated new fertilizer management practices. Elemental S is occasionally applied as soil amendment for the purpose of reducing pH and therefore increasing P and micronutrient availability (Gabriel, et al., 2008). The microbial oxidation of elemental S to SO_4 produces acidity which in turn releases P bound to Ca minerals, which may result in a pulsed flux of P to soil solution. Also, the reduction in pH creates a more favorable environment for the soil microbial community and may enhance nutrient availability through increases in organic matter mineralization rates. Thus, there is concern that widespread S application may stimulate nutrient export from these soils which can harm down gradient wetlands. However, the buffering capacity of these calcareous Histosols is strong and may counteract the acidifying effects of S oxidation, thus effects of amendments may only be temporary and minimally effective (Beverly and Anderson, 1986).

Increased nutrient availability resulting from S amendment is likely to stimulate microbial activity and subsequently alter nutrient cycling and organic mater turnover (Wright and Reddy, 2001; Castillo and Wright, 2008). Due to the increases in pH and the decreasing depth to bedrock of soils in the EAA, the need for S amendments may increase in the future. Investigations of the responses of microbial functional

activities to variable S application rates are necessary and would provide insight in understanding the response of this system to S application. These results can then be used to help to formulate fertilizer and nutrient management solutions for better soil management in the EAA.

Material and Methods

Site Description

The experimental site is located in the central EAA on Dania muck (euic, hyperthermic, shallow Lithic Haplosaprist) with a depth to bedrock of approximately 50 cm. The experimental design was a randomized complete block with four S application rates and four field replications. Each field plot measured 9 m x 13 m and consisted of 6 rows of sugarcane (*Saccharum spp.*). Sugarcane cultivar CP 89-2143 was planted in November 2007 and harvested in February 2009. Elemental granular S (90%) was applied at rates of 0, 112, 224, and 448 kg S ha^{-1} to the furrow and covered after planting. Other fertilization was provided using typical recommendations and guidelines for this region and soil type (Rice et al., 2006). All fertilizers were soil-applied prior to planting and included 17 kg N ha^{-1} and 37 kg P ha^{-1} as monoammonium phosphate, 228 kg K ha^{-1} as KCl, 8.5 kg Mn ha^{-1}, 4.5 kg Cu ha^{-1}, 5.6 kg Fe ha^{-1}, 2.8 kg Zn ha^{-1}, and 1.1 kg B ha^{-1}. All plots received common cultural practices including tillage and herbicide application. Water was applied as needed via seepage irrigation in field ditches approximately 182 m apart.

Soil Sampling and Analysis

Soil samples were collected before planting and fertilizer application and then in January 2008, May 2008, August 2008, and December 2008, corresponding to approximately 0, 2, 6, 9, and 13 months after planting, respectively. Twelve soil (0-15 cm) cores (2.54 cm diameter) were randomly collected from rows within each field plot and composited. Samples were homogenized after the removal of visible plant residues and stored at 4 °C.

The pH was measured using a soil to water ratio of 1:3 after equilibration for 30 min. Total organic C was measured by loss-on-ignition at 550°C for 4 hr after conversion to organic C with a coefficient factor of 0.51 (Wright et al., 2008). Dissolved organic C was measured by extraction with 0.5 *M* K_2SO_4 and analyzed with a TOC-5050A total organic C analyzer (Shimadzu, Norcross, GA). Total N was measured by Kjeldahl digestion followed by NH_4 analysis (Bremner, 1996). Extractable NH_4-N and NO_3-N were determined by extraction with 2 *M* KCl followed by colorimetric analysis (Castillo and Wright, 2008). Total P was determined using the ascorbic acid-molybdenum blue method after Kjeldahl digestion, and labile inorganic P measured after Mehlich-1 extraction (Kuo, 1996).

Microbial biomass C and N were measured by the fumigation-extraction method using a conversion factor of 0.37 and 0.54, respectively (Wright et al., 2009). The microbial biomass P was determined as the difference in total P of $NaHCO_3$ extracts between fumigated and unfumigated samples (Wright et al., 2009). Potentially mineralizable N and P were determined based on a 10-d incubation

followed by extraction with 2 *M* KCl (for N) and 1 *M* HCl (for P) and subtraction of initial N and P concentrations (Wright et al., 2009). Mineralized S was calculated as the difference in water-extractable SO_4 between residual soil and after a 10 d incubation, with SO_4 being analyzed by ion chromatography (Gharmakher et al., 2009). Microbial respiratory activities were measured as CO_2 production after 5 and 10 d incubation (Corstanje et al., 2007) and expressed as the slope of the regression of cumulative CO_2 production over the 10-d incubation period (Wright and Reddy, 2008).

In consideration of the potential acidification effect induced by S oxidation and in order to resemble real soil pH variations between treatments, all enzyme activities were assayed with non-buffered solutions. Approximately, 1 g moist soil was placed in polypropylene centrifuge tubes, mixed with 30 ml of sterile water, and shaken for 25 min. Homogenized samples were further diluted 5 times for enzyme assays. Enzyme assays were conducted using 6 replicates with controls to offset non-enzymatic production. Phosphatase, glucosidase, and leucine aminopeptidase assays were conducted in 96-well microtiter plates. The enzyme substrates were 1 *mM* 4-MUF-phosphate (Sigma, St. Louis, MO), 1 *mM* 4-MUF-*β*-D-glucopyranoside (Sigma, St. Louis, MO), and 2.5 *mM* L-Leucine 7-Amino-4-methylcoumarin (Biosynth, Naperville, IL), respectively. 200 µL of sample was incubated with 50 µl substrates at 20°C for 3 hr. The florescence readings were collected at 0.5 hr intervals using a Bio-TEK FL600 fluorescence plate reader (Bio-TEK Instruments Inc., Winooski, VT) at a setting of 365 ηm excitation and 450 nm emission. Enzyme

activity was determined by calculating the mean florescent reading changes over time. Sulfatase activity was determined colorimetrically according to the methods of Wright and Reddy (2001).

Statistical Analysis

A mixed model was fit using restricted maximum likelihood in the MIXED procedure of SAS (Littell et al., 2006). The fixed effects were S application rate, time and their interaction. Block was a random effect. Degrees of freedom were adjusted using the Kenward-Roger adjustment. An exponential covariance structure was used to model the correlation among observations taken from the same plot over time. Significant differences among individual treatments and time intervals were determined with Tukey's test at $\alpha = 0.05$. Pearson correlation analysis was performed to assess relationships between variables. All statistical analyses were carried out with SAS 9.1 (SAS Institute).

Results

Soil Physical and Chemical Properties

Soil chemical properties were listed on Table 5-1. Application of S at a range from 0 to 448 kg S ha^{-1} did not significantly affect soil pH, extractable NH_4-N, NO_3-N, and SO_4, and dissolved organic C. Dissolved organic C increased significantly from 2 (1346 mg kg^{-1}) to 6 months (1572 mg kg^{-1}), and then decreased toward the end of the season. Inversely, extractable NO_3-N decreased from 2 (383 mg kg^{-1}) to 6 months (16 mg kg^{-1}), but then increased at 13 months (49 mg kg^{-1}). Extractable NH_4-N and SO_4 both decreased gradually throughout the growing season. The

interaction of S application and time was significant for labile P. Further analysis revealed that labile P was substantially higher in soils amended with 448 kg S ha^{-1} (118 mg kg^{-1}), than soils receiving lower S rates at 2 months after application (49 mg kg^{-1}) (Fig. 5-1). However, the higher P concentrations were not observed at later months.

Extracellular Enzyme Activities

The S application effect on phosphatase activity was significant, yet the effect was only observed at 2 months after S application (Fig. 5-2a). Phosphatase activity at 2 months was considerably higher for soils receiving 448 kg S ha^{-1} (131 mg MUF kg^{-1} h^{-1}) than soils receiving 0, 112, and 224 kg S ha^{-1}, which averaged 61, 76, and 81 mg MUF kg^{-1} h^{-1}, respectively. Phosphatase activity also fluctuated seasonally, with the lowest activity observed at 6 months (52 mg MUF kg^{-1} h^{-1}) and the highest activity at 9 months (102 mg MUF kg^{-1} h^{-1}). Glucosidase activity significantly increased at 2 months, averaging 15, 56, 58, and 99 mg MUF kg^{-1} h^{-1} for the increasing S application rates (Fig. 5-2b). Glucosidase was also significantly higher at 2 and 9 months than at 6 and 13 months. Analysis of variance showed that leucine aminopeptidase activity was not affected by any S rate, averaging 68 mg MUF kg^{-1} h^{-1} (Fig. 5-3a). However, leucine aminopeptidase decreased significantly toward the end of the sugarcane growing season. Sulfatase activity did not respond to S application at any rate (Fig. 5-3b) and was largely unaffected by seasonality.

Microbial Biomass

Microbial biomass C was not altered as a result of S amendment, but did fluctuate during the growing season (Table 5-2). The highest concentration occurred at 9 months (17 g kg^{-1}), followed by 2 months (13 g kg^{-1}) and 6 and 13 months (11 g kg^{-1}). Similarly, the size of the microbial biomass N pool did not change after S amendment and was stable throughout the growing season. Microbial biomass P increased significantly at 2 months at the highest S rate (177 mg kg^{-1}), which was about 3 times higher than for lower S application rates (Fig. 5-4). However, the stimulating effect did not extend beyond 2 months.

Microbial-Mediated Mineralization

Aerobic CO_2 production rates did not differ between soils receiving variable S application rates (Table 5-2). The production was the highest at 9 months after S application (44 mg CO_2-C kg^{-1} d^{-1}) and lowest at 2 months (26 mg CO_2-C kg^{-1} d^{-1}), corresponding to temperature patterns. Similarly, N and P mineralization rates were not influenced by S amendment (Table 5-2). The highest overall rates of N mineralization were found at 9 months (10 mg kg^{-1} d^{-1}) and the lowest rates at 13 months (2 mg kg^{-1} d^{-1}). Sulfur mineralization rates significantly increased as a result of S application (Fig. 5-5). Overall, mineralized S was 1421% greater for soils receiving 448 kg S ha^{-1} than unamended soil, 375% greater than soils receiving 112 kg S ha^{-1}, and 219% greater for soils receiving 224 kg S ha^{-1}. Nonetheless, mineralized S rates significantly decreased throughout the growing season.

Discussion

Labile P is considered the most bioavailable form of P in soils. Sulfur application at 448 kg S ha^{-1} significantly increased concentrations of labile P at 2 months (Fig. 5-1), suggesting increased P availability to sugarcane as well as soil microorganisms (Codling, 2008). There are two primary mechanisms by which S influences P availability: lowering of soil pH (Gabriel, et al., 2008) and replacement of PO_4 with SO_4 and the release of P from association with Fe, Al, and Ca (Jaggi et al., 2005). These results are supported by the fact that labile P was significantly correlated with pH (R^2 = -0.35, p = 0.005) and extractable SO_4 (R^2 = 0.29, p = 0.019). However, increased P availability was not observed at later months indicating limited long-term effects of S on the reduction in soil pH due to the high buffering capacity of this calcareous organic soil (Jaggi et al, 2005; Snyder, 2005).

Enzymatic Activities

Extracellular enzymes are excreted by the microorganisms to the soil for the purpose of sequestering nutrients. The enzyme activities, especially hydrolases, are known to be involved in organic matter turnover and nutrient cycling in terrestrial systems (Wright and Reddy, 2001; Corstanje et al., 2007). Phosphatase catalyzes the hydrolysis of organic P ester resulting in the release of P, and thus plays an important role in P regeneration from soils (Wright and Reddy, 2001). Glucosidase catalyzes the hydrolysis of glycosides and its activity reflects the state of organic matter and processes occurring therein (Tejada et al., 2006). Our results showed that phosphatase activities were 115% higher for soils receiving the highest S rates

than unamended soils at 2 months after S application, while glucosidase activities were 573% higher (Fig. 5-2). High enzyme activity may indicate nutrient limitation (Sinsabaugh et al., 1993; Allison et al., 2007), and the highest activity occurred during the winter sampling when soil oxidation rates are typically lowest (Snyder, 2005). Thus, the nutrient-supplying capacity of this soil was low at this time. In fact, soil oxidation typically provides a major portion of the sugarcane nutrient requirements for this soil (Rice et al., 2006). Negative correlations between nutrient availability and related enzyme activities have been demonstrated (Wright and Reddy, 2001; Allison and Vitousek, 2005). However, no such correlations were observed in the present study, but instead, both phosphatase and glucosidase activities were positively correlated to the concentrations of labile P (Table 5-3), which together may suggest the P limitation for organic matter turnover in these high C soils. (Allison et al., 2007). The EAA soils are primarily Histosols with high organic matter content, approximately 85% by weight, which contain high N yet low P and micronutrient concentrations that require supplemental fertilization (Snyder, 2005; Castillo and Wright, 2008). The background C: N: P molar ratio at this site was 1274:98:1, indicating this soil was indeed P limited, which may constrain the activities and growth rates of microorganisms (Fontaine et al., 2003). As demonstrated previously, application of S increased the concentration of labile P at 2 months (Fig. 5-1) and both activities of phosphatase and glucosidase simultaneously increased (Fig. 5-2). It is plausible that the application of S had a transitory effect on soil pH which stimulated P release to the soil environment and hence enhanced the

microbial enzymatic activities. Thus, application of S at the current recommended rate had a short-lived effect on microbial activity. The combination of soil buffering capacity and sugarcane nutrient uptake likely minimized P release and accumulation in soil that was initiated by S application. These results indicate that higher S rates may be needed to prolong the response of the soil microbial community and maintain nutrient availability throughout the growing season.

Leucine aminopeptidase and sulfatase were also assayed in this study to represent N and S cycles. Leucine aminopeptidase is involved in the degradation of proteins (Larson et al., 2002), while sulfatase hydrolyzes aromatic ester sulfates (Knauff et al., 2003). Both enzyme activities were not influenced by any rate of S application (Fig. 5-3). Since enzymes are biological catalysts capable of catalyzing specific chemical reactions, their responses may vary based on substrate quality and real microbial community composition (Corstanje et al., 2007). Soil enzyme activities may also depend on other parameters, such as seasonal variation in soil moisture, pH and temperature (Knauff et al., 2003; Wallenstein et al., 2009), which may explain the lack of response to S amendment in this soil.

Microbial Biomass

Microbial biomass P was more sensitive to S addition than biomass C and N at 2 months, as biomass P for soils receiving 448 kg S ha^{-1} was 314% higher than soils receiving the lower rates (Fig. 5-4). Correlation analysis revealed that microbial biomass P was significantly correlated to labile P, total P, extractable SO_4, pH, NH_4-N, and NO_3-N (Table 5-3). Considering the fact that this Histosol is P limited, it was

likely that increased labile P at 2 months after S application caused P immobilization into microbial biomass, which may explain the lower labile P concentrations at subsequent sampling times during the growing season. Increasing biomass P as a result of increased P availability in Everglades soils has been well documented (Corstanje et al., 2007; Castillo and Wright, 2008; Wright et al., 2009).

Microbial-Mediated Organic Matter Mineralization

Agricultural practices in EAA soils, such as tillage and P fertilization, showed variable effects on organic C mineralization (Morris et al., 2004). The present study indicated that S amendment did not appear to influence microbial aerobic respiration rates indicating that S application will not further stimulate soil oxidation. Correspondingly, the metabolic coefficient (qCO_2), the proportion of aerobic respiration to microbial biomass C, did not change across S rates (Table 5-4), suggesting that application at rates up to 448 kg S ha^{-1} did not alter organic matter turnover. Glucosidase activity is known to reflect the state of organic matter cycling (Tejada et al., 2006). However, glucosidase activity was not correlated with C mineralization rates (Table 5-3). Glucosidase is one of several soil enzymes involved in C mineralization process and hence its activities alone may not be able to reflect the overall real C mineralization, which may explain why no increased CO_2 production was observed at 2 months at 448 kg S ha^{-1} when glucosidase activity was significantly enhanced.

Potentially mineralizable N and the quotient (mineralized N as a function of biomass N) characterize the potential N turnover in the system (Corstanje et al.,

2007; Castillo and Wright, 2008). No clear effects of S amendment on N turnover rates in this Histosol were observed (Table 5-2 and 5-4). Likewise, net P regeneration did not appear to be impacted by S, even after phosphatase activities were significantly increased at 2 months, suggesting that P mineralization was not limited by phosphatase activity (Carreira et al., 2000). Studies have shown no correlation between phosphatase activity and gross P mineralization, whereas others have shown positive relationships (Carreira et al., 2000). Agriculture in the EAA has been identified as a major source of P enrichment in Everglades wetlands, which damaged the ecosystem and impaired water quality (Wright et al., 2009). Our results indicated that S application under current guidelines may not enhance the microbial-mediated P regeneration in EAA soils and thus minimize the risk of P exports from agricultural fields.

Potential S mineralization was the only mineralization process showing a clear response to S amendment. It has been proposed that S mineralization involved two processes, biological and biochemical mineralization, in which SO_4 is released as a by-product of C oxidation and as a product of enzymatic hydrolysis (McGill and Cole, 1981; Chen et al., 2001). Nonetheless, in the present study, mineralized S was not correlated with CO_2 production or sulfatase activity. Instead, S mineralization was highly correlated with glucosidase activity (Table 5-3), which may suggest that net mineralization of S was not simply dependent on either biological or biochemical processes (Eriksen et al., 1998). Oxidation of elemental S may also contribute to the observed increased mineralized S rates with increasing S application rate.

