

SCREENING FORAGE CROPS SUITABLE FOR REMEDIATING P-IMPACTED SOILS
IN FLORIDA

By

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To my Mom and Dad

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LIST OF ABBREVIATIONS

BHG	Bahiagrass
BMD	Bermudagrass
DM	Dry Matter
FAWN	Florida Automated Weather Network
IFAS	Institute of Food and Agricultural Sciences
K	Potassium
M1P	Mehlich-1 Phosphorus
N	Nitrogen
STG	Stargrass
STP	Soil Test P
TKN	Total Kjeldahl Nitrogen
TKP	Total Kjeldahl Phosphorus
P	Phosphorus
WEP	Water-Extractable Phosphorus

Abstract of Thesis Presented to the Graduate School
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Phosphorus (P) is an essential element for all livings and represents an important input in agriculture. However, when improperly managed, P from intensive agriculture operations may contribute to the non-point source pollution and surface water eutrophication problems. Phytoremediation, defined as the use of plant to extract P from soils, is considered an environmental sound alternative to reduce the risks associated with soil P buildup and subsequent transport to water bodies. From the economic perspective, synergies between biomass and bioenergy production can further enhance the importance of phytoremediation as a cost-effective alternative to remediate P in P-enriched soils.

The objective was to evaluate suitable forage crops to remediate P-impacted soils in Florida. An experimental site impacted by continuous manure deposition for over fifty years was selected for the study. The project aimed to 1) screen an array of minor forage crops for their phytoremediation capacity potential, and to 2) evaluate the P removal capacity of four pre-chosen forage crops and their effects on groundwater quality. Fifteen forage species were selected based on dry matter yields, adaptability to Florida conditions and potential to be used as bioenergy crops. Soil samples were collected from the Ap, E, and Bh horizons and analyzed for Mehlich-1 and water-extractable P at the beginning and the end of the growing seasons. Forages were harvested periodically and analyzed for tissue P and N concentrations.

Water samples were collected every 2 wks at 60- and 90-cm soil depths throughout the growing season and analyzed for ortho-P.

Our data indicate significant effect of crop P uptake on Mehlich-1 soil P concentrations during the 2-year study. On average, Mehlich-1 P was reduced from 657 to 459 mg kg⁻¹. Elephantgrass (*Pennisetum purpureum*) exhibited the greatest P-removal potential (139 kg P ha⁻¹ yr⁻¹) compared with other forage species (average = 65 kg P ha⁻¹ yr⁻¹). Groundwater quality also showed treatment effect. Results indicated that elephantgrass has the highest P removal potential and can be also used as a renewable energy source. Further investigation is needed to address the long-term effects of forage crops on water quality.

CHAPTER 1 INTRODUCTION

Environmental Issues Associated With Phosphorus

Phosphorus plays an important role in agriculture. Adequate P supplies are necessary for seed and root formation, straw strength in cereals, crop quality, and synthesis of microbial biomass in ruminant animals (Whitehead, 2000; Havlin et al., 2004). Phosphorus fertilization, including the use of commercial fertilizers and animal waste, has been widely used in agriculture to supply adequate amounts of P to crops. However, when improperly managed P fertilizers can also pose a potential threat to the environment.

Once added to the soil, P can be sorbed by different soil constituents such as Al and Fe oxides (Schmidt et al., 1997). Although P mobility in most soils is generally low, P can be easily transported from agriculture areas when P additions exceed the maximum soil retention capacity. It is estimated that over 50% of US soils exhibit high P status (Fixen, 2002). The high P levels are generally associated with repeated applications of fertilizers and manure. Soil P buildup may pose a threat to the environment, as P may be easily transported to the water bodies (Sharpley et al., 1994).

Phosphorus losses from agricultural areas cause serious problems for water quality and the environment (Sharpley, et al., 1994). A major problem is the accelerated eutrophication in aquatic environments. Eutrophication occurs when a water body receives excess nutrient input, resulting in unwanted growth of algae and aquatic weeds, causing anoxic conditions. (Sharpley, et al., 1994; Brady and Weil, 1999). Increasing number of harmful algae blooms [such as a dinoflagellate (*Pfiesteria spp.*)] in water bodies has been linked to excess P input (Sharma et al., 2007). Eutrophication is an important environmental concern because it results in fish kills, unbalanced nutrients status, and can make the water unsuitable for drinking. These negative effects may also restrict the use of surface waters for aesthetics, fisheries, recreation, industry, and drinking, and thus have serious local and regional economic impacts,

including posing threats to human health (Burkholder and Glasgow, 1997). In most inland waters, P is the limiting nutrient for algae growth (Thomann and Mueller, 1987); thus even small P concentrations can negatively impact water quality and cause eutrophication problems (Sharpley, et al., 1994).

Phosphorus is transported to water bodies through two main pathways: i. runoff, and ii. leaching. Runoff is often the most important mechanism by which P is transported from agricultural land to surface water bodies (Sharpley, et al., 1994). Factors affecting the potential of P runoff include soil erosion, soil test level, and P fertilizer application rate (Sharpley, et al., 1994). Leaching also represents an important pathway through which P can pose a threat to the aquatic environment. Although P is relatively immobile in most soils, long-term application of manure and P fertilizer could increase P leaching potential (Eghballet al., 1996). Research has shown that P leaching from P-impacted soils could continue for many years even when P additions are reduced or ceased (Nelson et al., 2005).

Phosphorus leaching can be a serious environmental threat, especially in sandy soils with shallow water tables (Nelson, et al., 2005). Florida soils are particularly susceptible to P leaching due to their sandy texture, low P retention capacity, and fluctuating water table. Research has shown that P losses from spodosols represent an important environmental concern in Florida (Reddy et al., 1995). In addition, lateral movement of water and nutrients induced by artificial drainage also increases the potential risks of P transport to surface water bodies. Overland flow may also represent an important source of nutrient loss to nearby streams, rivers, and lakes during the rainy season (Graetz and Nair, 1995).

Phytoremediation of High Phosphorus Soils

Concept

Phytoremediation refers to the direct use of living plants for in-situ remediation of contaminated soils, sludge, sediments, and ground water through contaminant removal,

degradation, or containment (USEPA, 1999). Most of the research on phytoremediation has focused on the ability of plants to remediate trace metals or organic contaminants.

Hyperaccumulator species, which by definition are those that can accumulate certain target contaminants at very high tissue concentration (usually $> 10 \text{ g kg}^{-1} \text{ DM}$), have been identified for remediation of organic compounds and trace metals (Hogstad, 1996; Cunningham et al., 1997; Maet et al., 2001).

The most important advantages of phytoremediation strategies are: a. a cost-effective and environmental friendly approach to remediate soils, b. better public acceptance than traditional remediation approaches such as excavation, and c. capable of remediating a diverse range of contaminants (Macek et al., 2000). Estimated costs of phytoremediation for the organics and metal contaminated sites are \$25,000 to \$75,000 per ha, which is 20 to 50% of traditional capping (Suresh and Ravishankar, 2004).

There are also limitations associated with phytoremediation. Because phytoremediation relies on plant roots to absorb the contaminants, the extension of plant roots is critical to the success of phytoremediation. The concentration of contaminants below the root zone is usually not affected by phytoremediation strategies. Another limitation is that plants cannot grow in highly toxic levels of contaminants, so phytoremediation is only suitable when contaminant concentrations are not excessively high. Disposal of harvested plant biomass also represents difficulties. Perhaps the most important limitation associated with phytoremediation is that it is usually a time-consuming process, and may require years to decades to reduce contaminant concentrations to acceptable levels (Cunningham and Berti, 1993). Salt et al. (1995) reported that even when effective Ni and Zn hyperaccumulator species were used, phytoremediation required 13 to 14 yr to clean a contaminated site.

Phytoremediation of Phosphorus

Limited research has been conducted on the potential benefits of phytoremediation to minimize environmental problems associated with excess P (Delorme et al., 2000; Gastonet al., 2003). Before the 1980s, agronomists were primarily concerned about the limited mobility of P in the soil and its effects on plant P nutrition. Because P was perceived to be highly immobile, recommendations of animal waste applications at rates based on crop N requirements (McDowell et al., 2001). Decades of excessive manure and fertilizer applications resulted in increased soil P concentration in many agricultural areas.

Current approaches to reduce the off-site movement of P from agricultural areas include the utilization of soil chemical amendments and agricultural best management practices. Phosphorus can form insoluble complexes with a variety of metal ions including aluminum, calcium, and iron. Chemical amendments such as alum, ferric chloride and lime have been applied to reduce the availability of water-soluble P in manure-impacted soils (Moore and Miller, 1994; Dao, 1999; Douet et al., 2003). Recently, water treatment residuals have been evaluated as a potential soil amendment that can effectively decrease the water-soluble P concentration in P-impacted soils in both the short and long term (Silveira et al., 2006; Agyin-Birikorang and O'Connor, 2007). Riparian buffers are also used to reduce transport of particle P into streams (Novak and Chan, 2002). Agricultural practices that reduce surface runoff and erosion can also minimize P movement into surface water bodies (Brady and Weil, 1999; Novak and Chan, 2002).

Although many best management practices have been shown to effectively reduce off-site movement of P into surface water bodies, they generally fail to prevent P accumulation or reduce total P concentrations in the soil. Thus, the threat of P movement and subsequent solubilization remains after implementation.

Potential Phosphorus Hyperaccumulator Species

Delorme et al. (2000) suggested that various crop species exhibited extensive variation in the P-uptake, and suitable plants which have above-average tissue P concentrations could be used to remove P from the soil. The average P concentration of plant tissue ranges from 2 to 4 g kg⁻¹ of dry matter (Brady and Weil, 1999); and successful phytoremediation of excess soil P requires P-hyperaccumulator species that exhibit tissue P concentrations between 8 and 14.5 g kg⁻¹ (dry matter basis) (Novak and Chan, 2002). The majority of previous P hyperaccumulator studies were conducted under greenhouse conditions (Sharma and Sahi, 2005; Sharma, et al., 2007). However, development of P-hyperaccumulator plants under greenhouse or small plot conditions does not guarantee a successful performance at field scale (Novak and Chan, 2002). No P hyperaccumulator crop has been identified at the field scale (Pantet et al., 2004). Annual removal of P by typical forage species ranges from about 20 kg ha⁻¹ (by red clover, *Trifolium pratense*) to about 85 kg ha⁻¹ [by johnsongrass, *Sorghum halepense* (L.) Pers.] (Pierzynski and Logan, 1993). Table 1-1 compiles the annual yield and P removal potential of several crops (Pierzynski and Logan, 1993; Delorme, et al., 2000; Novak and Chan, 2002).

Gaston et al. (2003) studied the P removal potential of five cool-season and five warm-season forages on a P-impacted Ruston soil (fine-loamy, siliceous, thermic Typic Paleudult). Bahiagrass (*Paspalum notatum* Flugge) or bermudagrass [*Cynodon dactylon* (L.) Pers.] followed by annual ryegrass (*Lolium multiflorum* Lam.) seeded in the fall was identified as a potential strategy to remediate soil with elevated P concentrations. Ryan (2006) conducted a field study of the effectiveness of a double-cropped system (bermudagrass over-seeded with annual ryegrass) in reducing soil P from a P-impacted Ruston fine-sandy loam. Removal rates were nearly 200 kg P ha⁻¹ over 4 yr. Sharma et al. (2007) studied several species, including vegetables, legume and herb crops, for their suitability for P

phytoremediation in the greenhouse. Cucumber (*Cucumis sativus*) and yellow squash (*Cucurbita pepo* var. melopepo) accumulated P concentration $> 10 \text{ g kg}^{-1}$ dry weight, but no field evaluations were conducted to validate the greenhouse results. Woodard et al. (2007) evaluated five forage systems for their P-removal capacity on a Kershaw sand soil (thermic, uncoated Typic Quartzipsamment). The greatest P removal ($91 \text{ kg ha}^{-1} \text{ cycle}^{-1}$) was observed in a bermudagrass-rye (*Secale cereale* L.) system in the first two years. The authors concluded that due to its high yield and persistence, bermudagrass was likely the best warm-season forage to recover P from dairy sprayfields in northern Florida.