Elemental S is oxidized by soil microorganisms producing SO_4, which can accumulate in soil solution. The elemental S oxidation rate may be slow (Deluca et al., 1989) and SO_4 accumulation in this study is likely a result of both organic S mineralization and elemental S oxidation to SO_4. Our results demonstrated that mineralized S increased concurrently with higher S application rates and the effects continued throughout the growing season, although stimulatory effects diminished with subsequent sampling times (Fig. 5-5).

Seasonal Fluctuations in Microbial Indices

Seasonal variations in soil microbial activities are common (Wallenstein et al., 2009) and dependent on environmental factors such as rainfall and temperature. Soil disturbance resulting from agricultural practices are also known to pose impacts on microbial community composition and activity (Knauff et al., 2003; Morris et al., 2004; Wright and Reddy, 2008). During the growing season, tillage was applied to improve drainage and weed control, which has been found to affect the size of the microbial biomass pool and organic matter mineralization rates (Morris et al., 2004; Castillo and Wright, 2008a). Our results showed clear seasonal fluctuations for most of the microbial parameters, suggesting the net results of interactions between environmental factors, soil management, and the sugarcane growing season. Most effects occurred within 2 months of S application, suggesting a strong effect of fertilization for this site. Nutrient concentrations decreased during the growing season due to sugarcane uptake, thus nutrient limitations may have minimized microbial responses to S application at subsequent sampling times. In fact, these

soils are often low in plant-available nutrients with the exception of N (Rice et al., 2006).

Conclusions

Application of elemental S at 448 kg S ha^{-1} increased P availability at 2 months, which subsequently stimulated some enzyme activities and simultaneously promoted labile P to be immobilized in microbial biomass. However, these effects were temporary and not observed beyond 2 months. There was limited effect of S application on increasing the P availability due to the high buffering capacity of this organic soil against pH reduction. Overall, S amendment at rates up to 448 kg S ha^{-1} did not appear to pose significant impacts on organic matter turnover and N and P regeneration rates, suggesting that S application will not stimulate soil oxidation and result in large-scale nutrient flux from soil. Using the current recommended S application guidelines and rates, impacts on microbial activities and functions should be minimal. However, due to the increasing pH trend for these soils, there may be a need for higher S application rates in the future. These higher S rates may overcome the soil's buffering capacity and release large amounts of P, potentially stimulating microbial functional activities and altering organic matter dynamics. Additionally, oxidation of elemental S would produce large amounts of SO_4 and therefore may pose an environmental hazard to the nearby aquatic ecosystem.

Table 5-1. Extractable nutrients (mg kg^{-1}) and pH in soil amended with elemental S during the sugarcane growing season. Values denote means and the standard error is in parentheses.

	Dissolved organic C	Extractable NH_4-N	Extractable NO_3-N	Extractable SO_4	Labile P*i*	pH
Treatment						
0 kg S ha^{-1}	1315 (58)	12 (2)	110 (41)	107 (40)	47 (4)	6.2 (0.1)
112 kg S ha^{-1}	1583 (93)	10 (1)	115 (39)	145 (40)	46 (4)	6.5 (0.1)
224 kg S ha^{-1}	1419 (56)	10 (1)	120 (42)	141 (37)	56 (8)	6.3 (0.1)
448 kg S ha^{-1}	1381 (45)	12 (3)	121 (42)	179 (42)	66 (11)	6.1 (0.1)
Time						
2 months	1346 (47)	19 (3)	383 (19)	376 (32)	66 (12)	6.0 (0.1)
6 months	1572 (67)	10 (0)	16 (1)	129 (13)	48 (1)	6.3 (0.0)
9 months	1436 (80)	9 (1)	19 (1)	21 (3)	53 (4)	6.4 (0.1)
13 months	1344 (66)	6 (0)	49 (2)	47 (7)	46 (8)	6.4 (0.1)

Table 5-2. Potentially mineralizable C (Cmin), N (Nmin), P (Pmin), and microbial biomass C (MBC), N (MBN), P (MBP) in soil amended with elemental S during the sugarcane growing season. Values denote means and the standard error is in parentheses.

	Cmin ($mg\ kg^{-1}\ d^{-1}$)	Nmin ($mg\ kg^{-1}\ d^{-1}$)	Pmin ($mg\ kg^{-1}\ d^{-1}$)	MBC ($g\ kg^{-1}$)	MBN ($g\ kg^{-1}$)	MBP ($mg\ kg^{-1}$)
Treatment						
0 kg S ha^{-1}	34 (1.9)	6 (0.8)	6 (3.6)	12 (0.9)	0.28 (0.03)	33 (4.5)
112 kg S ha^{-1}	39 (2.7)	8 (1.1)	4 (1.5)	13 (0.8)	0.27 (0.03)	31 (2.8)
224 kg S ha^{-1}	38 (3.4)	7 (1.0)	5 (2.3)	13 (0.6)	0.27 (0.03)	29 (3.9)
448 kg S ha^{-1}	32 (2.0)	6 (0.9)	2 (1.2)	13 (0.8)	0.29 (0.03)	57 (13.2)
Time						
2 months	26 (1.3)	8 (1.1)	6 (2.8)	13 (0.4)	0.27 (0.01)	64 (13.2)
6 months	36 (1.8)	6 (0.3)	6 (2.7)	11 (0.9)	0.27 (0.02)	33 (4.0)
9 months	44 (3.3)	10 (0.3)	3 (2.3)	17 (0.2)	0.35 (0.04)	28 (4.1)
13 months	37 (1.2)	2 (0.2)	1 (0.6)	11 (0.2)	0.23 (0.01)	27 (1.1)

Table 5-3. Significant correlation coefficients ($p < 0.05$) between selected chemical properties and microbial functional activities (n = 64).

	pH	DOC	NH_4	NO_3	P*i*	SO_4	LAP	PHO	GLU	SUL	Cmin	Nmin	Pmin	Smin	MBC	MBN	MBP
pH	1																
DOC	0.63*	1															
NH_4	-0.42	NS	1														
NO_3	-0.51	NS	0.61	1													
P*i*	-0.35	NS	0.60	0.28	1												
SO_4	-0.46	NS	0.60	0.88	0.29	1											
LAP	0.42	0.57	NS	NS	NS	NS	1										
PHO	NS	NS	NS	NS	0.28	NS	NS	1									
GLU	-0.28	NS	0.30	0.41	0.35	0.35	NS	0.64	1								
SUL	NS	-0.29	NS	0.41	NS	NS	NS	NS	NS	1							
Cmin	0.52	0.50	-0.33	-0.51	NS	-0.45	NS	NS	NS	NS	1						
Nmin	NS	NS	NS	NS	NS	NS	0.44	NS	NS	0.29	NS	1					
Pmin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1				
Smin	-0.38	NS	0.31	NS	0.49	0.39	NS	0.28	0.41	NS	NS	NS	NS	1			
MBC	NS	NS	NS	NS	NS	NS	NS	0.34	NS	NS	NS	0.41	NS	NS	1		
MBN	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.36	NS	NS	NS	1	
MBP	-0.38	NS	0.57	0.49	0.63	0.47	NS	NS	0.35	NS	-0.37	NS	NS	0.61	NS	NS	1

*, significant at $p = 0.05$; NS, not significant; DOC, dissolved organic C; NH_4, extractable NH_4-N; NO_3, extractable NO_3-N; P*i*, extractable labile P; SO_4, extractable SO_4-S; MBC, microbial biomass C; MBN, microbial biomass N; MBP, microbial biomass P; LAP, leucine aminopeptidase; PHO, phosphatase; GLU, glucosidase; SUL, sulfatase; Cmin, potentially mineralizable C; Nmin, potentially mineralizable N; Pmin, potentially mineralizable P; Smin, potentially mineralizable S;

Table 5-4. Microbial metabolic coefficient (qCO_2), microbial biomass C to organic matter content ratio (MBC/OM), and potential N and P mineralization quotient (qPMN and qPMP) in soils amended with elemental S during the sugarcane growing season. Values denote means and the standard error is in parentheses.

	qCO_2 (×100)	MBC/OM (%)	qPMN (mg N g^{-1} MBN)	qPMP (mg P g^{-1} MBP)
Treatment				
0 kg S ha^{-1}	0.31 (0.04)	1.5 (0.1)	22 (3.3)	192 (130)
112 kg S ha^{-1}	0.31 (0.04)	1.7 (0.1)	28 (4.1)	136 (54)
224 kg S ha^{-1}	0.29 (0.02)	1.6 (0.1)	26 (4.4)	153 (71)
448 kg S ha^{-1}	0.28 (0.04)	1.6 (0.1)	23 (5.0)	42 (22)
Time				
2 months	0.20 (0.01)	1.6 (0.1)	31 (4.0)	160 (59)
6 months	0.39 (0.05)	1.3 (0.1)	24 (2.3)	212 (121)
9 months	0.27 (0.02)	2.1 (0.0)	36 (4.8)	109 (72)
13 months	0.33 (0.01)	1.4 (0.0)	8 (0.9)	45 (24)

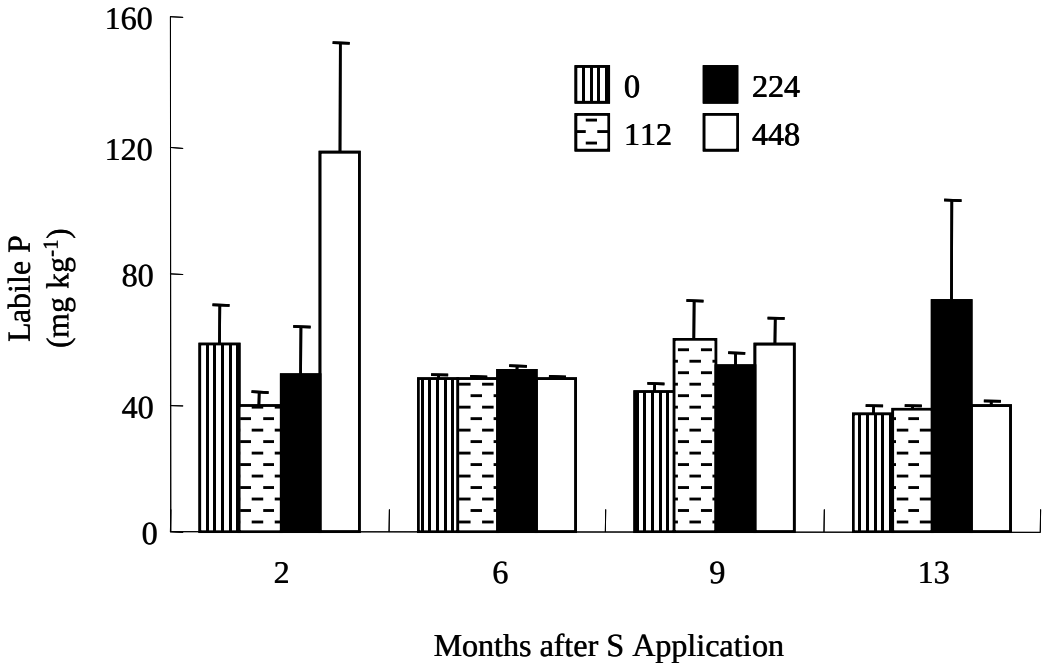

Fig. 5-1. Concentrations of labile P in soils at 2, 6, 9, and 13 months after elemental S application at different rates (0, 112, 224, and 448 S kg ha^{-1}). Error bars represent the standard error of the mean.

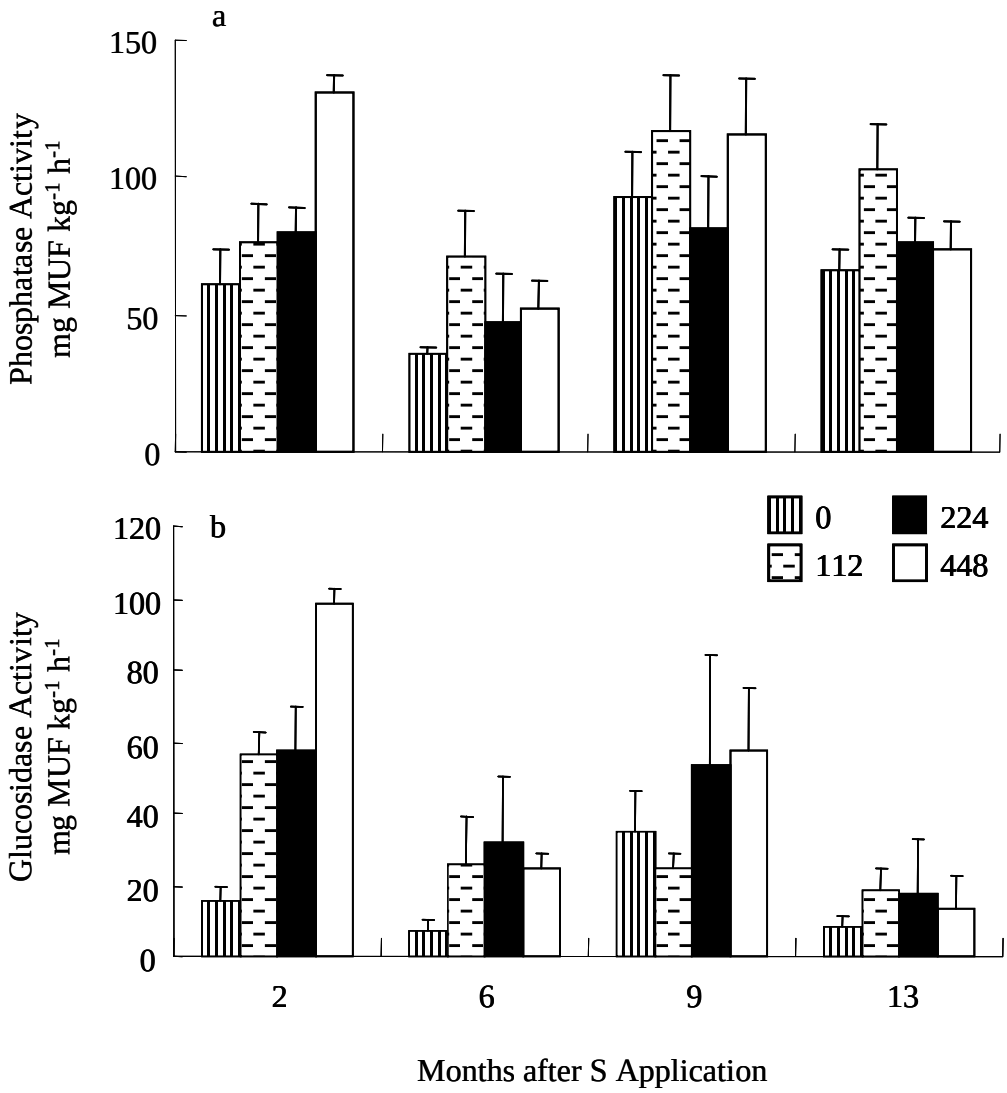

Fig. 5-2. Activities of phosphatase (a) and glucosidase (b) in response to different elemental S application (0, 112, 224, and 448 kg S ha^{-1}) throughout the sugarcane growing season. Error bars represent the standard error of the mean.

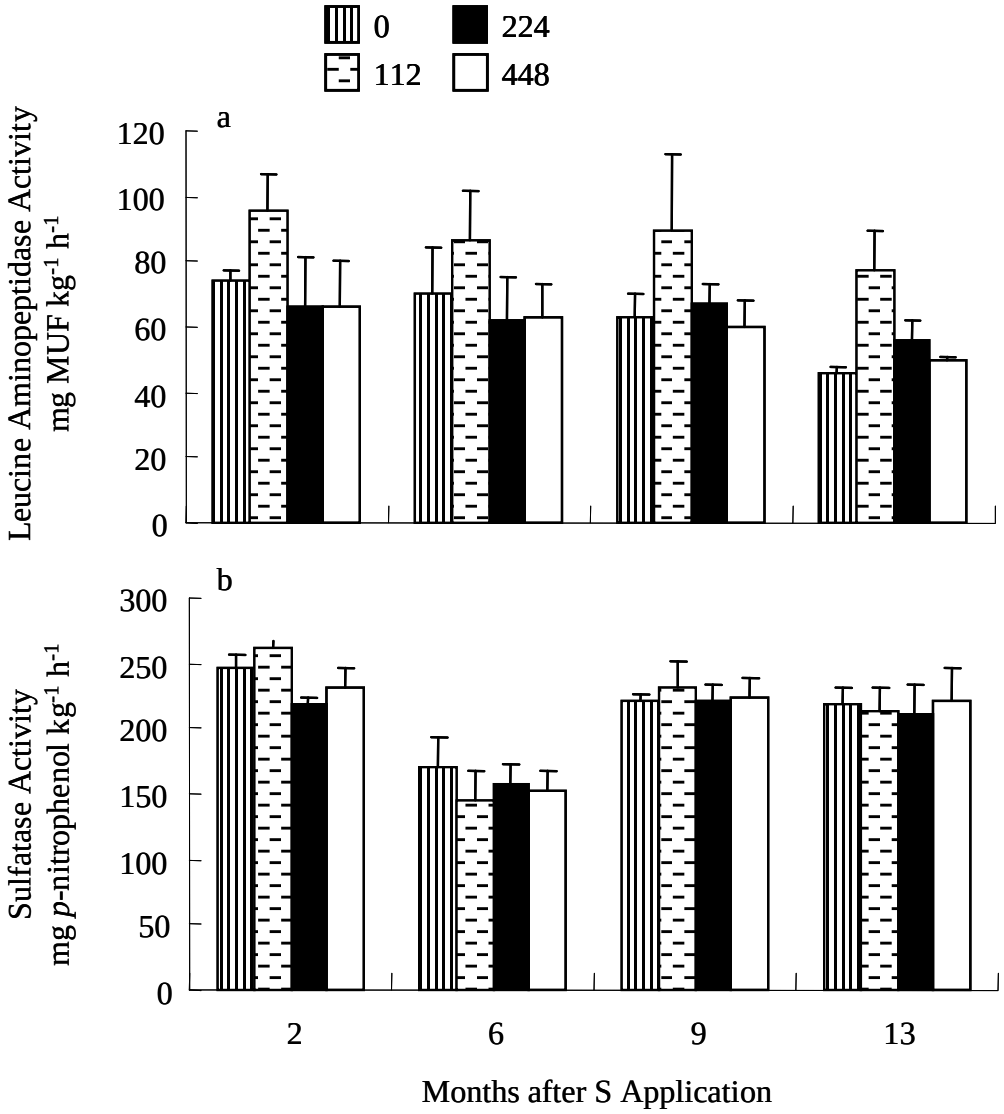

Fig. 5-3. Activities of leucine aminopeptidase (a) and sulfatase (b) in response to different elemental S application rates (0, 112, 224, and 448 kg S ha^{-1}) throughout the sugarcane growing season. Error bars represent the standard error of the mean.

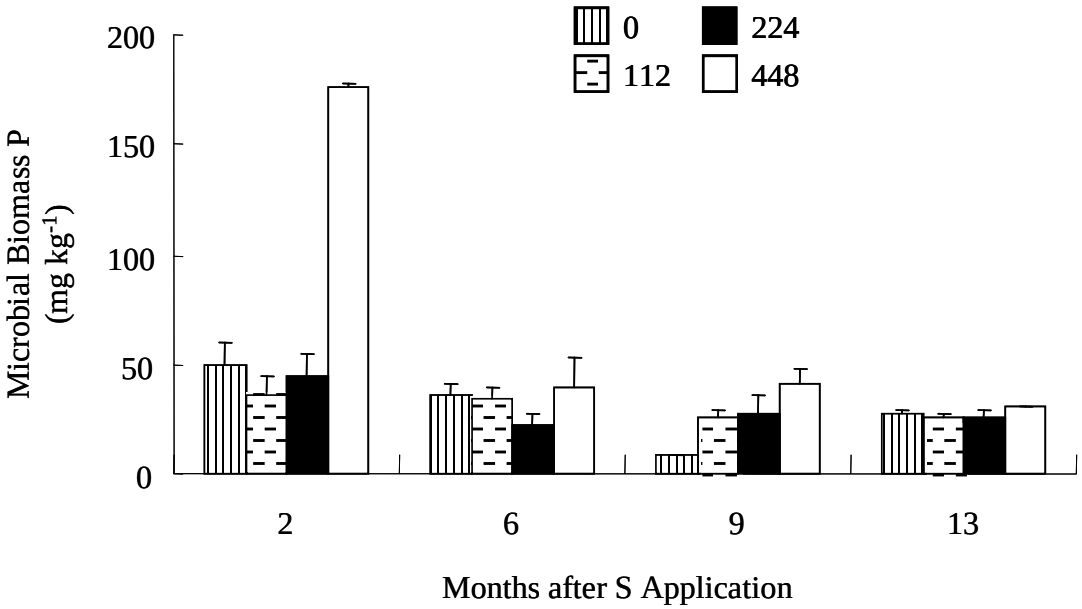

Fig. 5-4. Microbial biomass P in soils at 2, 6, 9, and 13 months after elemental S application at different rates (0, 112, 224, and 448 S kg ha^{-1}). Error bars represent the standard error of the mean.

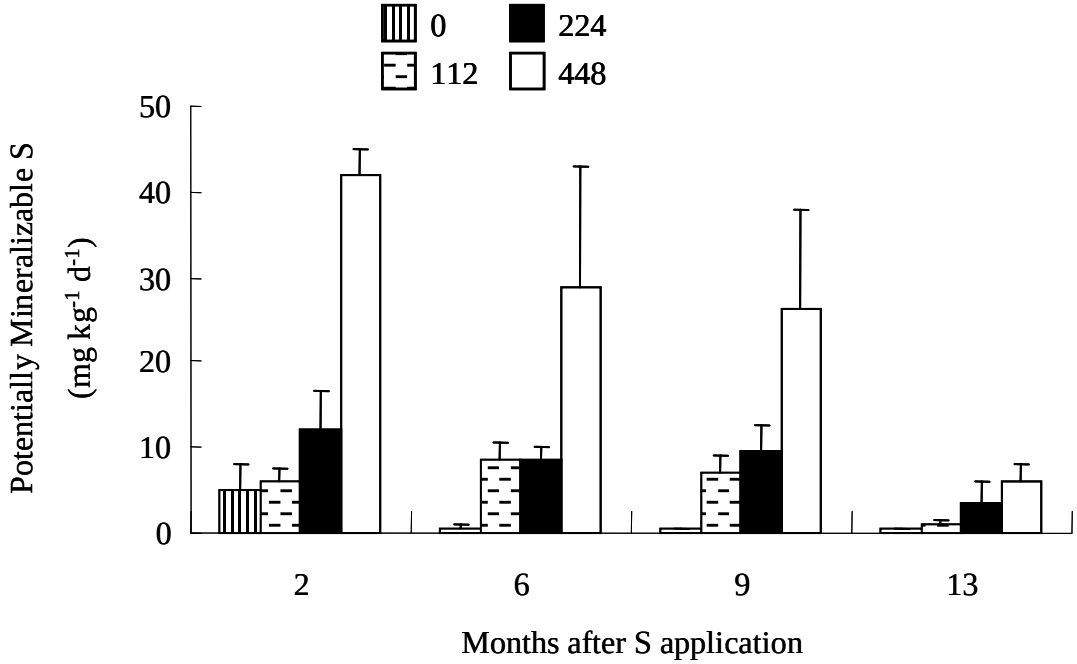

Fig. 5-5. Mineralized S in soils at 2, 6, 9, and 13 months after S application at different rates (0, 112, 224, and 448 S kg ha^{-1}). Error bars represent the standard error of the mean.

CHAPTER 6
SEASONAL CHANGES IN NUTRIENT AVAILABILITY IN SULFUR-AMENDED EVERGLADES SOILS UNDER SUGARCANE

Introduction

The availability of essential elements is known to affect the yield and quality of crops (Heitholt et al., 2002; Parsons et al., 2007). Soils are the major sources for plant nutrients; however, their nutrient availability varies during the growing season depending on characteristics such as soil organic matter content, pH, and cation-exchange capacity (Cancela et al., 2002; Strahm and Harrison, 2007). Soil management practices, such as fertilization and amendments, are commonly employed to enhance the nutrient supply and increase crop yields. Nonetheless, the status and behavior of nutrients in soil are difficult to predict (Moral et al., 2002; Moreno-Caselles et al., 2005; Herencia et al., 2008). Interactions among nutrients affect their availability to crops (Rice et al., 2006) as an over-abundance of one nutrient may lead to a deficiency of another. For instance, excessive P fertilization can decrease Zn availability due to precipitation of $Zn_3(PO_4)_2$ (Li et al., 2007). Thus, applying proper amounts of fertilizers or amendments minimizes nutrient imbalances, maximizes crop yields, and improves fertilizer use efficiency.

Soil testing is widely used for evaluating nutrient availability and justifying fertilizer application rates to maximize crop production while minimizing adverse environmental impacts, including the runoff or leaching of excess nutrients (Rice et al., 2006; Slaton et al., 2009). Various testing methods have been introduced, including acids, salts and chelates to assess nutrient availability in soils (Cancela et

al., 2002). However, no universal standards have been achieved (Wang et al., 2004). Disagreements on nutrient availability and fertilizer recommendations with similar soils and crops as a result of different extraction methods have been documented (Cancela et al., 2002; Wang et al., 2004). Extractants vary in their extracting capabilities and therefore dissimilar extraction methods account for different degrees of nutrient availability. A sound and acceptable soil testing should be correlated with crop yield (Korndörfer et al., 1995).

The Everglades Agricultural Area (EAA) in south Florida was historically a seasonally-flooded prairie ecosystem, but was converted to agricultural use by drainage in the early 1900s. The soils are primarily Histosols with high organic matter content, approximately 85% by weight, which contain high N yet low P and micronutrient concentrations that require supplemental fertilization (Snyder, 2005). Upon drainage and land use conversion, high decomposition rates of these drained Histosols resulted in subsidence and a decreased depth to the underlying bedrock. The current estimate of soil loss is 1.5 cm yr^{-1} and many soils are less than 51 cm in depth, such as those classified as the Dania series (Shih et al., 1998; Snyder, 2005). Long-term cultivation of these drained soils, specifically the use of tillage coupled with soil oxidation, has resulted in incorporation of bedrock $CaCO_3$ into surface soil and has gradually increased the pH from the historic 5.0-5.5 to approximately 7.0-7.5 today (Snyder, 2005). As a result, P and micronutrient availability to crops has decreased and necessitated new fertilizer management practices to maintain nutrients at concentrations sufficient for optimal crop growth. Application of

elemental S is recommended in the EAA when soil pH exceeds 6.6 for the purpose of reducing pH and therefore increasing P and micronutrient availability (Schueneman, 2001). The recommendation rate of 448 kg S ha^{-1} was initially established in 1985 (Anderson, 1985), but due to the changes in soil conditions since 1985, revision of this recommendation may be required. Strong buffering capacity of these calcareous Histosols is likely to counteract the acidifying effects of elemental S oxidation, and thus effects of amendments may only be temporary and minimally effective (Beverly and Anderson, 1986). There is a need to determine the level of S application producing favorable responses in terms of nutrient availability and sugarcane yield. These results can then be used to help formulate fertilizer and nutrient management solutions for better sugarcane management in the EAA. Thus, the objective of this research was to evaluate various S application rates for their effects on nutrient availability during the sugarcane growing season and to assess the effectiveness of three soil test extractants in predicting sugarcane yield.

Material and Methods

Site Description

The experimental field is located in the central EAA on Dania muck (euic, hyperthermic, shallow Lithic Haplosaprist) with a depth to bedrock of approximately 50 cm. The experimental design was a randomized complete block with four S application rates and four field replications. Each field plot measured 9 m x 13 m and consisted of 6 rows of sugarcane (*Saccharum spp.*). Sugarcane cultivar CP 89-2143 was planted in November 2007 and harvested in February 2009. Elemental

granular S (90%) was applied at rates of 0, 112, 224, and 448 kg S ha^{-1} to the furrow and covered after planting. Other fertilization was provided using the typical recommendations and guidelines for this region and soil type (Gilbert and Rice, 2006). All fertilizers were soil-applied prior to planting and all field plots received 17 kg N ha^{-1} and 37 kg P ha^{-1} as monoammonium phosphate, 228 kg K ha^{-1} as KCl, 8.5 kg Mn ha^{-1}, 4.5 kg Cu ha^{-1}, 5.6 kg Fe ha^{-1}, 2.8 kg Zn ha^{-1}, and 1.1 kg B ha^{-1}. All plots received common cultural practices including tillage and herbicide application. Water was applied as needed via seepage irrigation in field ditches approximately 182 m apart.

Soil Sampling and Analysis

Soil samples were collected before planting and fertilizer application and then in January, May, August, and December 2008, corresponding to approximately 0, 2, 6, 9, and 13 months after planting, respectively. Twelve soil (0-15 cm) cores (2.54 cm diameter) were randomly collected within each field plot and composited. Samples were homogenized after the removal of visible plant residues and stored at 4°C.

Soil pH was measured using a soil to water ratio of 1:3 after equilibration for 30 min. Organic matter content was determined by loss-on-ignition at 550°C for 4 hr (Wright et al., 2008). Dissolved organic C (DOC) was measured by extraction with 0.5 *M* K_2SO_4 and analyzed with a TOC-5050A total organic C analyzer (Shimadzu, Norcross, GA). Extractable NH_4-N and NO_3-N were determined by extraction with 2 *M* KCl followed by colorimetric analysis (Castillo and Wright, 2008). Water extractable SO_4-S was analyzed by ionic chromatography after shaking 3 g soil with

25 mL water for 0.5 hr, followed by filtering through Whatman No. 41 filter paper (Gharmakher et al., 2009).

Three different extractants were tested in this study: water, 0.5 *N* acetic acid, and Mehlich-3. Water and acetic acid are the soil test extractants for use on muck soils, and Mehlich-3 is the soil test extractant for sandy soils in Florida (Morgan et al., 2009; Mylavarapu, 2009). Phosphorus concentrations for different extracts were determined using the ascorbic acid-molybdenum blue method (Kuo, 1996) after shaking 4 mL air-dry soil with 50 mL of extractant for 50 min, followed by filtering through Whatman No. 2 (acetic acid extraction) and No. 5 (water and Mehlich-3 extraction) filter paper, respectively. Extractable macro- and micronutrients in different extractants were then analyzed by ICP.

Sugar Yield

Harvestable stalks were counted in 2 of the 4 middle rows of each plot in August. Stalk weights were determined by cutting and weighing 20 stalks from 2 of the middle 4 rows of each plot (40 stalks total) in February 2009. Sugarcane yield was calculated by multiplying stalk number by stalk weight, and dividing by unit area. Sugar yield (Mg ha^{-1}) was determined according to the theoretical recoverable sugar method utilizing sugar content of harvested cane and sugarcane yield (Glaz et al., 2002).

Statistical Analysis

A mixed model was fit using restricted maximum likelihood in the MIXED procedure of SAS (Littell et al., 2006). The fixed effects were S application rate, time

and their interaction. Block was a random effect. Degrees of freedom were adjusted using the Kenward-Roger adjustment. An exponential covariance structure was used to model the correlation among observations taken from the same plot over time. Significant differences among individual treatments and time intervals were determined with Tukey's test at $\alpha = 0.05$. Pearson correlation analysis was performed to assess relationships between variables. Stepwise multiple regression was conducted to evaluate the relative importance of extractable nutrients in predicting sugar yield. A *p* value of 0.1 and 0.05 was used as the entry and staying values, respectively, in the stepwise selection method (Majchrzak et al., 2001). All statistical analyses were carried out with SAS 9.1 (SAS Institute).

Results and Discussion

Soil pH

Soil pH was not affected by S application (Fig. 6-1). The background pH prior to S application was 6.2, which did not differ from soils collected during the growing season. The limited effect of acidification may result from a S application rate too low to cause a change in pH and from the high buffering capacity of this calcareous organic soil (Jaggi et al, 2005; Deubel et al., 2007). When the original S recommendation for sugarcane of 448 kg S ha^{-1} was established years ago, soil pH was considerably lower. However, the rise in pH and decrease in soil depth to bedrock likely increased the capacity of these soils to resist changes in pH. Thus, higher S application rates may be necessary to produce the same response as 448 kg ha^{-1} did in the 1980s (Anderson, 1985). Soils with high concentrations of

carbonates and bicarbonates are highly buffered against acidification (Rogovska et al, 2007). The buffering effects often take place more slowly than the formation of sulfuric acid from elemental S (Deubel et al., 2007). A limited reduction in soil pH after S application was also observed in other studies of calcareous soils (Hassan and Olson, 1966).

Dissolved Organic C and Extractable N

Application of S at a range from 0 to 448 kg S ha^{-1} did not affect DOC, but the concentrations varied seasonally (Fig. 6-2). Averaged across treatments, DOC significantly increased from 2 (1346 mg kg^{-1}) to 6 months (1572 mg kg^{-1}), but then decreased toward the end of the growing season. Extractable NH_4-N and NO_3-N were not influenced by S application, but concentrations fluctuated during the growing season (Fig. 6-2). Extractable NH_4-N significantly decreased from 2 (19 mg kg^{-1}) to 6 months (10 mg kg^{-1}), while extractable NO_3-N exhibited the same trend from 2 (383 mg kg^{-1}) to 6 months (16 mg kg^{-1}). Oxidation of the muck soil provides most of the N requirement for sugarcane grown in the EAA (Gilbert and Rice, 2006). However, the soil N pool can change rapidly under the impacts of environmental (precipitation, temperature) and management (tillage, irrigation) factors (Rice et al., 2006). Considering the fact that sugarcane biomass accumulation is the greatest during the summer months of the rainy season (Rice et al., 2006), it was likely that plant uptake and leaching losses contributed to the low NO_3-N concentrations as the growing season progressed.

Phosphorus

Sulfur application at 448 kg S ha^{-1} significantly increased concentrations of acetic acid, Mehlich-3, and water-extractable P at 2 months (Fig. 6-3), suggesting increased P availability to sugarcane caused by S application (Codling, 2008). There are two primary mechanisms by which S application could influence P availability: lowering of soil pH and replacement of PO_4 with SO_4 (Gabriel et al., 2008), or the release of P from association with Fe, Al, and Ca caused by pH reduction (Jaggi et al., 2005). However, increased P availability was not observed at later months indicating limited long-term effects of S on the reduction in soil pH due to the high buffering capacity of this calcareous organic soil (Snyder, 2005).