Time Required to Remediate P-Enriched Soils

Lacking a P hyperaccumulator species, phytoremediation of soils enriched with P is expected to require long times to reduce P to acceptable levels using common row crops and forage grasses (Sharpley, et al., 1994). For example, McCollum (1991) estimated that reducing Mehlich-3 P in a Portsmouth soil (fine sandy over sandy or sandy-skeletal, mixed, thermic Typic Umbraquult) from 100 mg P kg^{-1} to the threshold agronomic level of 20 mg P kg^{-1} would require 16 to 18 yr of consecutive cropping with corn (*Zea mays* L.) or soybean [*Glycine max* (L.) Merr.]. Mozaffari and Sims (1996) reported that 16 yrs of corn and soybean cropping was required to reduce Mehlich-1 P levels of 100 mg kg^{-1} to a crop response level on a Portsmouth fine sandy loam.

Synergies Between Phytoremediation and Bioenergy Production

As discussed before, phytoremediation can be a feasible technique to reduce soil P concentrations. However, serious limitations associated with phytoremediation of P are (i) the disposal of the harvested plant biomass, (ii) possible high establishment and maintenance costs, and (iii) long remediation time that may be required. One alternative to improve the economic sustainability of phytoremediation strategies is the use of plant biomass for

bioenergy production. Biomass produced in the processes of phytoremediation can potentially be used as a renewable energy source.

The concept of synergies between phytoremediation and bioenergy production is relatively new, especially for the phytoremediation of P. Van Ginneken et al. (2007) reported a phytoremediation project conducted in Belgium where plants were cultivated to extract metals from the soil, and then harvested for seed and biofuel production. The use of metal-accumulating plants for bioenergy production is promising, despite some concerns relative to the fate of metals in the plant tissue material. The plants accumulate heavy metals in the tissues which are further harvested and processed to produce biofuel. When the biofuel is exhausted, or the plant ash disposed, it is unknown if there will be environmental risks associated with hazardous metal emission (Van Ginneken, et al., 2007). Jasinskas (2008) conducted a 3-year study of the potential of 8 species of tall perennial grasses for use as energy crops, and concluded that biomass of perennial grasses can be used as fuel. Among 35 species chosen for evaluation, switchgrass (*Panicum virgatum*) was the native perennial grass with the greatest potential for bioenergy production. Results from studies conducted in Florida indicate that biomass and biofuel production vary considerably among forage species (Newman et al., 2008).

The focus of this project was to evaluate the phytoremediation potential of various forage crops that could also be used as a renewable energy source. The study was not designed to evaluate the biofuel production potential of the various species.

Hypotheses and Research Objectives

The research objectives of this study are to:

Specific Aim 1: Screen an array of minor forage crops for their phytoremediation capacity potential in soils with excessive P concentrations.

Specific Aim 2: Compare the P removal capacity of four forage crops [(elephantgrass (*Pennisetum purpureu*), sugarcane (*Saccharum officinarum*), switchgrass (*Panicum*

virgatum), and stargrass (*Cynodon nluemfuensis*)] grown on a manure-impacted site and the impacts of forage P uptake on groundwater quality.

Three hypotheses will be tested for the project:

Hypothesis 1: Soil test P concentrations will decrease over time in response to crop P uptake.

Hypothesis 2: P removal capacity will vary among different crop species

Hypothesis 3: Groundwater P concentrations will be reduced in response to P uptake of forage crops.

Table 1-1. Yield and P uptake for different crops, adapted from Pierzynski and Logan (1993), Delorme et al. (2000) and Novak and Chan (2002)

Crop	Yield (Mg ha ⁻¹ yr ⁻¹)	P uptake (kg ha ⁻¹ yr ⁻¹)
Corn silage (<i>Zea mays</i>)	67.2	39.2
Coastal Bermuda grass (<i>Cynodon dactylon</i>)	22.4	70.6
Red clover (<i>Trifolium pratense</i>)	9	22.4
Johnsongrass (<i>Sorghum halepense</i>)	26.9	93
Indian mustard (<i>Brassica juncea</i>)	18	84.6

Table 1-2. Biofuel crops adapted to Florida conditions (adapted from Newman et al., 2008)

Crop	Scientific name	Yield	Biofuel Production Potential	Environmental Concerns	Production Costs
Switchgrass	<i>Panicum virgatum</i>	4500 kg ha ⁻¹ yr ⁻¹ - 13000 kg ha ⁻¹ yr ⁻¹	combustion/lignocellulosic ethanol	little	Not well known
Miscanthus	<i>M. sacchariflorus</i> x <i>M. sinensis</i>	11200 kg ha ⁻¹ yr ⁻¹ - 33600 kg ha ⁻¹ yr ⁻¹	Combustion	could escape and spread	Not known
Sweet Sorghum	<i>Sorghum bicolor</i> (L.) Moench	2900 kg ha ⁻¹ yr ⁻¹ - 3100 kg ha ⁻¹ yr ⁻¹ in sugar	ethanol production	none	Limited information
Soybean	<i>Glycine max</i>	470 L ha ⁻¹ yr ⁻¹ - 560 L ha ⁻¹ yr ⁻¹ in biodiesel	Biodiesel	none	Varies
Peanut	<i>Arachis hypogaea</i>	1100 ha ⁻¹ yr ⁻¹ - 1400 L ha ⁻¹ yr ⁻¹ in biodiesel	Biodiesel	Little	\$2000 ha ⁻¹ - \$2500 ha ⁻¹
Canola	<i>Brassica napus</i>	1500 L ha ⁻¹ yr ⁻¹ in biodiesel	biodiesel	None	Varies
Elephantgrass	<i>Pennisetum purpureum</i>	31000 kg ha ⁻¹ yr ⁻¹ - 45000 kg ha ⁻¹ yr ⁻¹	methane generation/ co-firing with coal	nitrate leaching / invasiveness	\$1500 ha ⁻¹
Sugarcane	<i>Saccharum spp.</i>	2000 L ha ⁻¹ yr ⁻¹ - 2500 L ha ⁻¹ yr ⁻¹ in ethanol	ethanol	byproduct of vinasse	\$370 ha ⁻¹ - \$600 ha ⁻¹

CHAPTER 2 MATERIALS AND METHODS

Site Description

Field experiments were conducted from 2007 to 2008 on a P-impacted site, located in the Lake Okeechobee basin in South Florida (27° 32' 17"N, 81° 51' 31"E; Figure 2-1). The site is a private dairy farm and has been continuously occupied by dairy cows for over 50 yr. The area selected for the research project was approximately 0.6 ha in size. The study area was fenced to prohibit access of cattle, sprayed to kill the existing stargrass (*Cynodon nlemfuensis* Vanderyst) pasture before planting.

Forage Selection and Experimental Design

Small Plot Study

To accomplish the first specific aim, 15 forage cultivars adapted to South Florida conditions were selected for study (Table 2-1). Treatments consisted of the fifteen cultivars replicated three times in a completely randomized design for a total of 45 plots. Control plots (bare ground) were also included, one per replicate. Forage species used in the study were selected based on the following criteria:

- High forage production potential (yield and quality)
- Expected relatively high P uptake
- Adapted to South Florida conditions
- Seed/planting material widely available
- Potential to be used as bioenergy crops

Plot area was 3 x 2 m with a 2-m aisle between plots. Elephantgrass (*Pennisetum purpureum*) and sugarcane (*Saccharum officinarum*) were planted on 1 Feb. 2007. Bahiagrass (*Paspalum notatum*), switchgrass (*Panicum virgatum*), and mulato (*Brachiaria sp.*) were seeded on 5 Apr. 2007. The remaining species were established between late June and early July, 2007. Plots were closely monitored to assess need for weed control. Mechanical and chemical weed control was performed as needed during the 2-yr study.

Large Plot Study

The second specific aim was addressed using large scale (10 x 10m) plots to more accurately evaluate forage DM yields, P removal capacity of various species, and the impacts of P uptake on groundwater quality. Sixteen larger plots were established on the experimental site using four forage species believed to have the greatest P removal potential. The species include: (i) sugarcane (*Saccharum officinarum*) cv. CP 78-1620, (ii) elephantgrass (*Pennisetum purpureum*) cv. Merkeron, (iii) switchgrass (*Panicum virgatum*) cv. Alamo, and (iv) stargrass cv. Florona. Treatments consisted of the four forage species replicated four times on a completely randomized design for a total of 16 plots (Table 2-2), and no bare plots were included. Sugarcane and elephantgrass were established on 1 Feb. 2007. Switchgrass and stargrass planting was delayed because of drought conditions, and planting finally occurred between late June and early July of 2007. As in the small plot study, mechanical and chemical weed control was performed on a regular basis throughout the 2-yr study.

Two lysimeters were installed at 60- and 90-cm depths in the center of each large plot for a total of 32 sample points. The lysimeters were positioned at these depths to monitor water quality above and below the Bh horizon (66- to 90-cm), respectively. A pressure transducer was also installed in the field to monitor fluctuations in the groundwater level. Precipitation was monitored by Ona station of the Florida Automated Weather Network (FAWN).

Fertilization and Harvest Schedule

Crops were fertilized according to IFAS recommendations (Mylavarapuet al., 2009). All experimental units in both studies (excluding elephantgrass and sugarcane plots) were fertilized with ammonium nitrate (NH_4NO_3) at a rate of $90 \text{ kg ha}^{-1} \text{ N}$ after every harvest (every 6 wk). The elephantgrass plots received $180 \text{ kg ha}^{-1} \text{ N}$ after every harvest (every 12

weeks). The sugarcane plots received 200 kg ha⁻¹ N after every cut (every 12 months). No P and K fertilizer was added during the study.

During the growing seasons (May to November) each year, plots were periodically harvested to determine DM yields, and tissue N and P concentrations determined (Table 2-3). All the plots, except for the elephantgrass and sugarcane plots, were harvested at 6-wk intervals. Elephantgrass plots were harvested every 12 wk, and sugarcane was harvested once a year. Forage sub-samples were collected and placed in pre-weighed cloth bags and weighed for fresh weight. Sub-samples were oven-dried at 60°C for approximately 48 h until constant dry weight. Oven-dried samples were ground to pass through a 1-mm stainless-steel screen and stored for further analysis.

Soil Analyses

Soil in the plots was of the Pomona series (sandy, siliceous, hyperthermic Ultic Alaquods (United States. Natural Resources Conservation Service., 1996). Soil samples were taken from the Ap (0-15 cm) horizon in the small plot study at the beginning and the end of each growing season during the 2-yr study. In the large plot study, soil samples were collected from Ap (0-15 cm), E (15-60 cm) and Bh (66-90 cm) horizons Five 5-cm soil core samples (four from near each corner, one in the center) within each plot were taken and mixed thoroughly to constitute a representative composite sample. Soil samples were aired dried, sieved (2 mm) and stored for further analysis.

Soil P concentrations were determined using different extraction methods. Water extractable P (WEP) has been widely used in some European countries (Ehlert et al., 2003) as an indicator of runoff P potential (Kleinman et al., 2002). In this project, WEP was used as an indicator of the labile P that would be readily subject to leaching. Water-Extractable P was determined by adding 20 mL of water to 2 g of soil and shaken at 3.6 × g for 1 h. The mixture was centrifuged at 2147 × g for 10 min, and filtered through 0.45-µm membranes

(Luscombe et al., 1979; Pageet et al., 1982). Mehlich-1 P (Mehlich, 1953) was determined by adding 20 mL of Mehlich-1 extractant to 5-g soil samples and shaken at $3.6 \times g$ for 5 minutes, and filtered through Whatman grade No.2 filter paper (Mehlich, 1953). Phosphorus in the extracts was analyzed colorimetrically (EPA Method 365.1) using a Seal AQ-2 discrete analyzer (2006 SEAL Analytical Ltd. Mequon, WI).