Acetic acid extractable P decreased progressively during the season (Fig. 6-3). The concentrations at 13 months were 214% lower than those at 2 months, 107% lower than those at 6 months, and 75% lower than those at 9 months. Similarly, water extractable P decreased gradually from 2 months (15 mg kg^{-1}) to 13 months (3 mg kg^{-1}). Mehlich-3 extractable P did not change much during the same period. The EAA soils are traditionally P limited and sugarcane production requires supplemental P fertilization (Morgan et al., 2009). Therefore, the reduction in acetic acid and water extractable P during the season was likely a result of sugarcane uptake. Seasonal trends in P concentrations would be expected to show declines from planting to harvest, corresponding to uptake of extractable P by sugarcane. However, P mineralized from soil organic matter also contributes to the available P pool.

Across treatments and sampling times, acetic acid extracted 834% more P than water, while Mehlich-3 extracted 559% more P than water (Table 6-1). No significant difference was found between the amounts of P extracted by acetic acid and Mehlich-3. Water primarily extracts P in soil solution, while Mehlich-3 and acetic acid also extract P adsorbed or complexed with Ca, Mg, Fe, and Al, in addition to soluble P (Wright et al., 2007; Wright, 2009). Thus, it was not surprising that Mehlich-3 and acetic acid extracts contained more P than water.

Potassium

Sulfur application at the highest rates significantly increased K availability at 2 months (Fig. 6-3), but the treatment effect was not observed at subsequent sampling times. At 2 months, acetic acid extractable K for soils receiving 448 kg S ha^{-1} (1214 mg kg^{-1}) was significantly higher than for soils receiving 112 kg S ha^{-1} (647 mg kg^{-1}) and unamended soil (708 mg kg^{-1}). Likewise, Mehlich-3 extractable K for soils amended with the highest rates (949 mg K kg^{-1}) was significantly higher than soils receiving 112 kg S ha^{-1} (493 mg K kg^{-1}) and unamended soil (549 mg kg^{-1}). Water extractable K was only different between soils receiving 112 (395 mg kg^{-1}) and 448 kg S ha^{-1} (713 mg kg^{-1}). Acetic acid extractable K decreased 440% from 2 months to 13 months, while during the same period Mehlich-3 and water extractable K decreased 448% and 891%, respectively (Fig. 6-3). Despite the fact that EAA soils have high cation-exchange capacities, K is usually weakly held on the exchange sites (Gilbert and Rice, 2006). Therefore, K movement out of the soil profile occurs readily depending on precipitation patterns. The stimulatory effect of S on

extractable K concentrations was likely attributed to the replacement of K^+ by H^+ at adsorbing sites, while plant uptake and leaching were responsible for seasonal decreases in K availability.

Averaged across treatments and sampling times, acetic acid extracted the same amounts of K as Mehlich-3 (Table 6-1). However, acetic acid and Mehlich-3 extracted 106% and 66% more K than water. Correlation analysis revealed that water extractable K was highly correlated to acetic acid (R^2 = 0.98) and Mehlich-3 extractable K (R^2 = 0.96), while the later two were also strongly correlated (R^2 = 0.99), indicating that the three extractants may indeed extract the same pools of K.

Calcium

None of the extractable Ca concentrations exhibited S effects, but all displayed seasonal fluctuation (Fig. 6-4). Both acetic acid and water extractable Ca decreased significantly from 2 to 9 months and then increased to 13 months. Mehlich-3 extractable Ca was highest at 9 months (19516 mg kg^{-1}), followed by 13 (17359 mg kg^{-1}), 2 (14386 mg kg^{-1}), and 6 months (15224 mg kg^{-1}). Acetic acid extractable Ca was 3457% higher than water extractable Ca, while Mehlich-3 extracts had 3022% more Ca than water extracts.

Magnesium

Sulfur application did not affect Mg availability (Fig. 6-4). Acetic acid extractable Mg decreased significantly from 2 (1735 mg kg^{-1}) to 6 (665 mg kg^{-1}) months, but remained constant to 9 months (671 mg kg^{-1}). Mehlich-3 extractable Mg increased from 6 (580 mg kg^{-1}) to 9 months (774 mg kg^{-1}) and then decreased to 13

months (711 mg kg^{-1}). Water extractable Mg decreased gradually during the season, being 307% lower at 13 than 2 months. Acetic acid extracted 44% more Mg than Mehlich-3, while 1155% more than water (Table 6-1).

Sulfur

Sulfur application significantly increased SO_4 concentrations as a result of S oxidation (Jaggi et al., 2005) (Fig. 6-2). Extractable SO_4-S in soils receiving 448 kg S ha^{-1} was 131%, 201%, and 270% higher than unamended soils at 6, 9, and 13 months, respectively. Similar to extractable K, SO_4 concentrations decreased significantly from 2 (376 mg kg^{-1}) to 6 months (129 mg kg^{-1}) and continued to decrease from 6 to 9 months (21 mg kg^{-1}). In the EAA, soil oxidation generally supplies sufficient S to satisfy sugarcane nutrient requirements (Gilbert and Rice, 2006). Therefore, there is potential for S application at high rates to increase the risk of SO_4 export from fields. Lower SO_4-S concentrations at 6 and 9 months were likely due to SO_4 uptake by sugarcane and losses as runoff or leaching during precipitation events.

Copper

Extractable copper was not affected by S application at any time during the growing season (Fig. 6-5). Acetic acid extractable Cu decreased significantly from 2 (0.3 mg kg^{-1}) to 9 months (0.1 mg kg^{-1}), while water extractable Cu remained unchanged during the season. Mehlich-3 extractable Cu increased from 6 (0.1 mg kg^{-1}) to 9 months (0.2 mg kg^{-1}) and then to 13 months (1.6 mg kg^{-1}). Across

treatments and sampling times, acetic acid and water extracted similar amounts of Cu, but both extracted less than Mehlich-3 (Table 6-1).

Iron

Similar to Cu, extractable Fe did not respond to any rate of S application, but did fluctuate seasonally (Fig. 6-5). Acetic acid extractable Fe decreased significantly from 2 (13.6 mg kg^{-1}) to 6 months (9.4 mg kg^{-1}) and toward the end of the season (7.2 mg kg^{-1}). Inversely, Mehlich-3 extractable Fe increased from 2 (5.8 mg kg^{-1}) to 13 months (8.0 mg kg^{-1}). Water extracts contained similar amounts of Fe as acetic acid, but extracted 61% more Fe than Mehlich-3 (Table 6-1).

Manganese

The availability of Mn was not affect by S application, but varied during the season (Fig. 6-6). Similar to K and Fe, acetic acid extractable Mn decreased incrementally from 2 (23.9 mg kg^{-1}) to 13 months (9.4 mg kg^{-1}). Mehlich-3 extractable Mn decreased from 2 (4.2 mg kg^{-1}) to 6 months (3.7 mg kg^{-1}), and then increased form 6 to 9 months (5.3 mg kg^{-1}). Acetic acid extractable Mn was 7900% higher than water extractable Mn, while Mehlich-3 extracts had 2414% more Mn than water extracts.

Zinc

Acetic acid-extractable Zn in soils receiving 448 kg S ha^{-1} (7.2 mg kg^{-1}) was significantly higher than for unamended soils (2.5 mg kg^{-1}) at 2 months (Fig. 6-6). However, the stimulating effects were not observed beyond 2 months. Mehlich-3 extractable Zn did not exhibit any treatment effects during the growing season.

Interestingly, at 6 months after S application, water extractable Zn for soils receiving 448 kg S ha^{-1} (0.3 mg kg^{-1}) was significantly higher than for soils receiving 112 kg S ha^{-1} (0.2 mg kg^{-1}) and unamended soils (0.2 mg kg^{-1}). Similar to K, Fe, and Mn, acetic acid extractable Zn decreased gradually from 2 to 13 months. The decreases in nutrient availability can be attributed to losses of nutrient as leaching and sugarcane uptake. Meanwhile, fixation and chelating of Zn to organic matter, clay minerals, and carbonates may also remove it from the available pools. Zinc concentrations in acetic acid and Mehlich-3 extracts did not differ, but both extracted 943% and 817% more Zn than water extracts, respectively (Table 6-1).

Soil Properties and Micronutrient Availability

Organic matter and soil pH are two major properties that influence nutrient availability and mobility (Herencia et al., 2008; Provin et al., 2008). Organic matter provides ligands that chelate the micronutrients and promotes the formation of soluble micronutrient-organic matter complexes and therefore increases nutrient availability (Herencia et al., 2008). However, organic matter can also immobilize nutrients through the same complexation mechanism (Wei et al., 2006). In the present study, organic matter content was only significantly correlated with Mehlich-3 extractable Fe and acetic acid extractable Mn (Tables 6-2, 6-3, and 6-4), indicating that organic matter was unlikely the dominant factor influencing nutrient availability during the sugarcane growing season.

Changes in soil pH can mobilize nutrients from unavailable phases to available pools. Studies have shown that the availability of nutrients to crops depends on soil

pH (Wei et al., 2006). In the EAA, elemental S is introduced as soil amendment for the purpose of reducing pH and therefore increasing nutrient availability (Rice et al., 2006). Our results suggested that application of S up to 448 kg ha^{-1} introduced limited effects on soil pH and therefore had little influence on enhancing nutrient availability. Statistical analysis revealed that pH was significantly correlated with acetic acid extractable Fe and Zn and Mehlich-3 extractable Cu and Mn (Table 6-2 and 6-3). Nonetheless, no correlations were found between soil pH and any of water-extractable nutrients (Table 6-4), which suggests that soluble nutrients were not as sensitive as adsorbed or complexed nutrients to small changes in soil pH.

Phosphate can affect micronutrient availability by direct precipitation of nutrient cations. However, the effect varies among micronutrients and depends on other soil properties, such as water content (Li et al., 2007), pH (Wei et al., 2006) and metal solubility (Shuman, 1988), which helps to explain the varied relationships between P and micronutrient concentrations (Table 6-2, 6-3, and 6-4) in this study. Correlation analysis also revealed significantly negative correlations between water extractable Ca and Fe (R^2 = -0.86) and Mn (R^2 = -0.29) indicating that increasing $CaCO_3$ content in these soils was likely to decrease micronutrient availability. Calcium carbonate is able to precipitate micronutrient ions in soil solution during the formation of carbonate depending on desorption characteristics of micronutrients and the solubility of carbonate (Wei et al., 2006). Mehlich-3 extractable Ca was also significantly correlated to Mehlich-3 extractable Fe and Mn, which may further

suggest that Fe and Mn availability in these calcareous soils was affected by the $CaCO_3$ content.

Comparison of Soil Extractants

Nutrients exist in soils as water soluble, exchangeable, and non-exchangeable forms. The contribution of these pools towards nutrient availability to plants depends on the dynamic equilibrium among different fractions. Therefore, different extraction methods reflect the degree of nutrient availability (Wright et al., 2007). Water extracts represent the readily available chemical forms, whereas acetic acid and Mehlich-3, as acid solutions, extract the pool consisting of soluble, exchangeable, and some of non-exchangeable fractions (Cancela et al., 2002; Wright et al., 2007). Our results indicated that, as expected, acetic acid and Mehlich-3 extracted more P, K, Ca, Mg, Mn, and Zn than water (Table 6-1). However, acetic acid and Mehlich-3 did not extract more Fe than water, but in fact water extracted more Fe than Mehlich-3.

Acetic acid and Mehlich-3 have been deemed satisfactory extractants for soil testing on EAA soils (Korndörfer et al., 1995; Hochmuth et al., 1996). Mehlich-3 solution contains large amounts of salts, strong acids, and EDTA. Salts are present mainly for extracting major cation such as P, K, Ca, and Mg, while micronutrient extraction is accomplished by metal-EDTA complexation (Wang et al., 2004). Nonetheless, compared to 0.5 *N* acetic acid, Mehlich-3 extracted equal amounts of P, K, and Zn, but less Ca, Mg, Mn, and Fe (Table 6-1), indicating that acidity may in fact control the Ca, Mg, Mn, and Fe availability in this calcareous organic soil.

Strong acidity can help to dissolve Ca, Mg, Mn, and Fe from precipitates in soils. Acetic acid is more acidic than Mehlich-3 and appeared less affected by soil buffering capacity and the presence of free $CaCO_3$ (Korndörfer et al., 1995) and therefore extracted more Ca, Mg, Mn, and Fe. In other words, acetic acid method tends to extract relatively high amounts of non-exchangeable nutrients and thus may overestimate the concentrations of available nutrients. Mehlich-3 extracted more Cu than acetic acid and water, suggesting that extractable Cu was likely present in a complex with organic matter rather than as an insoluble precipitate. In fact, copper is often associated with dissolved organic matter (Wright et al., 2007).

Nutrient Availability and Sugar Yield

Sulfur amendment did increase the availability of P, K, and Zn at 2 months after application. Nonetheless, S application did not increase sugar yield. The yields for soils amended with 0, 112, 224, and 448 kg S ha^{-1} averaged 16, 17, 16, and 17 Mg sugar ha^{-1}, respectively. Results suggest that current recommended S application guidelines and rates in the EAA may not be high enough to achieve the projected goals. Higher application rates may be required to overcome the soil's buffering capacity and significantly increase nutrient availability and sugar yield. Our results also indicated that S application is likely to increase the risk of SO_4 export from fields. Large scale S application should be well evaluated, since SO_4 export from the EAA into Everglades wetlands has been implicated in causing stimulation of Hg methylation (Gabriel et al., 2008). Nonetheless, it has been reported that actual grower S application rates in the EAA are lower than the current recommended rates

(Schueneman, 2001). Everglades Agricultural Area growers tend to use micronutrient sprays to alleviate nutrient deficiency caused by elevated pH since it is more cost effective, so S application may not be considered necessary at this stage.

Significant linear regression equations for prediction of sugar yields with extractable nutrients are listed in Table 6-5. Stepwise multiple regressions identified the most significant model considering Mehlich-3 extractable P prior to planting as the main predictor, which explains 93% of the variation in sugar yield. Nonetheless, the correlation between Mehlich-3 extractable P and sugar yield was negative. As described previously, Mehlich-3 may not accurately reflect the P availability to sugarcane during the growing season. In fact, P measured as Mehlich-3 extractable may overestimate available P since it extracts P found in Ca and Fe-Al fractions which are considered unavailable to crops (Wright, 2009), which may explain the negative correlation between Mehlich-3 extractable P and sugar yield. Regression equations for 2, 6, and 9 months had low coefficients of determinant ranging from 0.30 to 0.60, suggesting important factors influencing sugar yield were not quantified (Anderson et al., 1999). Meanwhile, the response of sugar yield to a specific factor may not be linear. General linear models only offered rough approximations of the relationships and therefore may not be adequate in this case (Korndörfer et al., 1995; Anderson et al., 1999).

Conclusions

Sulfur application at rates up to 448 kg ha^{-1} had limited effects on the reduction in soil pH due to the high soil buffering capacities and generally failed to enhance the

nutrient availability. Correspondingly, S application at current recommendation rates did not increase sugar yield. Considering the increasing pH and the decreasing depth to bedrock of soils in the EAA, new S application guidelines with higher amendment rates may be needed. Sulfur application increased SO_4 concentrations in soils and also the risk for export from fields. Therefore, large scale of S application should be evaluated for their potential to adversely affect proximal sensitive wetland ecosystems. However, it may not be economically viable to increase S fertilizer recommendations because of the high cost of elemental S. An alternative, such as different P and micronutrient fertilizer application methods, timings, and sources, may be a better alternative to increase nutrient availability in these changing soils. Multiple regression analysis suggested that the parameter most influencing sugar yield was available P.

Table 6-1. Comparisons of soil test extractants on concentrations of available nutrients (mg kg^{-1}). Values denote the mean across S rates and time with standard error values in parenthesis.

	Acetic acid	Mehlich-3	Water
P	76 (11)	53 (4)	8 (1)
K	387 (41)	313 (32)	188 (27)
Ca	18936 (600)	16621 (287)	532 (54)
Mg	957 (59)	664 (12)	76 (8)
Cu	0.19 (0.02)	0.51 (0.08)	0.20 (0.01)
Mn	15 (0.8)	4.7 (0.1)	0.2 (0)
Fe	10 (0.4)	7 (0.2)	11 (0.6)
Zn	3.2 (0.3)	2.9 (0.2)	0.3 (0)

Table 6-2. Pearson correlation coefficients (r) between pH, organic matter content, and concentrations of acetic acid-extractable nutrients (n= 64).

	pH	OM	Ca	Cu	Fe	K	Mg	Mn	P	Zn
pH	1									
OM	-0.45*	1								
Ca	0.29	-0.71	1							
Cu	NS	NS	NS	1						
Fe	-0.51	NS	NS	0.41	1					
K	-0.61	NS	0.32	0.25	0.80	1				
Mg	-0.31	NS	0.66	0.38	0.78	0.79	1			
Mn	NS	-0.28	0.64	0.47	0.78	0.73	0.88	1		
P	-0.31	NS	NS	NS	0.37	0.60	NS	0.26	1	
Zn	-0.29	NS	NS	0.55	0.41	0.49	NS	0.38	0.66	1

Notes: OM, organic matter content; *, significant at $\alpha = 0.05$; NS, not significant.

Table 6-3. Pearson correlation coefficients (r) between soil pH, organic matter content, and concentrations of Mehlich-3-extractable nutrients (n=64).

	pH	OM	Ca	Cu	Fe		Mg	Mn	P	Zn
pH	1									
OM	-0.45*	1								
Ca	0.35	-0.29	1							
Cu	0.28	NS	NS	1						
Fe	NS	0.37	0.60	0.38	1					
K	-0.61	NS	-0.58	-0.44	-0.44	1				
Mg	0.38	NS	0.91	0.32	0.66	-0.54	1			
Mn	0.36	NS	0.67	0.57	0.71	-0.45	0.77	1		
P	-0.34	NS	NS	NS	NS	0.52	NS	NS	1	
Zn	NS	NS	NS	NS	0.46	NS	NS	0.26	0.36	1

Notes: OM, organic matter content; *, significant at α = 0.05; NS, not significant.

Table 6-4. Pearson correlation coefficients (r) between soil pH, organic matter content, and concentrations of water-extractable nutrients (n = 64).

	pH	OM	Ca	Cu	Fe	K	Mg	Mn	P	Zn
pH	1									
OM	-0.45*	1								
Ca	-0.39	NS	1							
Cu	NS	NS	NS	1						
Fe	NS	NS	-0.86	NS	1					
K	-0.56	NS	0.90	NS	-0.74	1				
Mg	-0.42	NS	0.99	NS	-0.86	0.90	1			
Mn	NS	NS	-0.29	0.26	NS	-0.27	-0.28	1		
P	-0.32	NS	0.42	NS	-0.26	0.63	0.39	NS	1	
Zn	NS	NS	NS	0.54	NS	NS	NS	0.42	NS	1

Notes: OM, organic matter content; *, significant at α= 0.05; NS, not significant;

Table 6-5. Multiple regression models relating soil nutrient concentrations (mg kg^{-1}) with sugar yield (Mg ha^{-1}) at different times.

Time	Equation	R^2
0 months	Y = 57 – 1.42 (M-P)	0.93
2 months	Y = 20 – 0.04 (W-Mg) + 0.01 (W-SO_4)	0.52
6 months	Y = 21 – 54.06 (W-Mn)	0.30
9 months	Y = 14 – 0.04 (A-K) + 0.17 (W-K)	0.60
13 months	Y = 10 + 0.13 (NO_3-N) + 15.09 (W-Mn) – 1.06 (W-P)	0.81

Notes: Y, sugar yield; M, Mehlich-3 extractable nutrient; W, water extractable nutrient; A, acetic acid extractable nutrient.

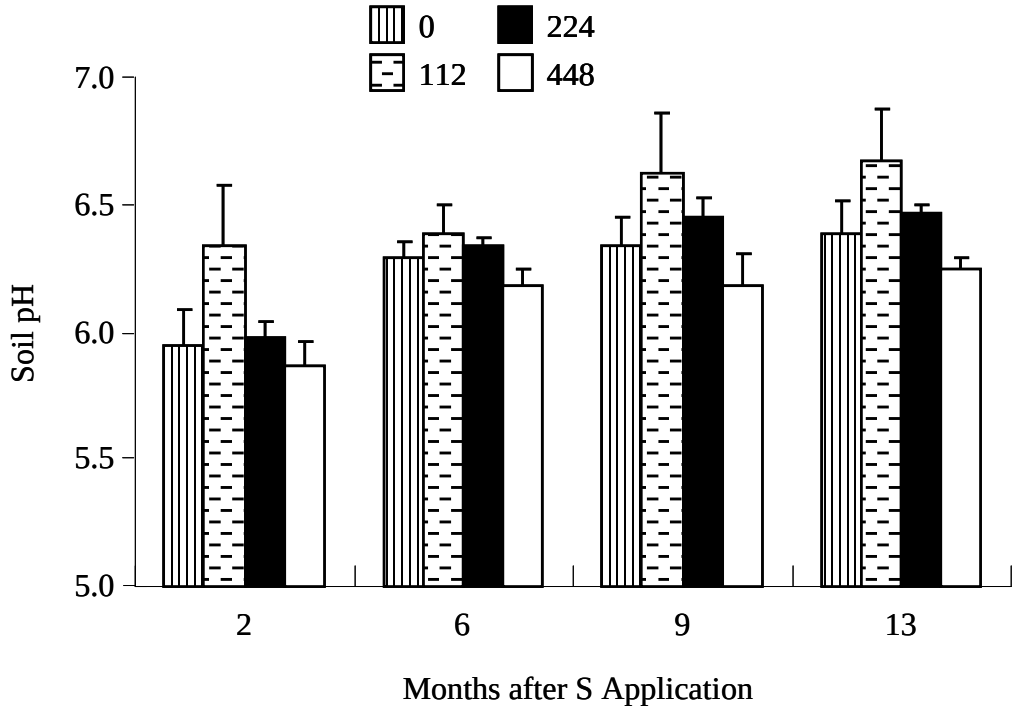

Fig. 6-1. Soil pH changes in response to different S application rates (0, 112, 224, and 448 kg S ha^{-1}) throughout the sugarcane growing season. Error bars represent the standard error of the mean.

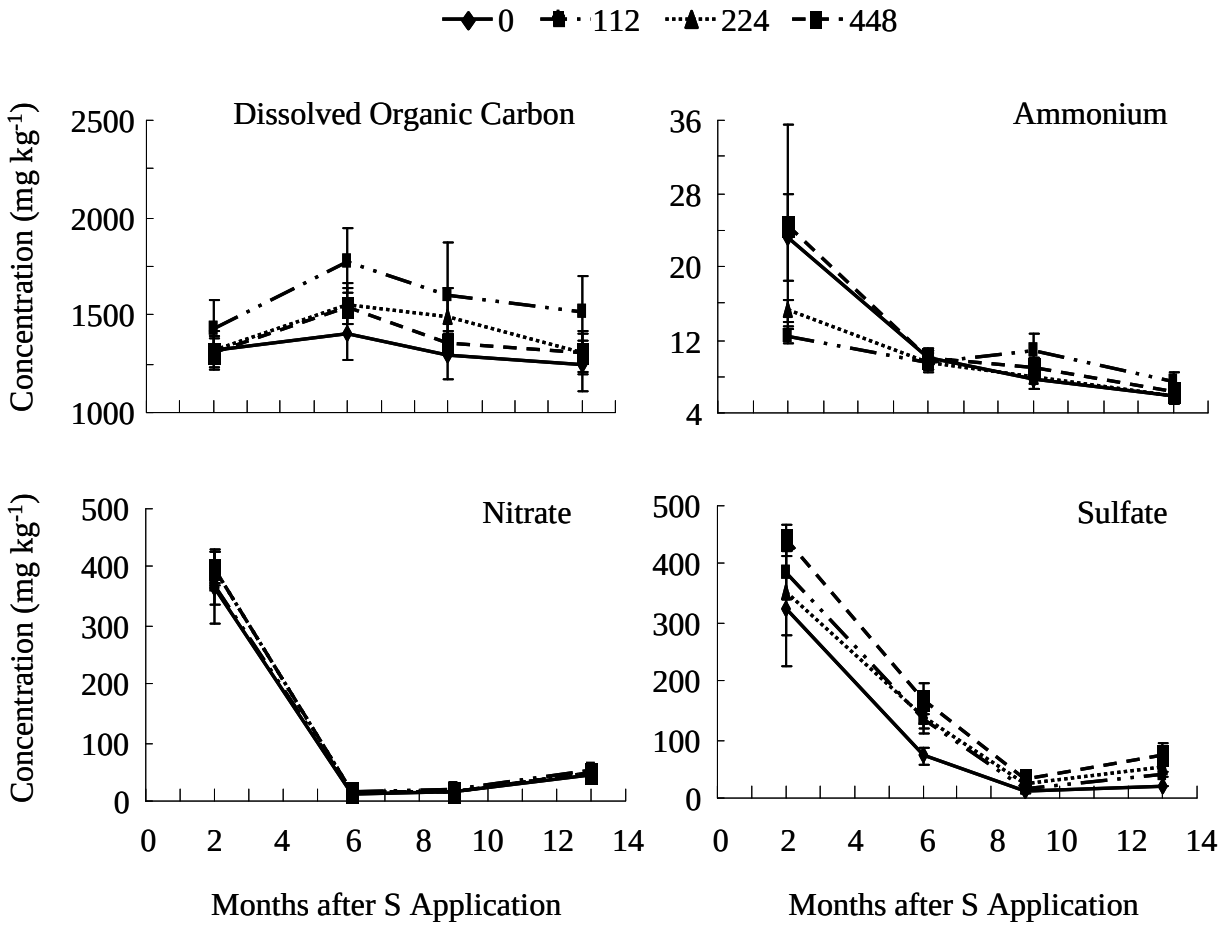

Fig. 6-2. Seasonal dynamics of dissolved organic C, extractable NH_4-N, NO_3-N, and SO_4-S after S application at 0, 112, 224, and 448 kg S ha^{-1}. Error bars represent the standard error of the mean.

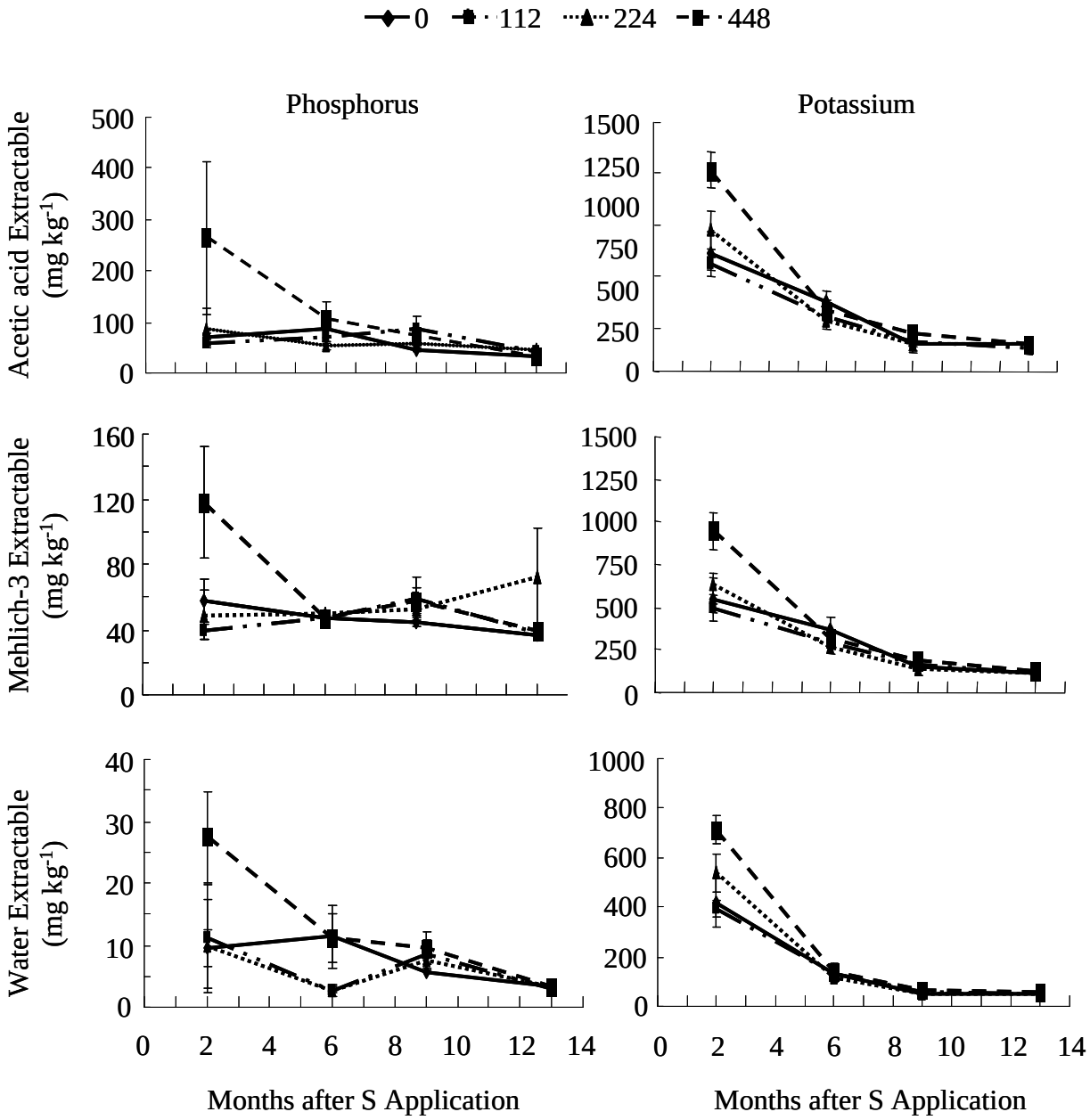

Fig. 6-3. Seasonal dynamics of acetic acid, Mehlich-3, and water extractable P and K after S amendment at 0, 112, 224, and 448 kg S ha^{-1}. Error bars represent the standard error of the mean.

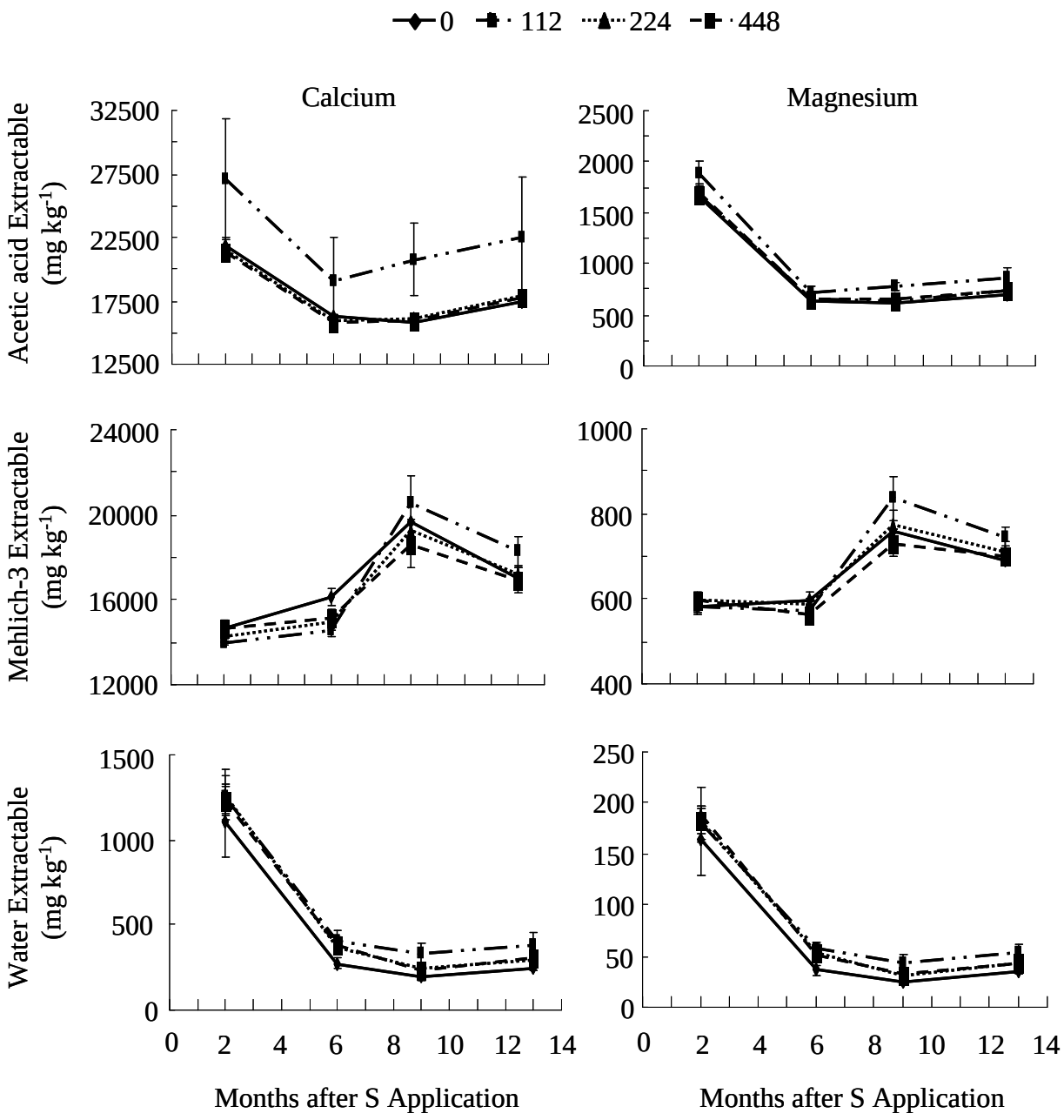

Fig. 6-4. Seasonal dynamics of acetic acid, Mehlich-3, and water extractable Ca and Mg after S amendment at 0, 112, 224, and 448 kg S ha^{-1}. Error bars represent the standard error of the mean.