Tissue Analyses

The forage tissue samples from each harvest were analyzed for total Kjeldahl P (TKP) (EPA Method 365.4), and total Kjeldahl N (TKN) (EPA Method 351.1). Briefly, tissue samples were heated in a digestion block in the presence of H_2SO_4 , K_2SO_4 and $CuSO_4$. The digestion converts P species to ortho-phosphate, and converts N compounds to ammonia. The residue from digestion was cooled and filtered through Whatman grade No.2 filter paper for analysis. Nitrogen and P analyses were performed using a Seal AQ-2 discrete analyzer. Phosphorus uptake by forage crops was calculated as the product of plant tissue P concentration and dry matter yield.

Water Sampling and Analyses

Lysimeter (water) samples were collected from 30 May 2008 to 23 Nov. 2008 at 14-d intervals. Samples were collected using hand-pumped syringes connected with long sampling tubes. Caps of the lysimeters were removed then sampling tubes were extended into the lysimeters for the water. Lysimeter samples were contained in 20-mL bottles in the field and were filtered through 0.45- μm membrane in the laboratory. Samples were analyzed for ortho-P (EPA Method 365.3), NO_2 -N plus NO_3 -N (EPA Method 352.1) and NH_4 -N (EPA Method 350.1) using a Seal AQ-2 discrete analyzer. At times in the beginning and the end of rainy season, less water was collected from the field and not enough for all the analyses; then ortho-P was analyzed as first priority.

Statistical Analysis

Data were analyzed using SAS software (Littelet al., 1996; SAS Inc., 2001).

Differences among treatments were statistically analyzed with a completely randomized design (CRD) using the general linear model (PROC GLM) of the SAS software.

The statistical model tested the effects of forage species, year and their interactions on soil P concentrations, P uptake, tissue P and N concentrations and water quality. Because initial soil P concentrations are expected to affect the effectiveness of crops to remediate P, initial P levels were included in the statistical analysis as a covariate. Mean separations were performed using Fisher's protected LSD (least significant difference) method at significance (α) level of 0.05.

Table 2-1. Species, cultivars, and plot numbers assigned to warm-season grasses screened for high capacity P-uptake in the small plot study

Treatment ID	Common name	Scientific Name	Cultivar	Plot No.
1	Bahiagrass	<i>Paspalum notatum</i>	Pensacola	11,29,37
2	Bahiagrass	<i>Paspalum notatum</i>	Argentine	6,22,38
3	Mulato	<i>Brachiaria sp.</i>	Mulato	5,32,42
4	Stargrass	<i>Cynodon nluemfuensis</i>	Ona	7,15,33
5	Stargrass	<i>Cynodon nluemfuensis</i>	Florico	4,24,27
6	Bermudagrass	<i>Cynodon dactylon</i>	Tifton 85	16,31,40
7	Bermudagrass	<i>Cynodon dactylon</i>	Jiggs	17,19,44
8	Bermudagrass	<i>Cynodon dactylon</i>	Florakirk	10,25,35
9	Bermudagrass	<i>Cynodon dactylon</i>	Coastcross-2	2,21,39
10	Elephantgrass	<i>Pennisetum purpureum</i>	Merkeron	1,14,34
11	Sugarcane	<i>Saccharum officinarum</i>	CP 78-1620	9,28,41
12	Limpograss	<i>Hermathria altissima</i>	Floralta	8,13,43
13	Guineagrass	<i>Panicum maximum</i>	Mombaca	12,20,30
14	Digitgrass	<i>Digitaria eriantha</i>	Pangola	18,23,36
15	Switchgrass	<i>Panicum virgatum</i>	Alamo	3,26,45

Table 2-2. Species, cultivars, and plot assigned to warm-season grasses selected for field evaluation of P accumulation in the large plot study

Treatment ID	Common name	Scientific Name	Cultivar	Plot No.
1	Sugarcane	<i>Saccharum officinarum</i>	CP 78-1620	3,10,11,16
2	Elephantgrass	<i>Pennisetum purpureum</i>	Merkeron	2,7,12,13
3	Switchgrass	<i>Panicum virgatum</i>	Alamo	1,6,8,14
4	Stargrass	<i>Cynodon nlemfuensis</i>	Ona	4,5,9,15

Table 2-3. Harvest protocols for the small plot and large plot studies.

Harvest No.	Date	Protocol	Note
1	7 Sep. 2007	II.	
2	17 Oct. 2007	I	
3	27 Nov. 2007	II	
4	12 Mar. 2008	III	
5	24 Apr. 08	I	In 2008, several plots in the small plot study were infested with excessive weed, and were not harvested. Switchgrass plots were not harvested throughout the year.
6	5 Jun. 08	II	
7	17 Jul. 08	I	
8	28 Aug. 08	II	
9	9 Oct. 08	I	
10	20 Nov. 08	II*	Sugarcane plots were harvested on 12 Mar. 2009

I denotes that all plots except sugarcane and elephantgrass plots were harvested.

II denotes that all plots except sugarcane plots were harvested.

III denotes that all plots were harvested.

Location of the Experiment Site

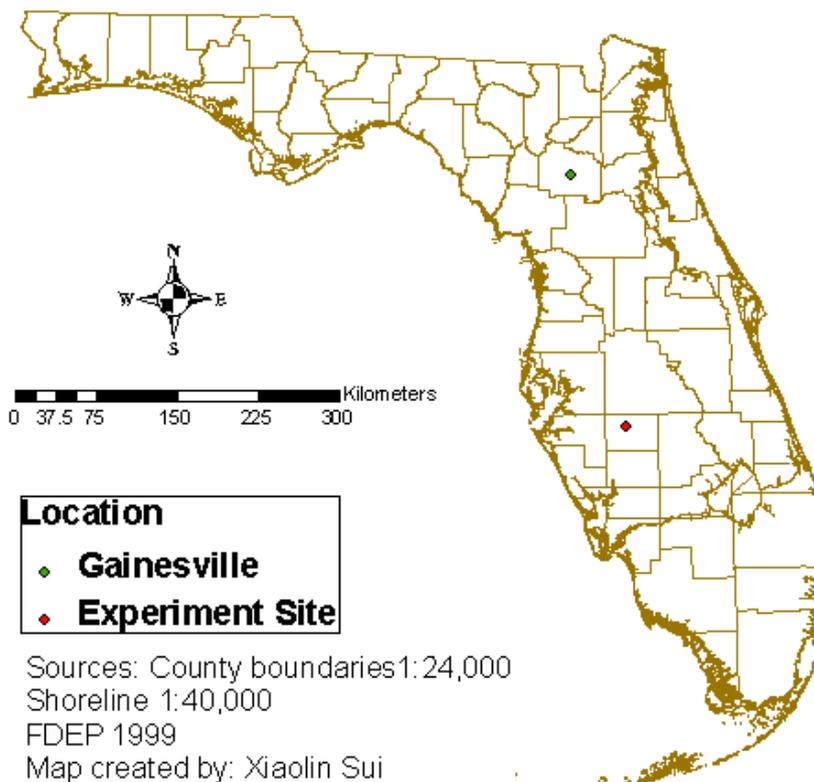


Figure 2-1. Location of the experiment site

CHAPTER 3 RESULTS

Small Plot Study

Mehlich-1 Phosphorus

There was considerable spatial variability associated with the initial soil P concentrations in the Ap horizons of the small plots (Figure 3-1). This was likely due to pasture management (i.e. location of feed and water trough) and non-uniform manure deposition over the field by the dairy operations.

Average initial soil test P in the Ap horizon (695 mg kg^{-1}) was considered “very high” according to the IFAS soil test interpretation (Mylavarapu and Kennelley, 2002). Mean Mehlich-1 P (M1P) concentrations decreased from 695 to 506 mg kg^{-1} (as much as 27%) over the 2-yr study period ($P < 0.01$; Figure 3-2A). Greater decreases in soil-test-P values were observed in 2008 compared to 2007 ($P = 0.02$). There was no significant effect of the interaction between forage species and year ($P = 0.83$).

During the 2-yr study, plots cultivated with forage species exhibited an average M1P decrease of 198 mg kg^{-1} compared to 55 mg kg^{-1} for the control plots ($P = 0.01$; Figure 3-3A). Significant differences in M1P were observed among the cultivars (Figure 3-4A). Mulato plots exhibited the largest decreases in M1P values, whereas sugarcane and limpgrass plots exhibited the smallest decreases.

Water-Extractable Phosphorus

Average WEP across all 48 of the small plots decreased from 97.1 to 53.0 mg kg^{-1} from the beginning of 2007 to the end of 2008 ($P < 0.01$; Figure 3-2B). A larger decrease in WEP values was observed in 2007 than in 2008 ($P < 0.01$). Similar to M1P data, the interaction between species and year was not significant ($P = 0.69$).

In contrast to the Mehlich-1 P data, there was no treatment effect on WEP values ($P = 0.14$; Figure 3-3B). Regardless of the forage species, water-extractable P values in all plots

was reduced after the 2-yr study (Figure 3-4B). The lack of WEP response to forage uptake was likely because WEP was designed as an environmental soil test, not an agronomical soil test related to the crop response.

Because WEP is an indicator of soil labile P, the decrease of soil WEP across the study area may suggest that labile P may have been leached out of the surface horizon. The fact that the decrease in 2007 was greater than 2008 suggests that a significant amount of labile P was leached out in the first year and less labile P was available to be leached out in the second year. The hypothesis that significant amounts of P were leached from the Ap horizon was further investigated in the large plot study.

Dry Matter Yield

Average crop DM yields by year varied considerably among the various species ($P < 0.01$; Figure 3-5). Average DM yield ranged from 11 Mg ha⁻¹ yr⁻¹ for Pensacola bahiagrass to 44 Mg ha⁻¹ yr⁻¹ for elephantgrass. Dry matter yields were, in general, consistent with those reported by Woodard and Prine (1993), Newman et al. (2008), Newman (2008a,b), Mislevy (2006), Woodard et al. (2007) and Legendre and Burner (1995). Switchgrass showed poor persistence and plots were not harvested in 2008 because of excessive weed infestation. The two cultivars of bahiagrass (Pensacola and Argentine) did not exhibit significant differences in DM yield; neither did the two cultivars of stargrass (Florico and Ona stargrass) or the four cultivars of bermudagrass. Elephantgrass and sugarcane, also referred to here as tall grasses, had significantly greater DM yields (44 Mg ha⁻¹ yr⁻¹ and 42 Mg ha⁻¹ yr⁻¹ respectively) than the other species. The effect of year and the interaction between year and species were not significant ($P = 0.14$).

Tissue Phosphorus Concentrations

Tissue P concentration is a useful indicator of P removal potential. Total tissue P concentrations varied from 1.5 g P kg⁻¹ for sugarcane to 4.4 g P kg⁻¹ for guineagrass (Figure

3-6). On average, shorter grasses exhibited tissue P concentrations of around 4 g P kg⁻¹. The two tall-grass species had significantly lower tissue P concentrations than the other forage species. Elephantgrass had an average tissue P concentration of 2.8 g P kg⁻¹, and sugarcane averaged 1.5 g P kg⁻¹. Goorahoo et al. (2005) evaluated the nutrition and growth of elephantgrass (*Pennisetum* sp.) on 1.2-ha plots in California and reported an average tissue P concentration of 7 g P kg⁻¹ in a 60-d study. However, the DM yields of 3800 kg ha⁻¹ observed by Goorahoo et al. (2005) were much less than observed here, which could explain the much greater tissue P concentration than what was found here.

Tissue Nitrogen Concentrations

The shorter-growing grasses and elephantgrass were fertilized at 360 kg N ha⁻¹ in 2007 and at 540 kg N ha⁻¹ in 2008, whereas sugarcane plots received 200 kg N ha⁻¹ yr⁻¹ in both years. The relatively high levels of N fertilization recommended by IFAS are typically not used by the majority of producers because of costs. The high N fertilization rates used in this study were intended to maximize DM yield and, consequently, P removal rates. Total tissue N concentrations varied from 7 g N kg⁻¹ for sugarcane to 23 g N kg⁻¹ for guineagrass (Figure 3-7). Similar to tissue P data, tissue N concentrations for the shorter-growing grasses (around 20 g N kg⁻¹) were greater than those for the taller grasses. Elephantgrass tissue N concentrations averaged 16 g N kg⁻¹, and sugarcane averaged 7 g N kg⁻¹. The tissue N concentrations for elephantgrass were similar to values reported by Goorahoo et al. (2005), average tissue N concentration of 20 g N kg⁻¹ during a 60-d growth period.

Phosphorus Uptake

Phosphorus uptake was significantly affected by forage species and varied from 24 kg ha⁻¹ yr⁻¹ for switchgrass to 126 kg ha⁻¹ yr⁻¹ for elephantgrass (Figure 3-8). As observed by Gaston et al. (2003), P uptake varied among species and was not necessarily related to tissue P concentration. Although elephantgrass exhibited lower tissue P concentrations than the

shorter-growing grasses, P uptake was greater because elephantgrass produced greater DM yields than the shorter grasses. Sugarcane, however, did not exhibit greater P removal compared to the shorter grasses. The phytoremediation benefit of high DM yield of sugarcane was undermined by its low tissue P concentrations. Gaston et al. (2003) observed that bahiagrass (*Paspalum notatum* Flugge), bermudagrass [*Cynodon dactylon*(L.) Pers.] and switchgrass (*Panicum virgatum* L.) removed 122, 128, and 146 kg P ha⁻¹ yr⁻¹ P respectively. The P removal rates observed by Gaston et al. (2003) were much greater than those found in our study, possibly due to optimal conditions for DM production and P uptake under greenhouse conditions.

Phosphorus Mass Balance

The overall results from the small plot study were compiled into Table 3-2, including DM yield, P uptake, and gross P change from soil for 10 forage species. Based on the P removal rate, elephantgrass appears to be the more suitable species in removing P from the soils. Roughly 10 yr ($695 \text{ mg kg}^{-1} \text{ observed STP} - 50 \text{ mg kg}^{-1} \text{ target STP} / 67 \text{ mg P kg}^{-1} \text{ yr}^{-1} \approx 10 \text{ yr}$) is the estimated time required to lower the soil test P to the agronomy threshold.

Large Plot Study

Mehlich-1 Phosphorus

Unlike in the small plot study, there was no significant spatial variability associated with M1P concentrations was observed in the large plot study (data not presented). On average, the initial soil-test-P value in the Ap horizons was 232 mg kg⁻¹, which is considered “very high” according to the IFAS soil test interpretation (Mylavarapu and Kennelley, 2002).

Mean Mehlich-1 P concentrations in the Ap horizons decreased from 232 mg kg⁻¹ to 119 mg kg⁻¹ (a 49% decrease) over the 2-yr study period ($P < 0.01$; Figure 3-9A). Sugarcane plots exhibited smaller decrease of M1P values in the Ap horizons than other treatments ($P = 0.03$; Figure 3-10A). This trend is consistent with the small plot study (Figure 3-4A). There

were no differences observed among species in M1P decrease in the Bh and E horizons (Figure 3-10A).

Water-Extractable Phosphorus

Mean WEP values for Ap horizons across all 16 plots decreased from 50 to 24 mg kg⁻¹ from the beginning of 2007 to the end of 2008 ($P < 0.01$; Figure 3-9B). Conversely, significant increases in average WEP values (from 16 to 24 mg kg⁻¹) were observed in the Bh horizons ($P = 0.01$). The simultaneous decreases of WEP values in the Ap horizon and increase of WEP values in the Bh horizon supported the hypothesis that a significant amount of P was leached to deeper soil depths. No significant change in WEP in the E horizon was observed from the beginning to the end of the study, because E horizons have the least P retaining capacity (Brady and Weil, 1999). Sugarcane plots exhibited the smallest decreases in WEP values in the Ap horizon (Figure 3-10B). No grass species effects were observed in the Bh and E horizons.

Crop Yield, Tissue Phosphorus/Nitrogen Concentration and Phosphorus Uptake

As in the small plots, elephantgrass and sugarcane produced greater yields than the other forage species (Figure 3-11). Dry matter yields by elephantgrass, sugarcane and stargrass (Ona) were similar with those observed in the small plot study, and comparable with the literature. Unlike in the small plots, switchgrass survived the 2-yr large-plot study, therefore DM yield by switchgrass in the large plots were greater than those observed in the small plots. Year significantly affected DM yields ($P < 0.01$), and the year by species interaction was significant ($P < 0.01$; Table 3-3). Elephantgrass and stargrass yielded more in 2008 than 2007, likely occurred because more harvests were conducted in 2008. Sugarcane was harvested once per year and DM yields in 2007 and 2008 were similar. More harvests in 2008 did not increase switchgrass DM yields possibly due to poor persistence.

Data from the large-plot study were consistent with the small-plot study and confirmed that tall grasses (elephantgrass and sugarcane) have significantly lower tissue P and N concentrations than other switchgrass and stargrass (Figure 3-12, Figure 3-13).

Phosphorus uptake was significantly affected by forage species. Phosphorus uptake was greater by elephantgrass than the other species (Figure 3-14). Despite high DM yields, P uptake by sugarcane was less than by stargrass, because of low sugarcane tissue P concentrations. Year and the interaction “year x species” significantly affected P uptake (Table 3-3). The species P uptake followed a similar pattern as species DM yield. The overall results from the large plot study are compiled into Table 3-4, including DM yield, P uptake, and soil P change by the four forage species.

In summary, the large-plot study confirmed the findings observed in the small plot study that elephantgrass produced greater DM yields and reduced soil P concentrations. Sugarcane, due to its low tissue P concentration, exhibited P removal capacity similar to the small grasses. The study also confirmed that after the 2-yr study, soil P remained above the agronomic sufficiency level. No P addition was needed to maintain adequate forage production.

Leachate P Concentrations

There were significant differences in mean ortho-P concentrations in the samples collected from the 60- and the 90-cm depth across the sampling dates, where ortho-P concentrations at the 60-cm depth were on average greater than those at the 90-cm depth. ($P < 0.01$; Figure 3-16). At the 60-cm depth, ortho-P concentrations varied from 0.012 to 7.9 mg L⁻¹, and were not affected by sampling dates. Species effects were significant for ortho-P concentrations at the 60-cm depth ($P = 0.01$; Figure 3-17). At the 90-cm depth, ortho-P concentrations ranged from 0.0057 to 6.6 mg L⁻¹, and were not affected by species or by sampling dates. The pattern of ortho-P concentrations at the 60-cm depth appeared inversely

correlated with the pattern of P uptake by the species (Figure 3-14). This suggested that the P uptake by species did reduce the leachate P concentration. In contrast, no relationship between leachate-P and forage P uptake was observed at the 90-cm depth.

Leachate N Concentrations

In 2008, small-grass plots and elephantgrass plots received a total of 540 kg N ha⁻¹ fertilization and sugarcane plots received 200 kg N ha⁻¹. These are the highest levels of N fertilization recommended by IFAS and are greater than those used by forage producers. Such N fertilization levels were chosen to maximize the DM yield and, consequently, P uptake rates. Such N application rates could result in contamination of groundwater, so the impact of high-level N fertilization on N losses was investigated.

Leachate NH₄ concentrations remained relatively low across the sampling period (Figure 3-18). Species and the sampling events had no significant effects on NH₄ concentrations at the 60- and 90-cm depth.

The levels of NO_x at both depths were very high, ranging from 32 to 150 mg L⁻¹ for the 60-cm depth and from 32 to 121 mg L⁻¹ for the 90-cm depth. The NO₃ concentrations observed in this study far exceed the U.S. Environmental Protection Agency drinking water standard of 10 mg L⁻¹. Baker and Johnson (1981) summarized results of a 4-yr study with corn that related the tile drainage NO₃ concentrations to the N fertilization rate. Nitrate concentrations for the plot receiving 45 to 50 kg N ha⁻¹ yr⁻¹ averaged 20 mg L⁻¹; for the plot receiving 120 kg N ha⁻¹ yr⁻¹, the NO₃ concentration was 40 mg L⁻¹. In our project, the N fertilization rate was 540 kg N ha⁻¹ yr⁻¹, four times greater than the high-level rate used by Baker and Johnson (1981), thus the high NO₃ concentrations are expected.

With exception of the data collected in 7 August, 2008 when the field was flooded (Figure 3-15), the NO₃ concentrations at both depths appear to follow a decreasing trend over time (Figure 3-19). However, due to the high variability within the data, statistical analysis

failed to confirm the trend. Species had an effect on NO_3 concentrations at either depth ($P < 0.01$). Switchgrass plots exhibited the greatest NO_3 concentrations of all treatments at the 60-cm depth. This could be partially due to the low DM yield resulting in low N uptake of switchgrass. At the 90-cm depth sugarcane plots had the greatest NO_3 concentrations of all treatments. Sugarcane plots were fertilized once in March 2008 at the 200 kg N ha^{-1} rate, whereas the other treatments received 90 kg N ha^{-1} (elephantgrass plots received 180 kg N ha^{-1}) per harvest, therefore a significant amount of N had leached into deep horizons after the fertilization for the sugarcane plots.

To summarize the water quality data, we observed that ortho-P concentrations in the leachates collected at the 60-cm depth appeared to be related to the P uptake of the species. The more P a species could take up, the greater ability it had to lower the ortho-P in the shallow (60-cm) water. The high N rate of fertilization used in this study led to substantial N leaching, which could cause other issues such as N contamination of the groundwater. Further studies on the long-term effects of N fertilization on N losses are needed.

Table 3-1. Summary of the initial soil test P values across the small plot study area (n = 48)

Soil P	Mean	Std error	Median	Std dev	Minimum	Maximum	Range
	mg kg ⁻¹						
Water-Extractable P	97	4.3	93	30	42	159	117
Mehlich-1 P	695	56	652	389	190	1520	1330

Table 3-2. Average dry matter yield, P uptake and soil P removal from soil for ten forage species in the 2-yr small plot study

Crop	No. of Harvests	Dry matter Yield (Mg ha ⁻¹)	Tissue P conc. (g kg ⁻¹)	P removal (kg ha ⁻¹)	STP decrease [‡] (mg kg ⁻¹)
Bahiagrass	10	22 (±1) [¶] cd [†]	4.4 (±0.1) abc	96 (±5) cd	166 (±34) b
Bermudagrass	10	47 (±2) b	4.1 (±0.1) cd	184 (±7) b	228 (±40) ab
Digitgrass	10	24 (±4) cd	4.7 (±0.2) ab	108 (±13) c	180 (±51) ab
Elephantgrass	6	89 (±4) a	3.0 (±0.2) e	253 (±9) a	160 (±83) b
Guineagrass	10	26 (±8) cd	4.8 (±0.3) a	113 (±33) c	195 (±54) ab
Limpograss	10	24 (±8) cd	4.3 (±0.2) bcd	98 (±28) c	96 (±48) b
Mulato	10	34 (±4) bc	4.3 (±0.2) bcd	128 (±14) c	347 (±61) a
Stargrass	10	33 (±5) bc	4.3 (±0.1) bc	138 (±17) bc	244 (±46) ab
Sugarcane	2	83 (±11) a	1.7 (±0.1) f	128 (±29) c	99 (±62) b
Switchgrass	4	13 (±3) d	3.9 (±0.3) d	47 (±11) d	161 (±70) b

[¶]Values followed by the plus/minus sign represents one standard error.