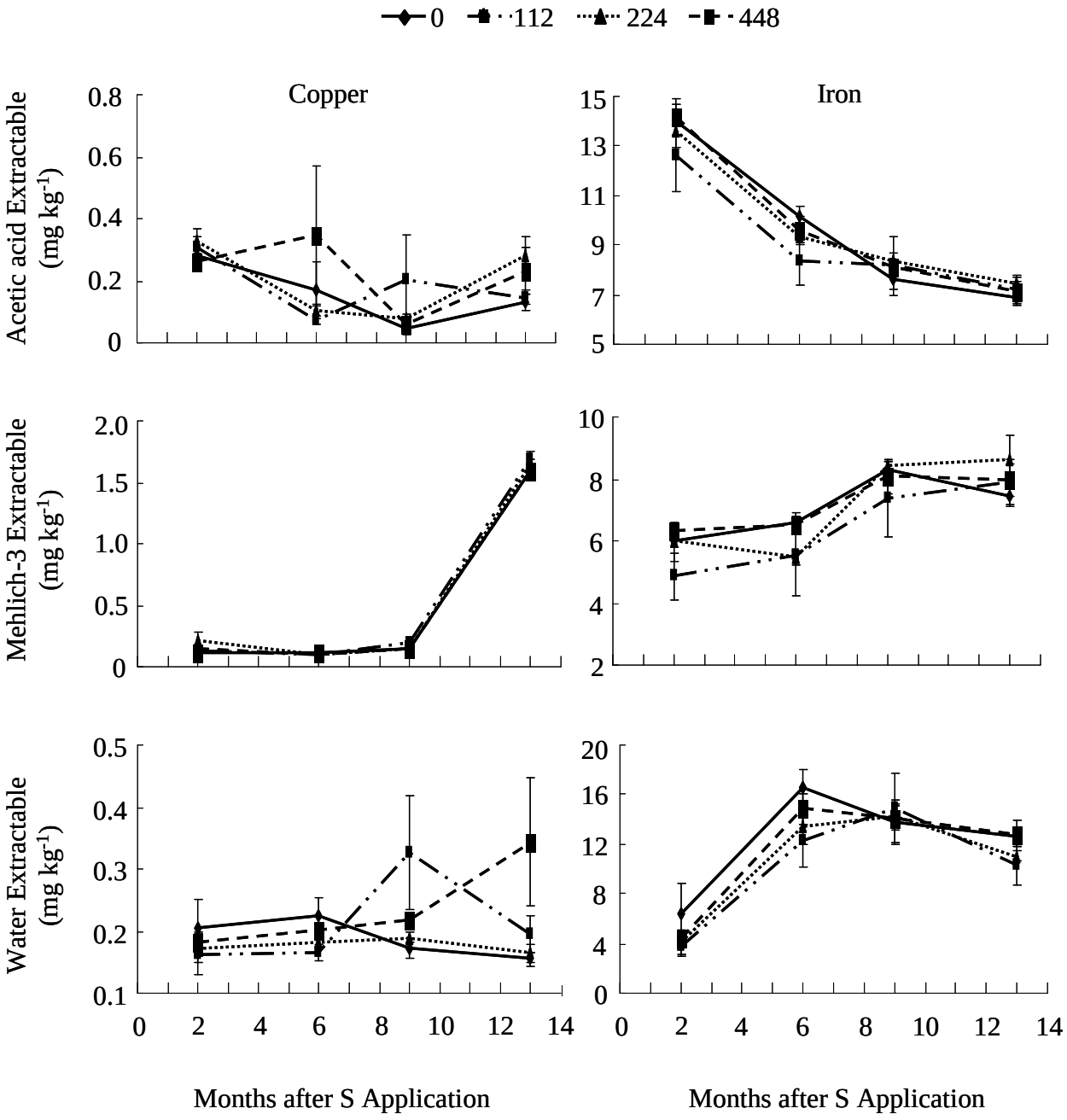

Fig. 6-5. Seasonal dynamics of acetic acid, Mehlich-3, and water extractable Cu and Fe after S amendment at 0, 112, 224, and 448 kg S ha^{-1}. Error bars represent the standard error of the mean.

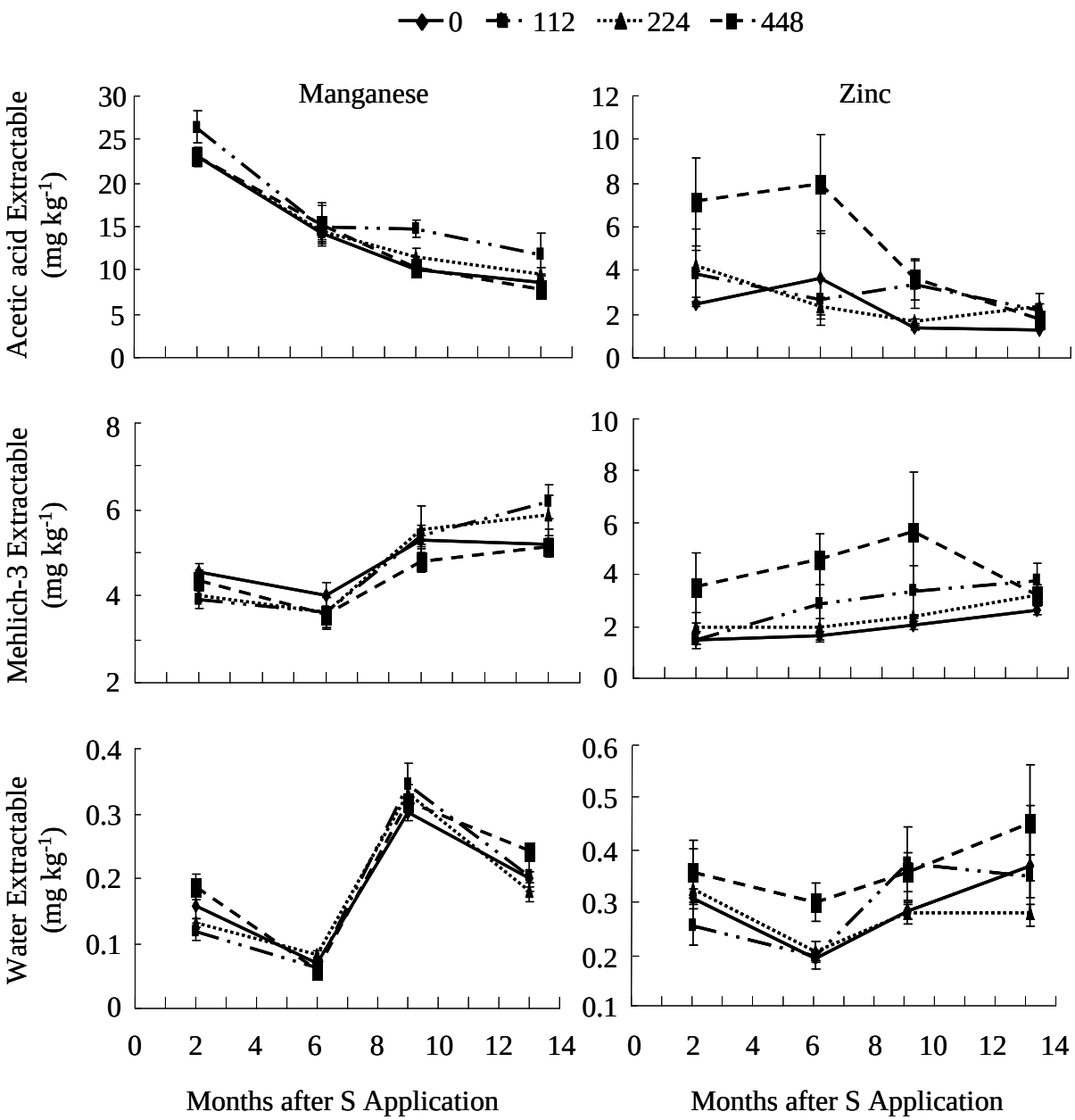

Fig. 6-6. Seasonal dynamics of acetic acid, Mehlich-3, and water extractable Mn and Zn after S amendment at 0, 112, 224, and 448 kg S ha^{-1}. Error bars represent the standard error of the mean.

CHAPTER 7
SULFUR POOLS, TRANSFORMATIONS, AND MINERALIZATION IN EVERGLADES AGRICULTURAL AREA SOILS

Introduction

Sulfur is an element required by all living organisms as an essential macronutrient (Wang et al., 2006). Sulfur exists in soils in various forms, each of which play important biological and chemical functions. Sulfate is the most abundant form of inorganic S found in most soils, as well as the main form available to plants, although reduced forms, such as elemental S, thiosulfate, and sulfide, are important for anaerobic soils (Zhou et al., 2005). However, the bulk of soil S in natural and managed ecosystems is in organic form, which is directly impacted by microbial activity through decomposition processes (Solomon et al., 2001). Sulfur dynamics is widely variable among soils and often closely associated with other nutrient cycles. It is widely believed that S mineralization in soils involves both biological and biochemical processes (McGill and Cole, 1981). As in biological process, SO_4 is released as a by-product during organic matter decomposition, while biochemical mineralization releases SO_4 through hydrolysis of ester SO_4, which is catalyzed by extracellular enzyme activity, primarily sulfatase (McGill and Cole, 1981; Wright and Reddy, 2001).

Water quality is a critical issue facing rehabilitation of the Florida Everglades (Gabriel at al., 2008), as evidenced by SO_4 contamination of the northern Everglades, which has been implicated in the stimulation of MeHg formation in soils and water (Bates et al., 2002). Methylmercury is a neurotoxin that is bioaccumulated

in higher organisms and found at high concentrations in fish and other wildlife in the Everglades (Orem, 2007). Potential sources contributing to the SO_4 enrichment in Everglades wetlands include groundwater, rainwater, sea aerosol, internal S flux from sediments, and surface water inputs from Lake Okeechobee and the Everglades Agricultural Area (EAA) (Schueneman, 2002). It has been reported that S from the EAA is the likely key contributor (Schueneman, 2001; Bates et al., 2002; Orem, 2007).

The EAA is located south of Lake Okeechobee and north of the Water Conservation Areas (WCA) of south Florida. Historically, it was a seasonally-flooded prairie ecosystem, but was converted to agricultural use by drainage in the early 1900s. The soils of the EAA are predominately Histosols with high organic matter content and but low P and micronutrient concentrations that require supplemental fertilization (Snyder, 2005; Ye et al., 2009). Since this area has changed from the wetland to agricultural ecosystem in the 1920s, several nutrient deficiencies were evident, mainly P but also micronutrients, particularly Cu. Thus, $CuSO_4$ was used to alleviate Cu deficiency to crops (Allison et al., 1927). Sulfur has also been applied to soils as part of pesticides that were commonly used to support sugarcane and vegetable production. In recent years, formation of shallow soils resulting from soil subsidence (Snyder, 2005; Wright, 2009), and increasing pH due to incorporation of limestone bedrock into soils, has increased the need for amendments to decrease soil pH. Elemental S is recommended to reduce soil pH when it exceeds 6.6 for the purpose of improving the availability of P and micronutrients to sugarcane

(Anderson, 1985; Schueneman, 2001). The microbial oxidation of elemental S to SO_4 produces acidity which reacts with the soil and reduces pH, which in turn increases P and micronutrient availability. However, SO_4 is soluble in water and susceptible to export from the field as runoff during precipitation events to down stream Everglades wetlands.

In consideration of the adverse impacts that S may pose to Everglades wetlands, reducing potential S export from the EAA is beneficial for protecting water quality and ecosystem health (Gabriel at al., 2008). Nonetheless, explicit quantification of S budgets and transformations within EAA soils is rare. Due to the increasing pH trend for EAA soils, demand for S application may continue to exist or increase in the future. Minimal research is available for S cycling in Everglades soils, such that there is a strong need to determine the influence of elemental S application on soil pH, S distribution, and transformation in soils.

Materials and Methods

Site Description

The experimental field is located in the central EAA on Dania muck (euic, hyperthermic, shallow Lithic Haplosaprist) with a depth to bedrock of approximately 50 cm. The experimental design was a randomized complete block with four S application rates and four field replications, with four sampling times encompassing the entire growing season. Each field plot measured 9 m x 13 m and consisted of 6 rows of sugarcane (*Saccharum spp.*) cultivar CP 89-2143 planted in November 2007 and harvested in February 2009. Elemental granular S (90%) was applied at rates of

0, 112, 224, and 448 kg S ha^{-1} to the furrow and covered after planting. Other fertilization was provided using typical recommendations and guidelines for this region and soil type (Gilbert and Rice, 2006). Fertilizers were soil-applied prior to planting and all field plots received 17 kg N ha^{-1} and 37 kg P ha^{-1} as monoammonium phosphate, 228 kg K ha^{-1} as KCl, 8.5 kg Mn ha^{-1}, 4.5 kg Cu ha^{-1}, 5.6 kg Fe ha^{-1}, 2.8 kg Zn ha^{-1}, and 1.1 kg B ha^{-1}. All plots received common cultural practices including tillage and herbicide application. Water was applied as needed via seepage irrigation in field ditches approximately 182 m apart.

Soil Sampling and Laboratory Analysis

Soil samples were collected before planting and fertilizer application and then in January 2008, May 2008, August 2008, and December 2008, corresponding to approximately 2, 6, 9, and 13 months after planting, respectively. Twelve soil (0-15 cm) cores (2.54 cm diameter) were randomly collected from rows within each field plot and composited to yield one sample per plot. Soils were homogenized after the removal of visible plant residues and stored at 4°C until analysis.

Soil pH was measured using a soil to water ratio of 1:3 after equilibration for 30 min. Total organic C was measured by loss-on-ignition at 550°C for 4 hr after conversion to organic C with a coefficient factor of 0.51 (Wright et al. 2008). Total N was measured by Kjeldahl digestion followed by NH_4 analysis (Bremner, 1996). Extractable NH_4-N and NO_3-N were determined by extraction with 2 M KCl followed by colorimetric analysis (Ye et al., 2009). Total P was measured using the ascorbic acid-molybdenum blue method after Kjeldahl digestion, and labile inorganic P was

measured after Mehlich-3 extraction (Ye et al., 2009). Acetic acid extractable nutrients were measured according to guidelines for muck soils (Sanchez 1990) by extracting 4 g soil with 25 mL of 0.5 *N* acetic acid for 1 hr, then filtering through Whatman #42 filters. Extracts were analyzed for Ca, Mg, Fe, and Al concentrations by ICP using EPA method 200.7. Select soil nutrient concentrations and properties before S application are listed on Table 7-1.

Water extractable SO_4-S was analyzed by ionic chromatography (Perkin-Elmer, Waltham, MA) after shaking 2 g field soil with 25 mL water for 0.5 hr, followed by filtering through Whatman No. 42 filter paper. Aliquots of extracts were analyzed for total extractable S by ICP. Extractable organic S was calculated by subtracting extractable SO_4-S from total extractable S. Elemental S was determined as described by Pansu and Gautheyrou (2006), with slight modification. Approximately 5 g soil was extracted with 10 mL acetone for 30 min and centrifuged at 5,000 g for 15 min. Extracts were then analyzed for S content colorimetrically at 420 nm. To avoid interference by organic matter, unamended soils were used as controls to calibrate all readings.

Potentially mineralizable S was measured based on the methods of White and Reddy (2000) using a 10-day incubation followed by extraction with water. Extracts were analyzed for SO_4-S as previously described. The sulfatase activity in soils was assayed using the colorimetric methods described by Wright and Reddy (2001).

Statistical Analysis

A mixed model was fit using restricted maximum likelihood in the MIXED procedure of SAS (Littell et al., 2006). The fixed effects were S application rate, time and their interaction. Block was a random effect. Degrees of freedom were adjusted using the Kenward-Roger adjustment. An exponential covariance structure was used to model the correlation among observations taken from the same plot over time. Significant differences among individual treatments and time intervals were analyzed with Tukey's test at $\alpha = 0.05$. Pearson correlation was employed to determine relationships between variables. All statistical analysis was carried out with SAS 9.1 (SAS Institute).

Results and Discussion

Soil pH

Elemental S is commonly considered beneficial in alkaline soils to lower soil pH, supply SO_4 to plants, and increase P and micronutrient availability (Lindemann et al., 1991; Schueneman, 2001; Yang et al., 2008). However, the effectiveness of elemental S is not observed until elemental sulfur is oxidized, which depends on factors such as application rates, soil properties, and the activity of S-oxidizing microorganisms (Yang et al., 2008). In the present study, S application did not significantly reduce soil pH during the growing season (Fig. 7-1). A limited reduction in soil pH after S application was also observed in other studies of calcareous soils (Hassan and Olson, 1966). The limited effect of acidification may result from a S application rate too low to cause a change in pH and from the high buffering capacity

of this calcareous organic soil. Soils with high concentrations of carbonates and bicarbonates are highly buffered against acidification (Rogovska et al, 2007). When the original S recommendation for sugarcane of 448 kg S ha^{-1} was established years ago (Anderson, 1985), soil pH in the EAA was considerably lower. However, the rise in pH and decrease in soil depth to bedrock since that time (Snyder, 2005) increased the capacity of these soils to resist changes in pH. Thus, higher S application rates may be necessary to produce the same response as 448 kg ha^{-1} did in the 1980s (Anderson, 1985).