[†]Means with the same letter in a column are not significantly different (Fisher's LSD test, P<0.05)

[‡]Note: Soil-test-P change was measured by the gross change of Mehlich-1 P from the beginning of 2007 to the end of 2008

Table 3-3. Mean dry matter yields, tissue P concentrations, P uptake by year and by species in the large plot study

Species	Dry matter (Mg ha ⁻¹)		Tissue P conc. (g kg ⁻¹)	P uptake (kg ha ⁻¹)	
	2007	2008		2007	2008
Elephantgrass	35 (±1.4) [¶] a [†]	63 (±1.0) b	2.8 (±0.2) b	102 (±4) a	176 (±6) b
Stargrass	15 (±0.3) a	30 (±0.7) b	4.0 (±0.1) a	64 (±3) a	116 (±4) b
Sugarcane	38 (±1.2) a	35 (±1.2) a	1.8 (±0.1) c	71 (±1) a	58 (±2) a
Switchgrass	15 (±0.3) a	17 (±0.4) a	3.9 (±0.1) a	59 (±1) a	64 (±2) b

[¶]Values followed by the plus-minus sign represents one standard error.

[†]Year means within a row and response variable are not different if followed by the same letter (Fisher's LSD test, P > 0.05)

Table 3-4. Average dry matter yield, P uptake and soil P removal from soil for four forage species in the 2-yr large plot study

Species	Dry Matter Yield (Mg ha ⁻¹)	Tissue P conc. (g kg ⁻¹)	P removal (kg ha ⁻¹)	STP decrease [‡] (mg kg ⁻¹)
Elephantgrass	98 (±1.0) [¶] a [†]	2.8 (±0.2) b	279 (±9) a	125 (±25) a
Stargrass	45 (±0.9) c	4.0 (±0.1) a	180 (±6) b	137 (±18) a
Sugarcane	73 (±1.4) b	1.8 (±0.1) c	129 (±3) c	76 (±13) b
Switchgrass	32 (±0.4) d	3.9 (±0.1) a	123 (±2) c	113 (±29) a

[¶]Values followed by the plus/minus sign represents one standard error.

[†]Means within a column followed by the same letter are not significantly different (Fisher's LSD test, P > 0.05)

[‡]Note: Soil-test-P change was measured by the gross change of Mehlich-1 P from the beginning of 2007 to the end of 2008

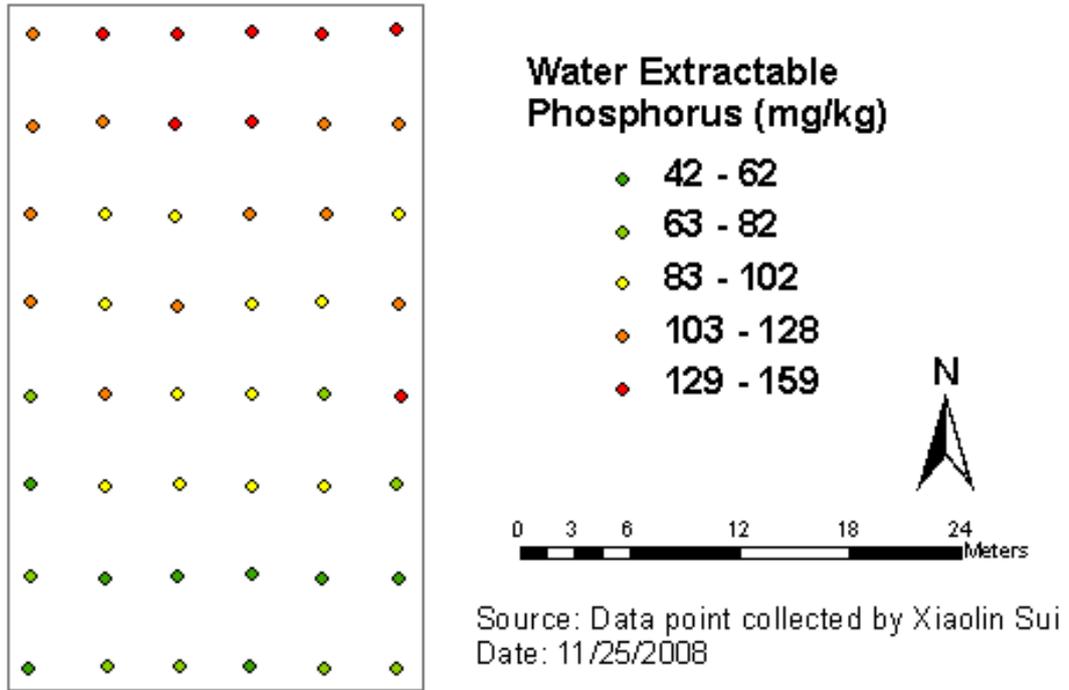


Figure 3-1. Initial water-extractable P concentration in the small-plot study. Map created using ArcMap™ 9.2 software package.

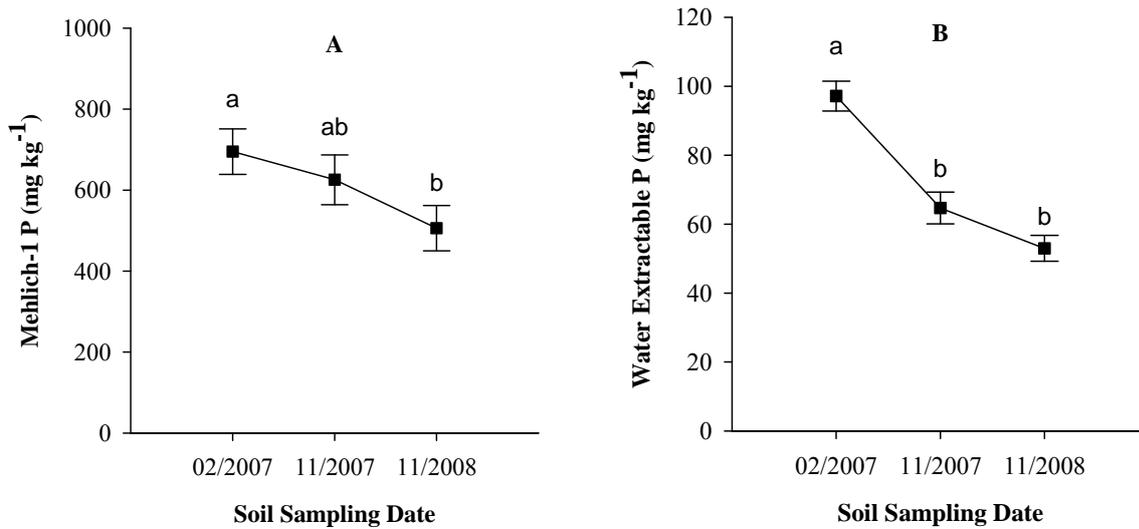


Figure 3-2. Average soil P concentrations in the small plot study over the study period measured as A) Mehlich-1 P and B) Water-Extractable P. Each data point represents the average of 48 samples. Error bars indicate one standard error. Values labeled with the same letters are not statistically different ($P > 0.05$; Fisher's Least Significant Difference).

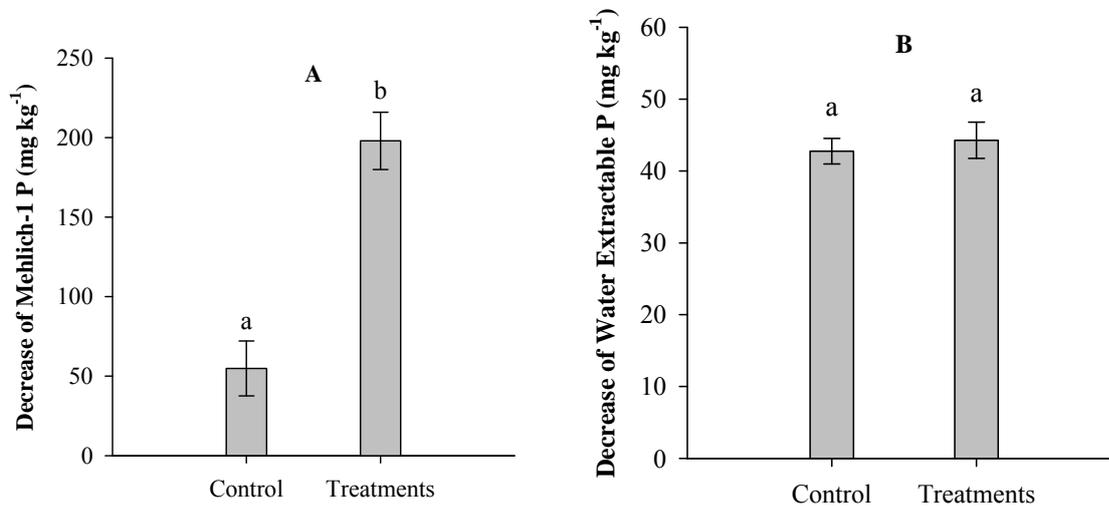


Figure 3-3. Average decreases of soil test P values during the 2-yr study by different treatment groups. A) Decrease of Mehlich-1 P and B) Decrease of Water-Extractable P. Means followed by the same letter are not significant different ($P > 0.05$) (Fisher's Least Significant Difference). Bars represent the average of 3 samples (control) and 45 samples (treatments), respectively. Error bars indicate standard error.

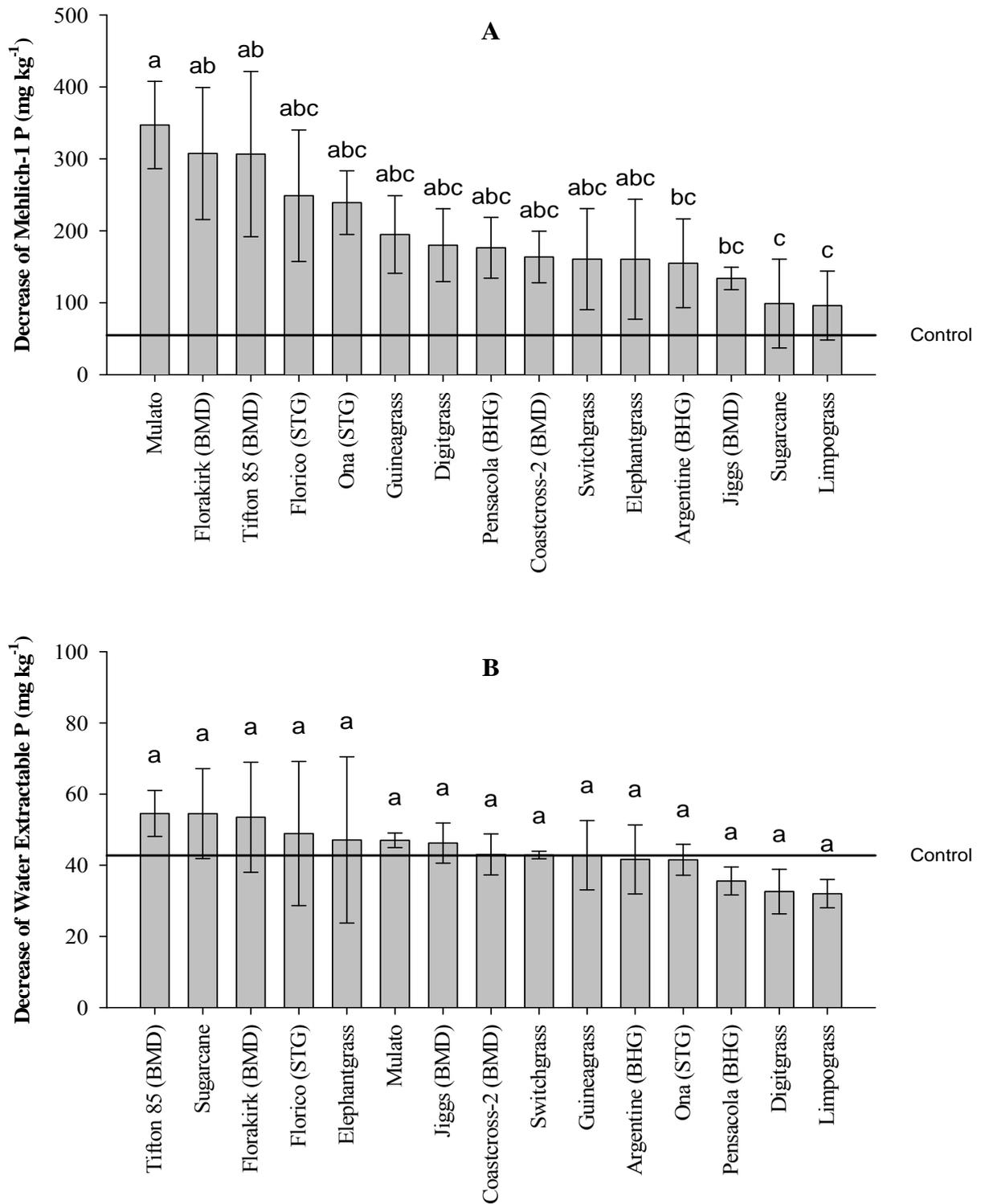


Figure 3-4. Average small plot soil test P decreases after 2 years as affected by 15 forage cultivars. A) Decrease of Mehlich-1 P and B) Decrease of Water-Extractable P. Means followed by same letter are not significantly different ($P > 0.05$). Error bars indicate standard error of the mean within the group. Control lines represent the average decrease of soil P in the control plots.

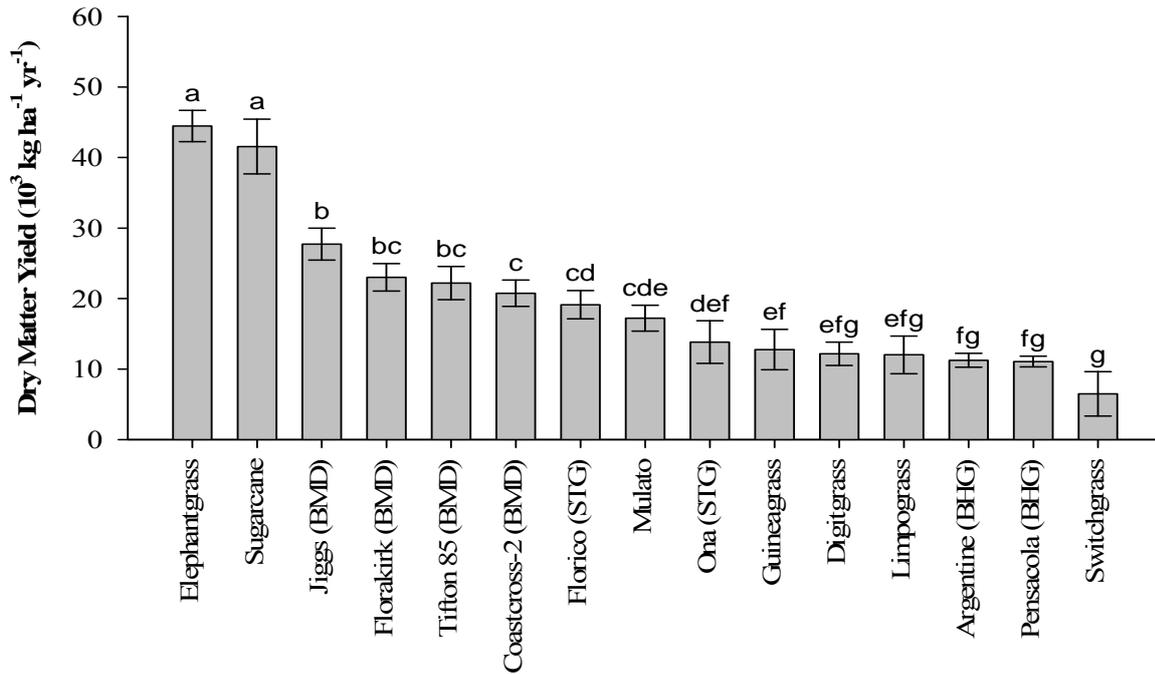


Figure 3-5. Average dry matter yields of 15 forage cultivars in the small plot study. Bars represent the average of all samples collected in all harvests over 2 yr. Means followed by same letter are not significantly different ($P > 0.05$). Error bars indicate one standard error of the mean within the group.

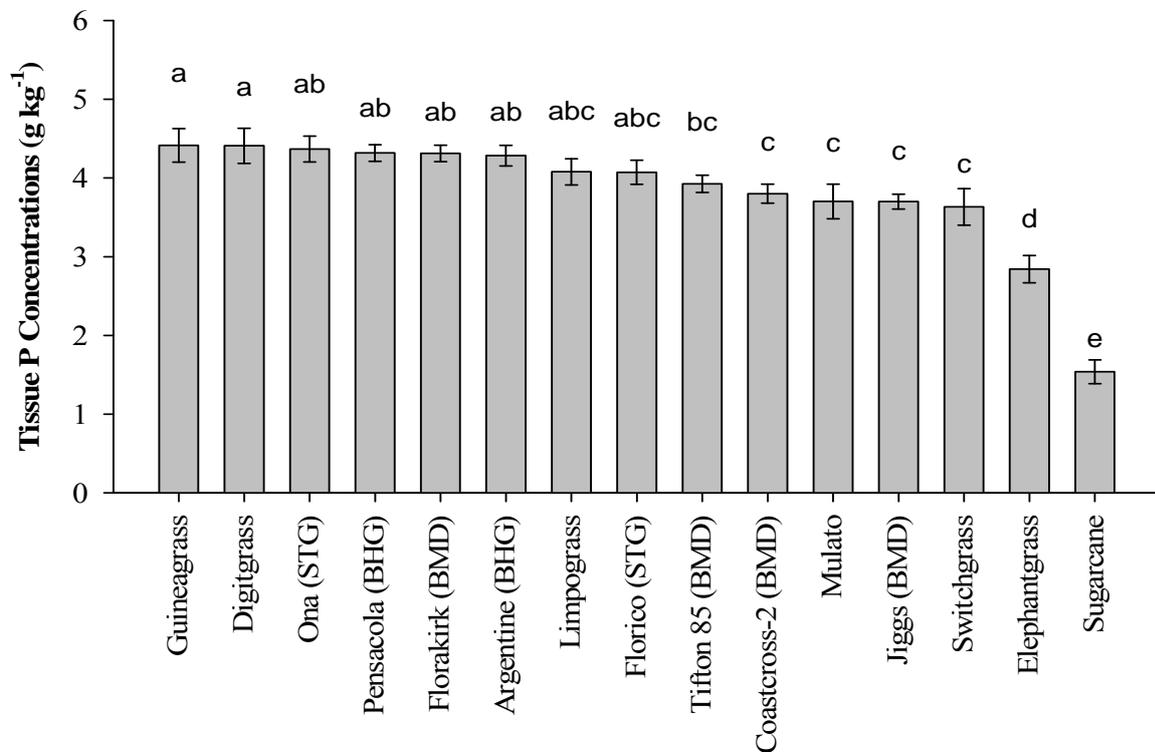


Figure 3-6. Tissue total Kjeldahl P of 15 forage cultivars. Bars represent DM-weighted average TKP of all samples collected in all harvests over 2 yr. Means followed by same letter are not significantly different ($P > 0.05$). Error bars indicate one standard error of the mean within the group.

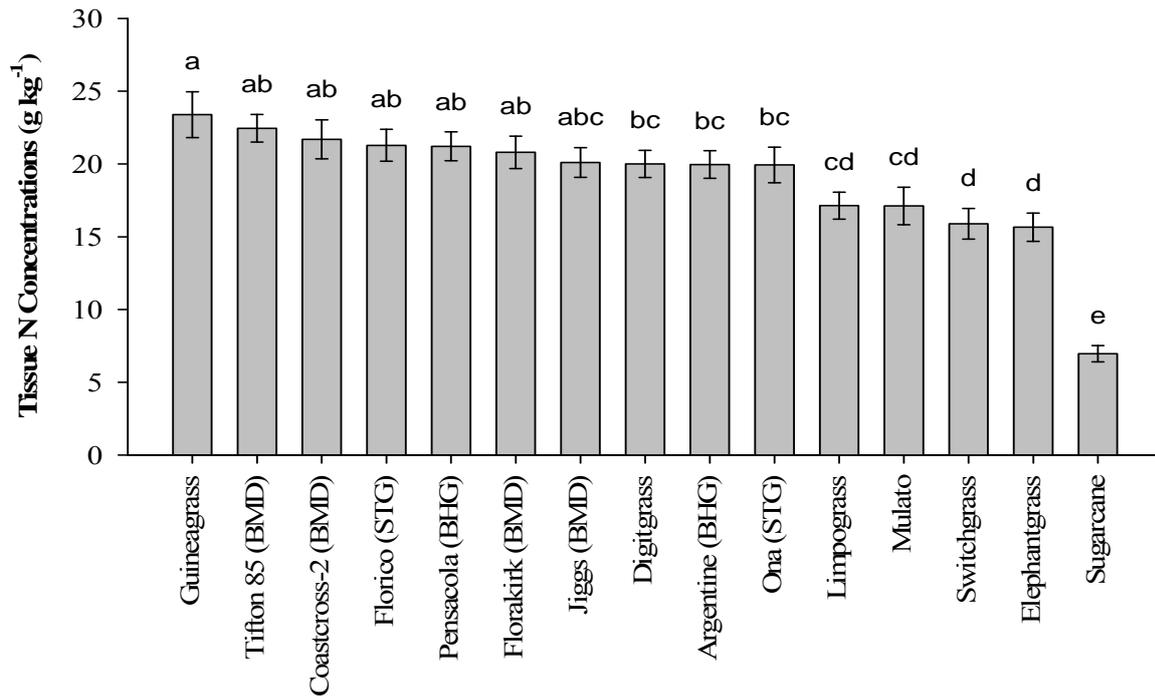


Figure 3-7. Tissue total Kjeldahl N concentrations of 15 forage cultivars in the small plot study. Bars represent DM-weighted average TKN of all samples collected in all harvests over 2 yr. Means followed by same letter are not significantly different ($P > 0.05$). Error bars indicate one standard error of the mean within the group.

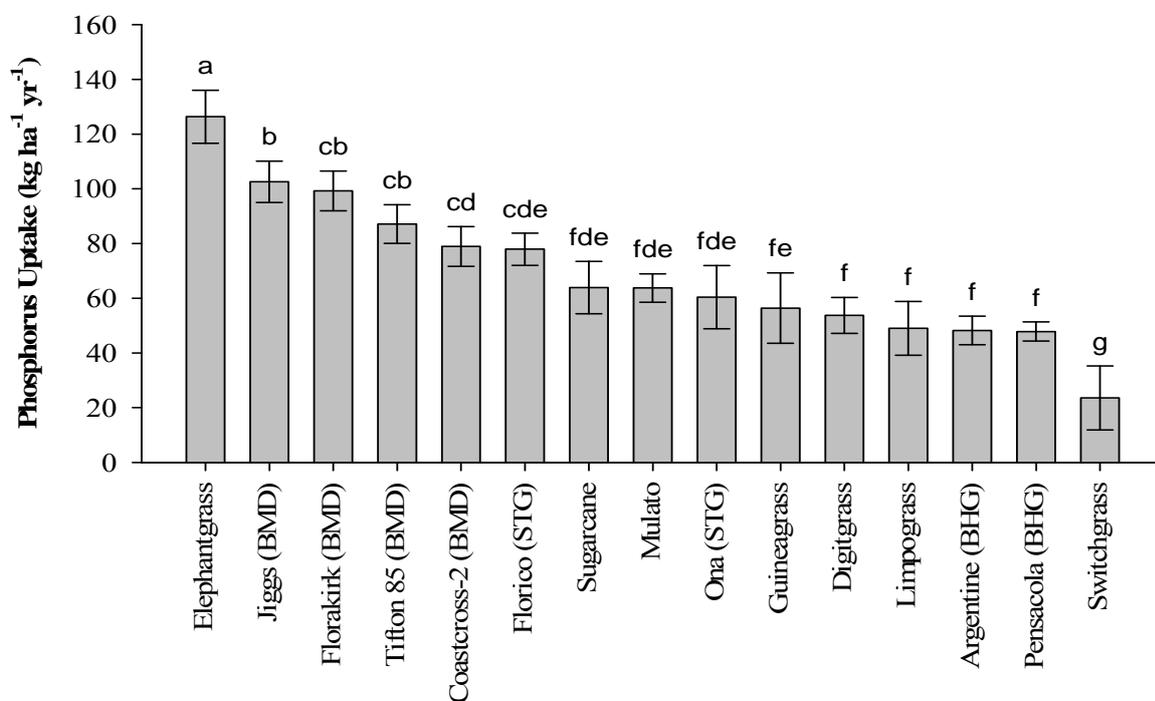


Figure 3-8. Phosphorus uptake by 15 forage cultivars in the small plot study. Bars represent the average of all samples collected in all harvests. Means followed by same letter are not significantly different ($P > 0.05$). Error bars indicate one standard error of the mean within the group.