Extractable SO_4-S

Elemental S application significantly increased SO_4-S concentrations in soils throughout the growing season (Fig. 7-2). Extractable SO_4-S in soils receiving 448 kg S ha^{-1} was 36%, 131%, 201%, and 270% higher than unamended soils at 2, 6, 9, and 13 months, respectively. Sulfate-S concentrations significantly decreased from 2 (376 mg kg^{-1}) to 6 months (129 mg kg^{-1}), and continuing to 9 months (21 mg kg^{-1}). The declining trend in SO_4-S concentrations was likely due to SO_4 uptake by sugarcane and loss as runoff or leaching during precipitation events. Extractable SO_4-S was significantly correlated to extractable organic S (R^2 = 0.89), elemental S (R^2 = 0.52), and potentially mineralizable S (R^2 = 0.39), which may suggest that microbial oxidation of elemental S and mineralization of organic S were two major sources of soil SO_4-S (Jaggi et al., 2005; Zhou et al., 2005). In the EAA, soil oxidation generally supplies sufficient SO_4 to satisfy sugarcane nutrient requirements (Gilbert and Rice, 2006), therefore S application at high rates is likely to increase

SO_4 concentrations in the soil and thus the risk of export from fields and into sensitive Everglades wetlands.

Extractable Organic S

Extractable organic S contains S associated with particular organic matter and generally accounts for only a small proportion of the total soil S (Dias et al., 2003). However, it is likely to be readily mineralized to SO_4 and as such this pool is considered an important source of available S to crops, especially in soils containing low inorganic SO_4 (Dias et al., 2003; Kaiser and Guggenberger, 2005). Extractable organic S was not affected by S application during the growing season, but exhibited a similar decreasing pattern as extractable SO_4-S, indicating that extractable organic S was as mobile as SO_4 in these organic soils (Fig. 7-3). Extractable organic S averaged 58, 22, 4, and 16 mg S kg^{-1} at 2, 6, 9, and 12 months, respectively, and was significantly correlated to elemental S (R^2 = 0.44) and potentially mineralizable S (R^2 = 0.26), and extractable SO_4-S (R^2 = 0.89), suggesting that production of extractable organic S and S mineralization were controlled by the same factors (Valeur et al., 2000).

Elemental S

Elemental S was not detected in unamended soils during the growing season, but it was significantly higher in soils receiving 448 kg S ha^{-1} (215 mg kg^{-1}) than soils receiving 112 (44 mg kg^{-1}) and 224 kg S ha^{-1} (20 mg kg^{-1}) (Fig. 7-4). Elemental S contents in soils receiving the highest S rate was 771% and 334% higher than soils amended with 112 and 224 kg S ha^{-1} at 2 months, respectively. However, at 13

months after S application, elemental S was only detected in soils receiving 448 (9 mg kg^{-1}) and 224 kg S ha^{-1} (0.5 mg kg^{-1}). The decreasing patterns of elemental S in soils throughout the season were due to the oxidation of elemental S to SO_4. It has been reported that oxidation of elemental S in some calcareous soils is slow and may take several years (Lindemann et al., 1991; Cifuentes and Lindermann, 1993). Our results showed that a relatively high concentration of elemental S persisted in soils receiving 448 kg S ha^{-1} by 13 months after application, suggesting that higher S application in these calcareous organic soils is likely to maintain high levels of elemental S and SO_4 in soils for long periods of time.

Sulfatase Activity

Sulfatase is an enzyme that hydrolyzes ester S and releases SO_4, and hence plays an important role in organic S mineralization (Chen et al., 2001). Sulfur application at a range from 0 to 448 kg ha^{-1} had minimal effects on sulfatase activity during the growing season (Fig. 7-5). Sulfatase activity can be influenced by several soil properties, such as SO_4 concentration, organic matter, pH, and seasonal variations in soil moisture (Knauff et al., 2003). Decomposition of organic matter in this Histosol typically supplies enough S needed for crop growth (Snyder, 2005), thus sulfate is probably at a high enough concentration to minimize sulfatase activity (Wright and Reddy, 2001). Sulfatase activity averaged 240, 157, 223, and 216 mg *p*-nitrophenol kg^{-1} h^{-1} at 2, 6, 9, and 13 months after S application.

Organic S Mineralization

Potential S mineralization rates increased concurrently with increasing S application rates, and the effects continued throughout the growing season (Fig. 7-6). Overall, mineralized S was 1421% greater for soils receiving 448 kg S ha-1 than unamended soil, 375% greater than soils receiving 112 kg S ha-1, and 219% greater for soils receiving 224 kg S ha^{-1}. However, mineralized S rates significantly decreased from 2 (16 mg kg^{-1} d^{-1}) to 6 months (12 mg kg^{-1} d^{-1}), and continued to decrease from 9 (11 mg kg^{-1} d^{-1}) to 13 months (3 mg kg^{-1} d^{-1}). It has been well recognized that organic S is mineralized to SO_4 by hydrolysis of ester SO_4 catalyzed by sulfatase or by mineralization of C-bound S due to microbiological activity (McGill and Cole, 1981; Chen et al., 2001; Gharmakher et al., 2009). In the present study, no significant correlation between mineralized S and both sulfatase activity and C mineralization rates was found (data not shown). Instead, S mineralization rates were significantly correlated to S application rate (R^2 = 0.60), elemental S concentrations (R^2 = 0.85), extractable SO4-S (R^2 = 0.39), and extractable organic S (R^2 = 0.26). It was likely that oxidation of elemental S to SO_4 was primarily responsible for the increased mineralized S rates rather than organic S mineralization (Eriksen et al., 1998).

Conclusions

Sulfur application under current recommendations and guidelines for sugarcane had limited effects on the reduction of soil pH, thus its use for enhancing soil nutrient availability appears limited. Higher rates than currently recommended

may be needed to affect a change in soil pH, which would then have the effect of increasing nutrient concentrations in soil for longer duration during the growing season. However, S application at 448 kg ha^{-1} significantly increased elemental S and SO_4 concentrations in soil solution. Sulfur application did not stimulate the sulfatase activities during the growing season, whereas it significantly enhanced potential S mineralization rates, which was largely attributed to the oxidation of elemental S. Both extractable SO_4 and dissolved organic S decreased significantly throughout the growing season likely due to uptake by sugarcane, but also potentially by runoff or leaching through the shallow soils. Large-scale S amendment of EAA soils, or an increase in S application rates, is likely to increase SO_4-S concentrations in soil, which may enhance the potential for S export to sensitive Everglades wetlands during field drainage or precipitation events, leading to S enrichment of downgradient wetlands and contributing to the stimulation of MeHg.

Table 7-1. Chemical properties of the Histosols in the Everglades Agricultural Area before fertilizer application.

Soil Property	Unit	Concentration
Total organic C	g kg^{-1}	416
Total N	g kg^{-1}	38
Total P	mg kg^{-1}	850
Extractable NO_3-N	mg kg^{-1}	290
Extractable NH_4-N	mg kg^{-1}	16
Extractable P	mg kg^{-1}	48
Extractable Ca	mg kg^{-1}	720
Extractable Mg	mg kg^{-1}	105
Extractable Fe	mg kg^{-1}	13
Extractable Al	mg kg^{-1}	1.1

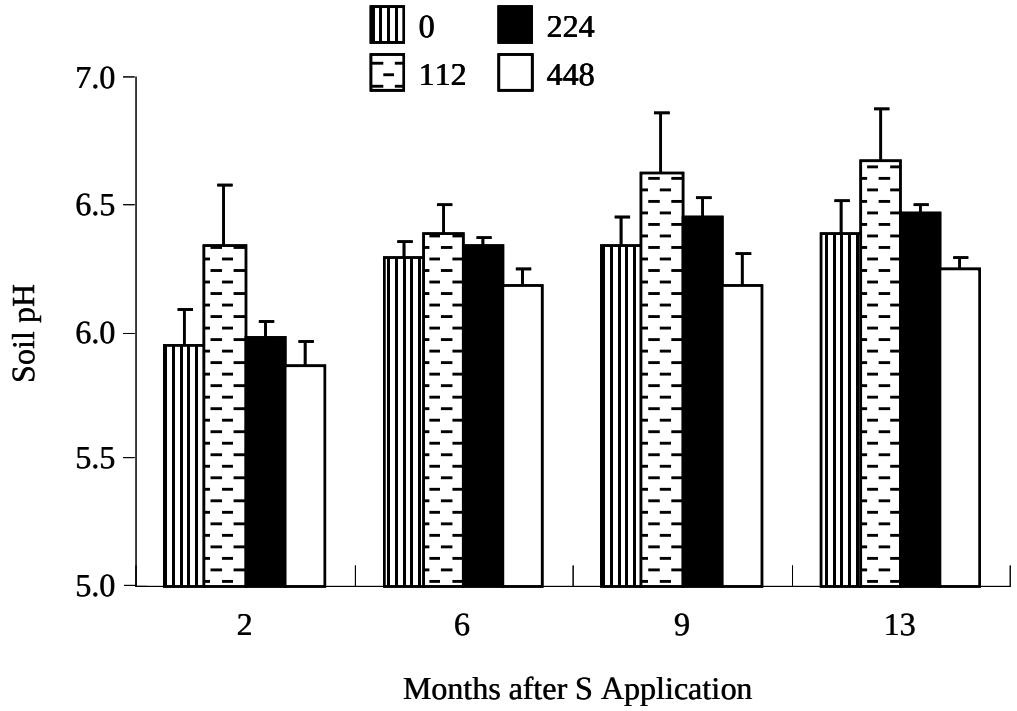

Fig. 7-1. Soil pH changes in response to different S application rates (0, 112, 224, and 448 kg S ha^{-1}) throughout the sugarcane growing season. Error bars represent the standard error of the mean.

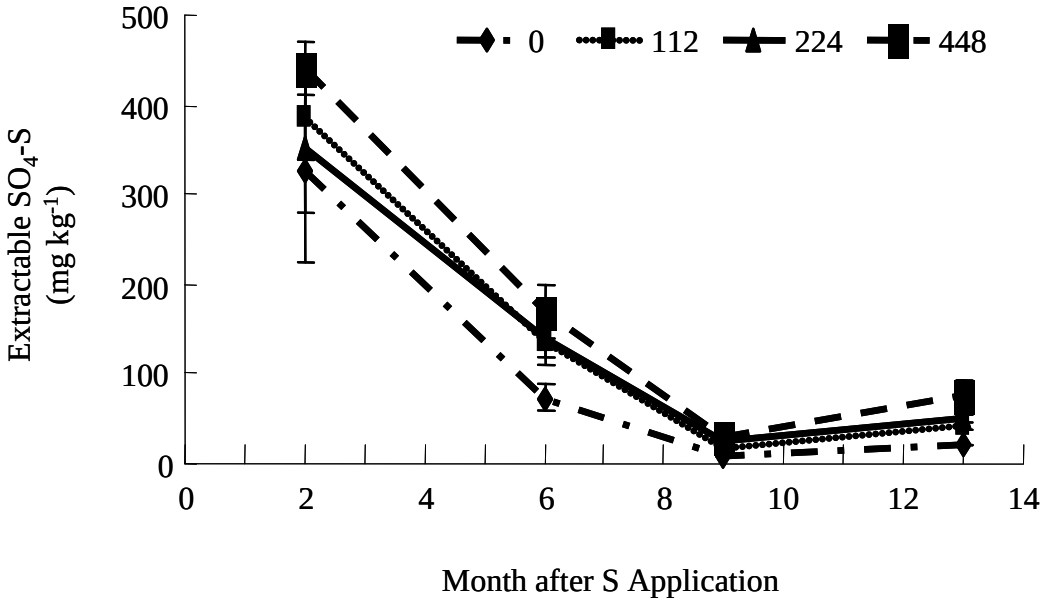

Fig. 7-2. Seasonal dynamics of extractable SO_4-S after S application at 0, 112, 224, and 448 kg S ha^{-1}. Error bars represent the standard error of the mean.

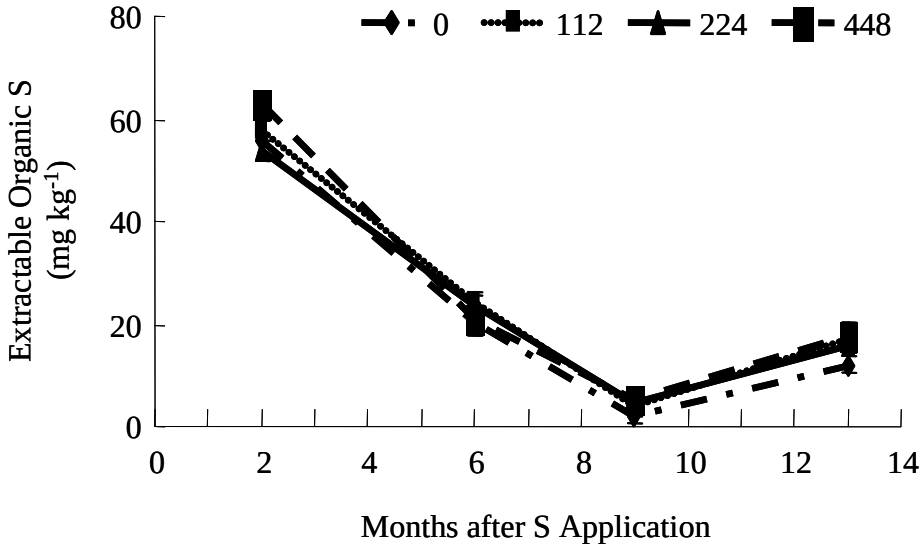

Fig. 7-3. Seasonal dynamics of extractable organic S after S application at 0, 112, 224, and 448 kg S ha^{-1}. Error bars represent the standard error of the mean.

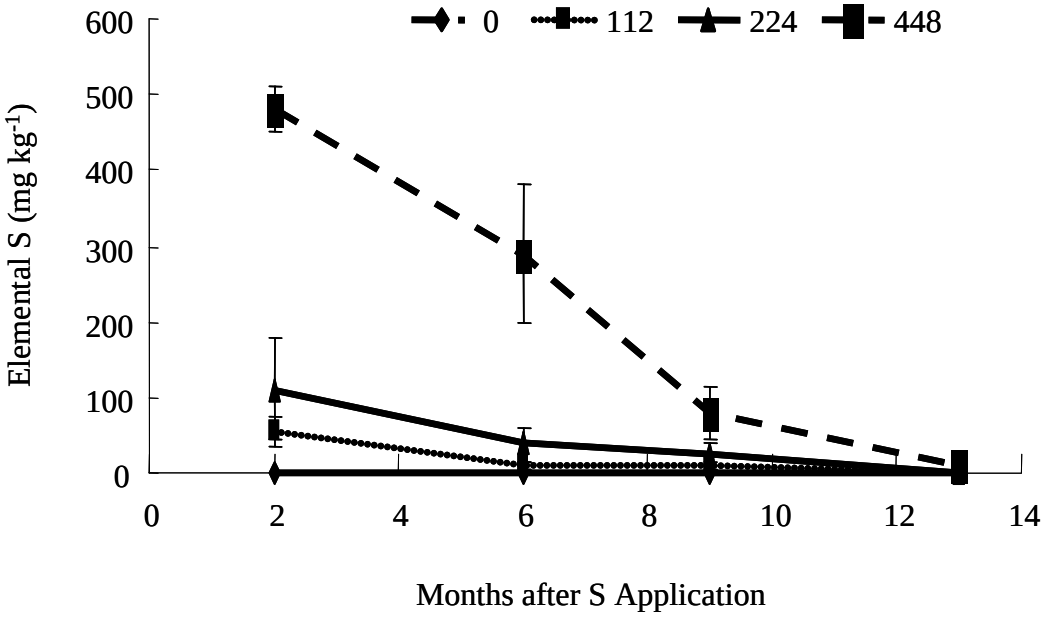

Fig. 7-4. Seasonal dynamics of elemental S after S application at 0, 112, 224, and 448 kg S ha^{-1}. Error bars represent the standard error of the mean.

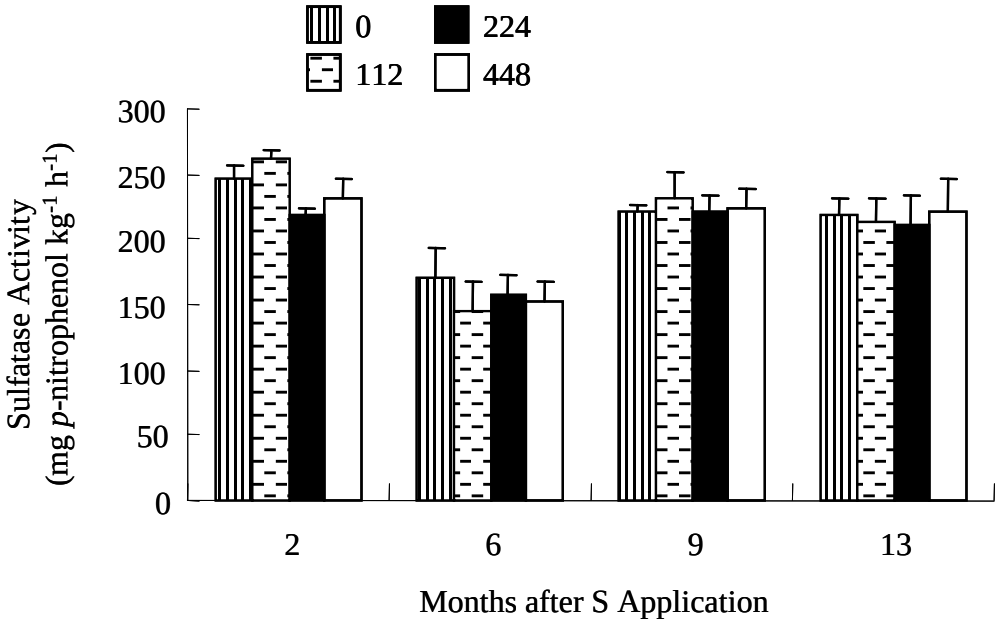

Fig. 7-5. Sulfatase activities in response to different elemental S application rates (0, 112, 224, and 448 kg S ha^{-1}) throughout the sugarcane growing season. Error bars represent the standard error of the mean.

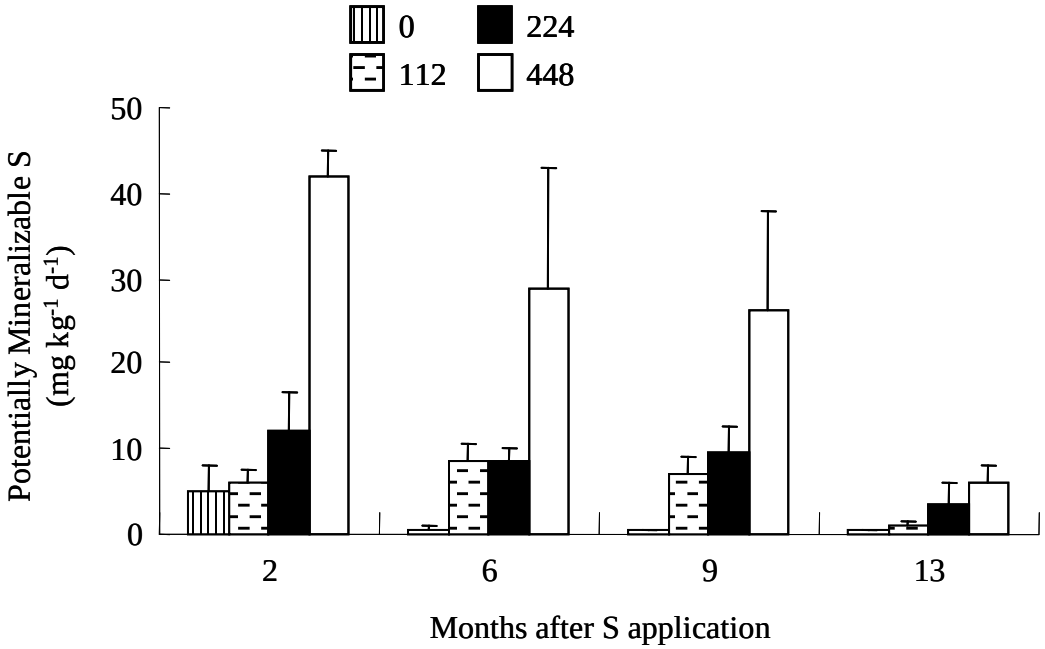

Fig. 7-6. Potential S mineralization in soils at 2, 6, 9, and 13 months after S application at different rates (0, 112, 224, and 448 S kg ha^{-1}). Error bars represent the standard error of the mean.

CHAPTER 8
SYNTHESIS

Laboratory and field studies were conducted to characterize chemical properties and microbial activities of EAA soils with different management history (Chapter 2). Multivariate analytical methods were utilized to assess differences between land uses and soils (Chapter 3). The impacts of S amendment on P distribution, availability, and stability were investigated for soils under sugarcane production (Chapter 4). The effects of S on soil microbial eco-physiological response, as measured using extracellular enzyme activities and potential mineralization rates, were determined for sugarcane soils (Chapter 5). The effectiveness of S amendment on enhancing macro- and micronutrient availability during the sugarcane growing season was evaluated (Chapter 6). Sulfur distribution and transformations were quantified for S-amended soils under sugarcane production (Chapter 7). A summary and implications of the experimental results related to objectives is present below.

Land Use Effects on Soil Nutrient Cycling and Microbial Community Dynamics in the Everglades Agricultural Area, Florida

Four land uses (sugarcane, turfgrass, forest and uncultivated soil) were characterized for nutrient cycling and microbial activity. Long-term cultivation and management significantly altered nutrient distribution and cycling in soil profiles, as well as the microbial community composition and activity. Turf soils, followed by forest and sugarcane, had the highest diversity of C sources and utilization rates of C resources, while the uncultivated soils had the lowest diversity. The uncultivated soils were the least managed, but the most P-limited and therefore had the smallest size of microbial population and the lowest C and N utilization efficiency. Labile inorganic P played an important role in regulating organic matter decomposition and microbial community

structure and function. Land use changes from sugarcane cropping to turf increased microbial activity and organic matter decomposition rates, indicating that changes from agricultural to urban land uses may further contribute to soil subsidence. Nonetheless, land use change from sugarcane cropping to uncultivated sites tends to slow down the oxidation rates of organic matter and subsequently may minimize soil subsidence.

Multivariate Analysis of Chemical and Microbial Properties in Histosols as Influenced by Land-Use Types

Soils from three land uses with different management intensity and history were analyzed for chemical and microbial properties. Cluster analysis on chemical properties demonstrated that soils from different land uses were perfectly clustered into their own groups, which were distinguished by labile inorganic P and total P. Likewise, integrated soil microbial characteristics were distinctive between land uses. Microbial biomass C and N, community-level physiological profile components, and potentially mineralizable N contributed most to such discriminations. Canonical correlation analysis suggested that variations in microbial properties between land uses were largely explained by difference in soil chemical properties, especially P availability. Long-term agricultural management, especially P fertilization, altered soil nutrient availability and consequently modified the microbial community structure and function. Intensive P fertilization is likely to stimulate microbial community and alter microbial processes, so future land use changes should consider the role of labile P on microbial community function and its control of nutrient cycling.

Sulfur-Induced Changes in Phosphorus Distribution in Everglades Agricultural Area Soils

Phosphorus fractionation procedures were performed to quantify P distribution in soil during the sugarcane growing season. The majority of P in this soil was retained in

organic forms (63%), followed by Ca-bound (32%), Fe-Al bound (4%) and labile fractions (1%). Labile and Fe-Al bound P comprised of the majority of available P for crops, and the size of these pools decreased throughout the growing season. Under current sugarcane production, P storages in the organic pools are susceptible to oxidation and a potential source for P loss from fields.

Sulfur application within a range from 0 to 448 kg S ha^{-1} did not significantly decrease soil pH due the high buffering capacity against acidification. As a result, the stimulatory effects of S on increasing labile and Fe-Al bound P were limited and temporary, whereas S addition did not impact the sizes of Ca-bound and organic P fractions. Therefore, S application under current recommendation guidelines and rates has minimal impact on increasing P availability beyond a short-term response (2 months) and is unlikely to enhance the potential for P export from agricultural fields into wetlands. Higher S application rates may overcome the soil's buffering capacity and consequently release large amounts of P from the Ca-bound fractions and pose an environmental hazard.

Microbial Eco-Physiological Response of a Calcareous Histosol to Sulfur Amendment

Application of elemental S at 448 kg S ha^{-1} stimulated the activities of phosphatase and glucosidase and simultaneously promoted labile P to be immobilized into microbial biomass. However, these effects were temporary and not observed beyond 2 months due to the high buffering capacity of this organic soil against pH reduction. Microbial biomass C and N were not affected by S amendment. The C, N, and P mineralization rates were independent of S addition, though all rates varied seasonally, suggesting that S application did not stimulate soil oxidation. Using the

current recommended S application guidelines, impacts on microbial activities and functions should be minimal. However, due to the increasing pH trend for these soils, there may be a need for higher S application rates in the future. These higher S rates may overcome the soil's buffering capacity and release large amounts of P, potentially stimulating microbial functional activities and altering organic matter dynamics.

Seasonal Changes in Nutrient Availability in Sulfur-Amended Everglades Soils under Sugarcane

Soils under sugarcane cultivation were amended with elemental S at four rates up to 448 kg S ha^{-1} to decrease pH and enhance nutrient availability. Water extractable P and K for soils receiving the highest S rate were significantly higher than for unamended soils only at 2 months after application, indicating a short-term enhancement of macronutrient availability. Similarly, soils amended with 448 kg S ha^{-1} contained more acetic acid extractable Zn than unamended soil, but the stimulatory effects did not extend beyond 2 months. The failure of S to enhance nutrient availability throughout the growing season indicates the limited benefit of applying elemental S to reduce pH and increase nutrient availability to sugarcane. As a result, S application did not increase sugar yield. Considering the trends of increasing pH and the decreasing depth to bedrock of soils in the EAA, new S application guidelines with higher amendment rates may be needed. Sulfur application increased SO_4 concentrations in soils and also its risk of export from fields. Therefore, large scale of S application should be evaluated for its potential to adversely affect proximal sensitive wetland ecosystems. It may not be economically viable to increase S fertilizer recommendations because of the high cost of elemental S. An alternative, such as different P and micronutrient fertilizer

application methods, timings, and sources, may be a better alternative to increase nutrient availability for these changing soils.

Three extractants, water, Mehlich-3, and acetic acid, were evaluated for their potential to extract plant-available nutrients and their relationship with sugarcane yield. Generally, acetic acid and Mehlich-3 extracted more nutrients from these calcareous organic soils than water, whereas acetic acid extracted more non-exchangeable nutrients and may not actually reflect nutrient availability to sugarcane. Mehlich-3 extractable P was identified as the single parameter most significantly correlated with sugar yield, so this extractant should be further evaluated for its potential to replace acetic acid as the extractant used for nutrient assessment for sugarcane grown on muck soils.

Overall Conclusions

Long-term cultivation and management has significantly altered the soil chemical properties, especially P availability, and microbial community composition and function in EAA soils. Long-term P fertilization has resulted in accumulation of P in soil profile and enhanced P availability, which consequently stimulated the microbial activity and function and organic matter turnover rates (Fig. 8-1). Current land use as sugarcane cropping requires P fertilization (Rice et al., 2006) and therefore would continue to promote organic matter mineralization. Future land use and management should consider the impacts of P on microbial communities and their control of nutrient cycling.

Elemental S application under current recommendation rates introduced temporary and limited effects on increasing nutrient availability to sugarcane and posed minimal impacts on microbial activity and function. Therefore, elemental S application is not beneficial at this stage under the current recommendations. Large scale application

is likely needed to overcome the high soil buffering capacity against acidification and produce desirable responses in terms of micronutrient availability and crop yield. However, S application at a large scale may stimulate nutrient regeneration rates and microbial activity and increase the risk of SO_4 export from the fields into adjacent wetlands (Fig. 8-2).

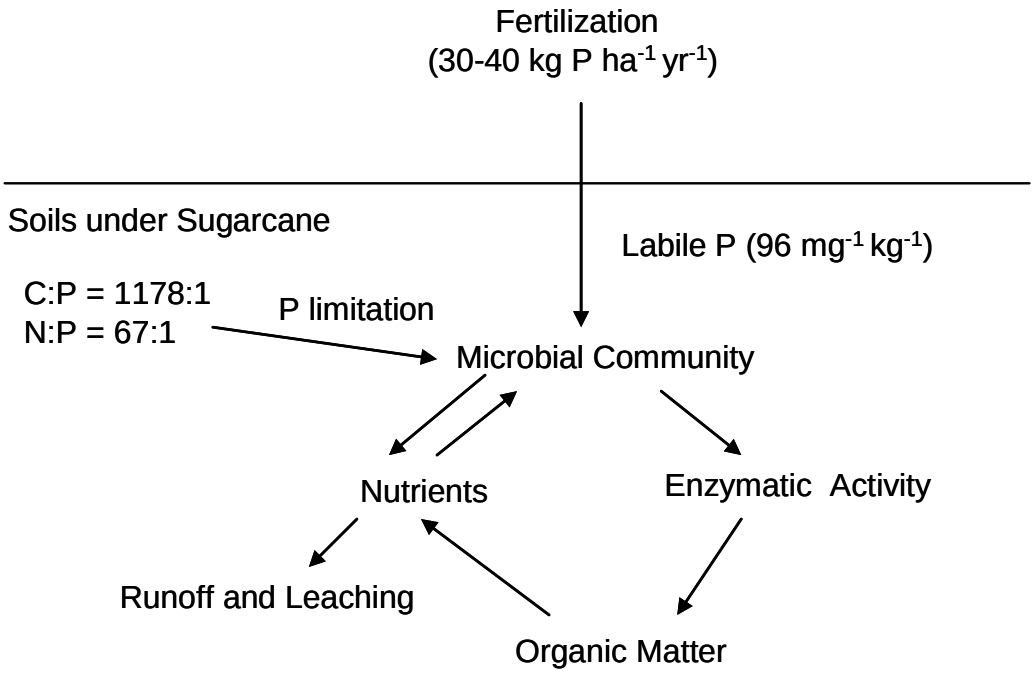

Fig. 8-1. Conceptual model of the microbial response to P fertilization in EAA soils under sugarcane. C:P, molar ratios of total carbon to phosphorus; N:P, molar ratios of total nitrogen to phosphorus.

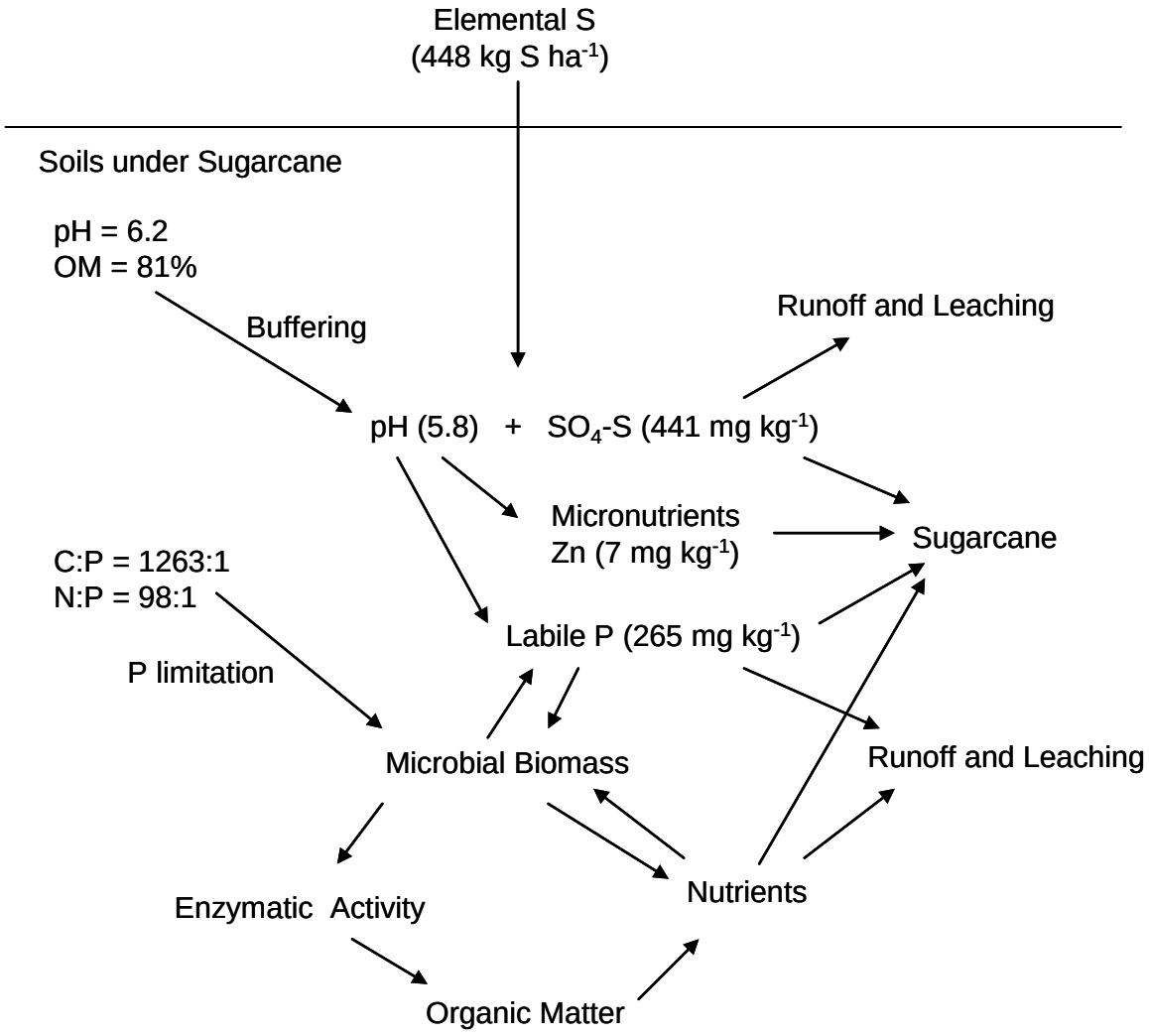

Fig. 8-2. Summary of biogeochemical processes in EAA soils after S applications at 448 kg S ha^{-1} at 2 months. C:P, molar ratios of total carbon to phosphorus; N:P, molar ratios of total nitrogen to phosphorus; OM, organic matter content; SO_4-S, water extractable sulfate; Labile P, acetic acid extractable phosphorus.

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BIOGRAPHICAL SKETCH

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