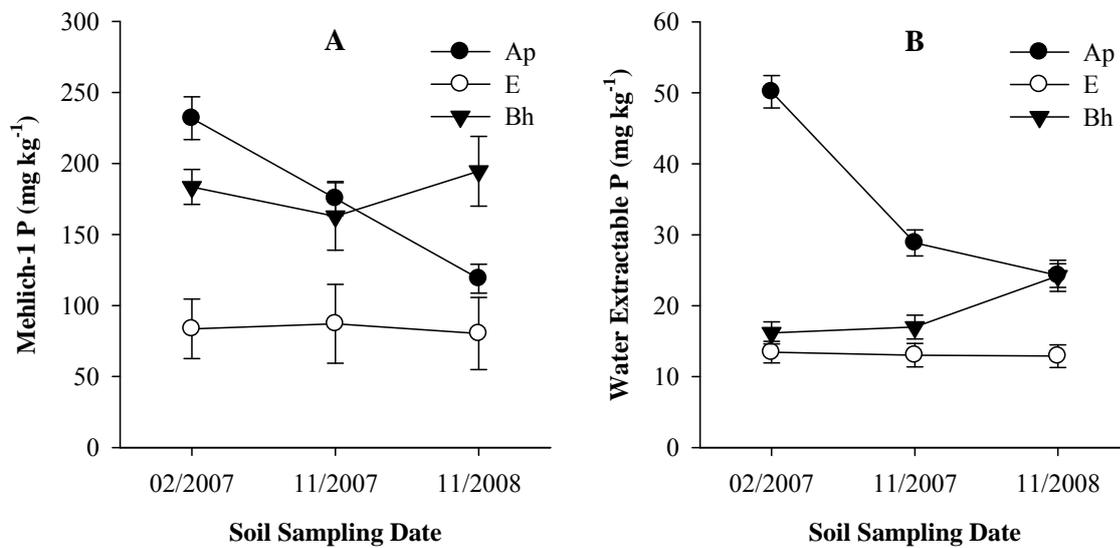


Figure 3-9. Average soil P concentrations in the large plot study across the study period measured as A) Mehlich-1 P and B) Water-Extractable P. Each date point represents the average of 16 samples. Error bars indicate one standard error.

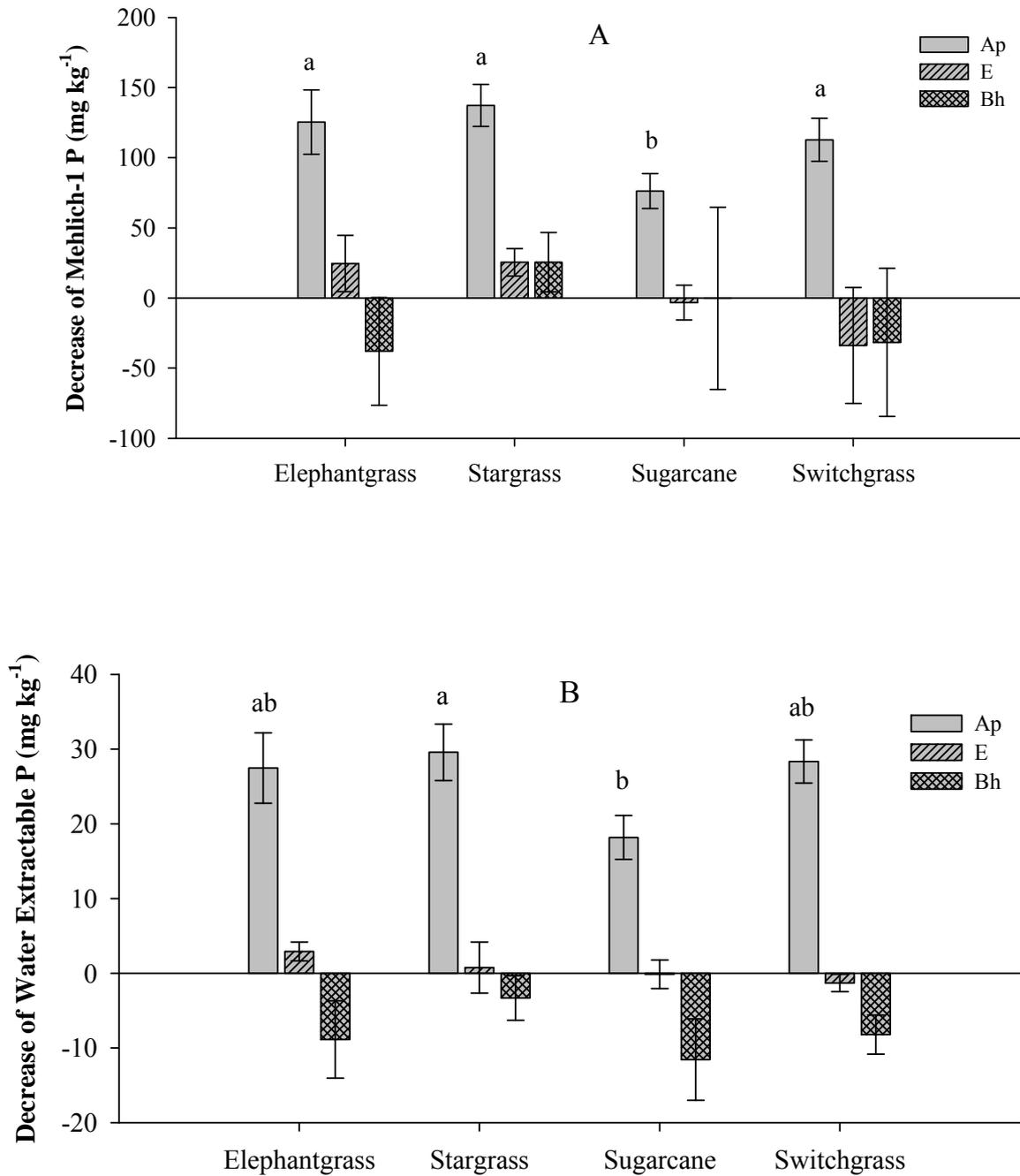


Figure 3-10. Average soil test P decrease in different horizons after the 2-yr large plot study as affected by species. A) Decrease of Mehlich-1 P and B) Decrease of Water-Extractable P. Means followed by same letter are not significantly different ($P > 0.05$) in Ap horizon. There were no significant differences in Bh and E horizons ($P > 0.05$). Error bars indicate standard error of the mean within the group.

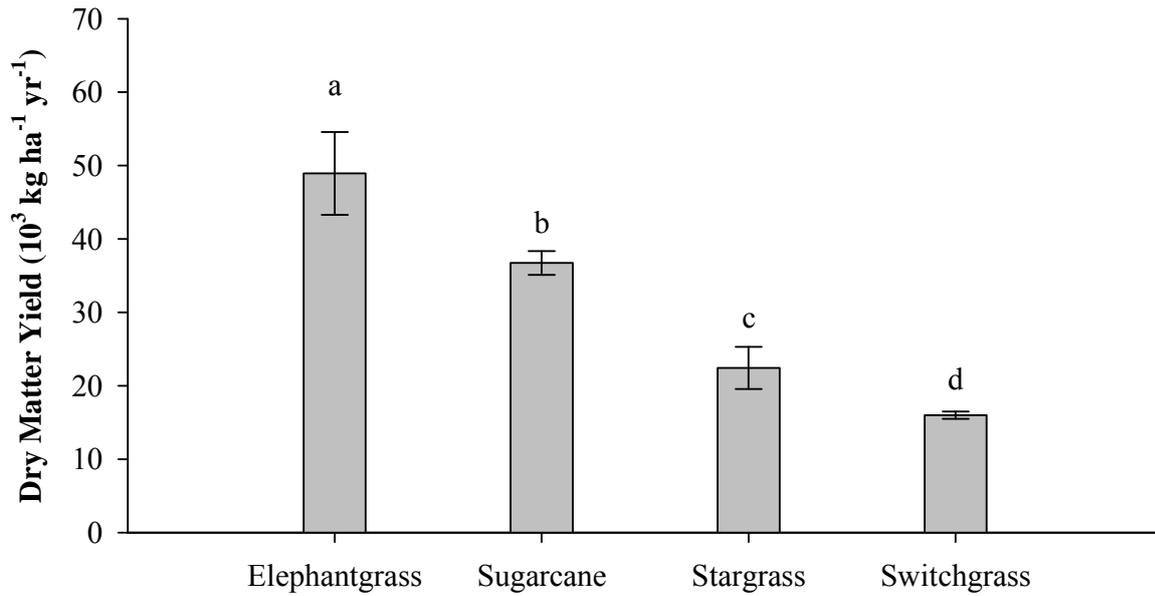


Figure 3-11. Average dry matter yields for the four treatments in the large plot study. Bars represent the average of all samples collected in all harvests over 2 yr. Means followed by same letter are not significantly different ($P > 0.05$). Error bars indicate one standard error of the mean within the group.

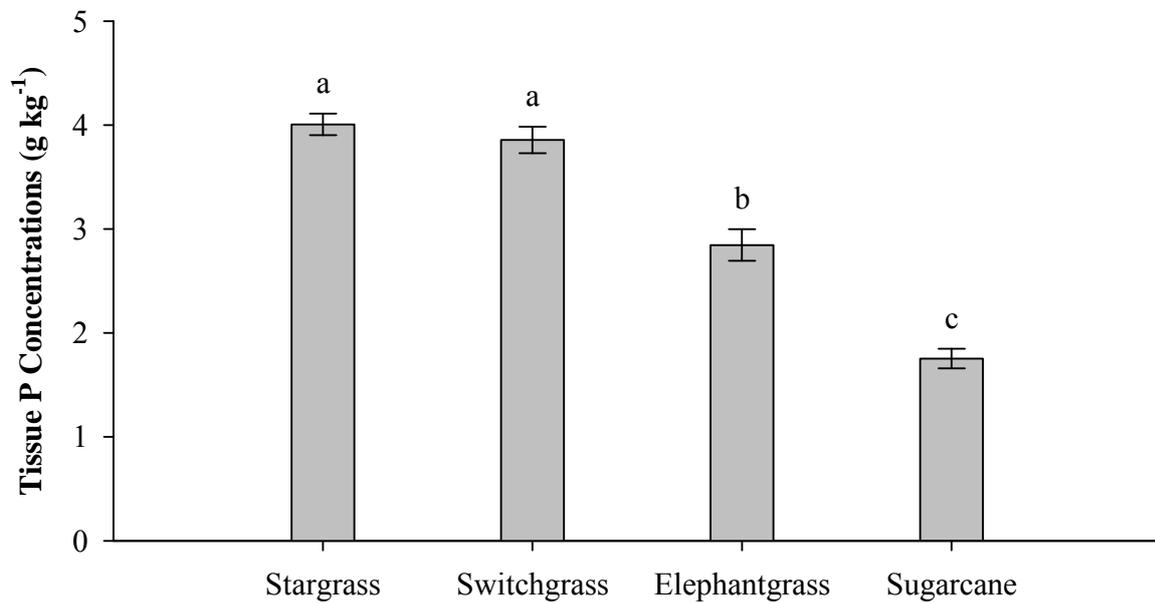


Figure 3-12. Tissue Total Kjeldahl P concentrations of the four forage cultivars in the large plot study. Bars represent DM-weighted average TKP of all samples collected in all harvests over 2 yr. Means followed by same letter are not significantly different ($P > 0.05$). Error bars indicate one standard error of the mean within the group.

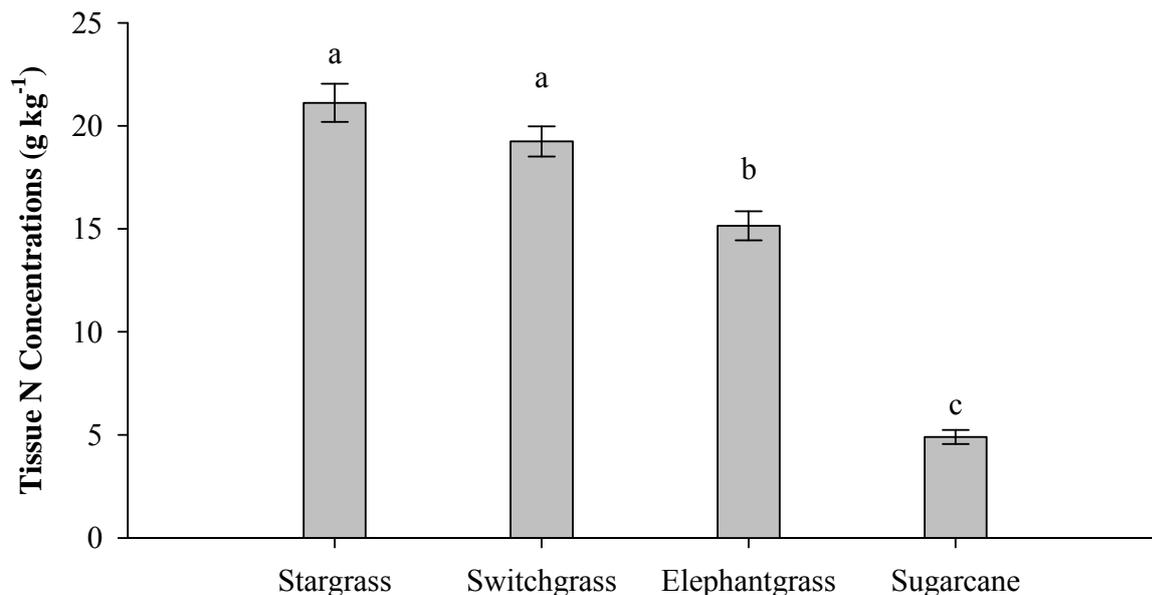


Figure 3-13. Tissue Total Kjeldahl N concentrations of the four forage cultivars in the large plot study. Bars represent DM-weighted average TKN of all samples collected in all harvests over 2 yr. Means followed by same letter are not significantly different ($P > 0.05$). Error bars indicate one standard error of the mean within the group.

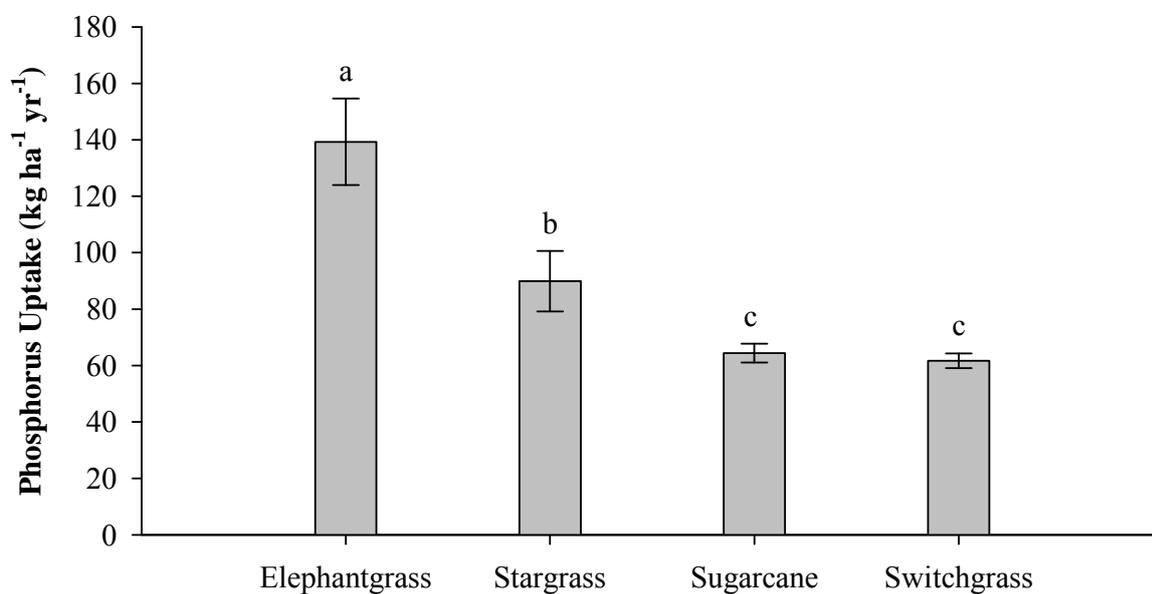


Figure 3-14. Phosphorus uptake values for the four forage cultivars in the large plot study. Bars represent the average of all samples collected in all harvests over 2 yr. Means followed by same letter are not significantly different ($P > 0.05$). Error bars indicate one standard error of the mean within the group.

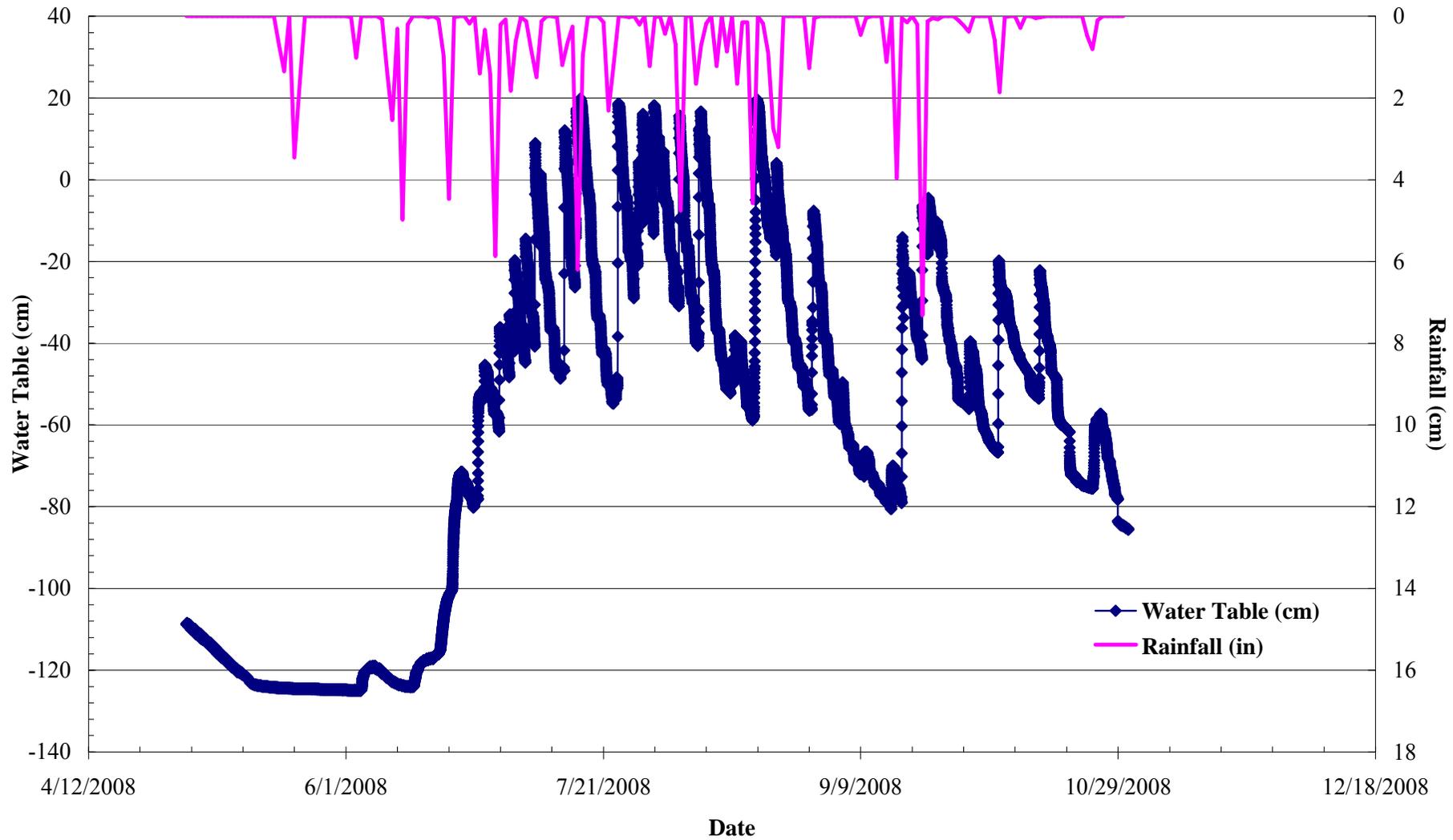


Figure 3-15. Precipitation and water-table depth during the growing season of 2008.

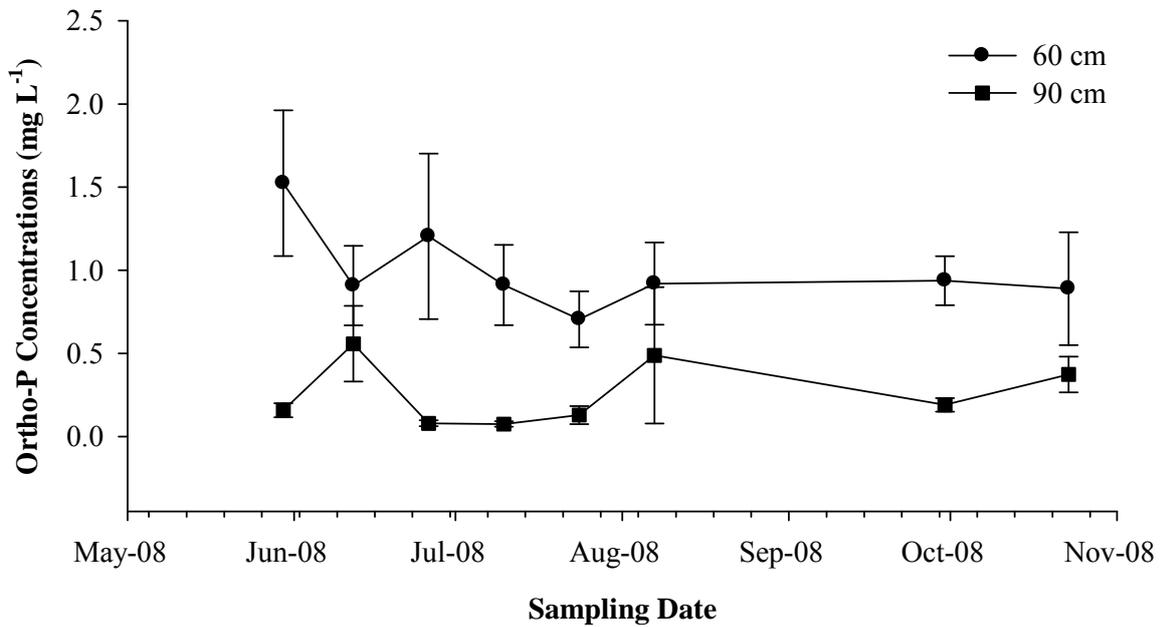


Figure 3-16. Average leachate ortho-P concentrations at two depths collected across the growing season in the large plot study. Each data point represents 16 samples collected in every sampling event. Error bars indicate one standard error of the mean within the group. Differences among sampling events are not significant for both depths ($P > 0.05$).

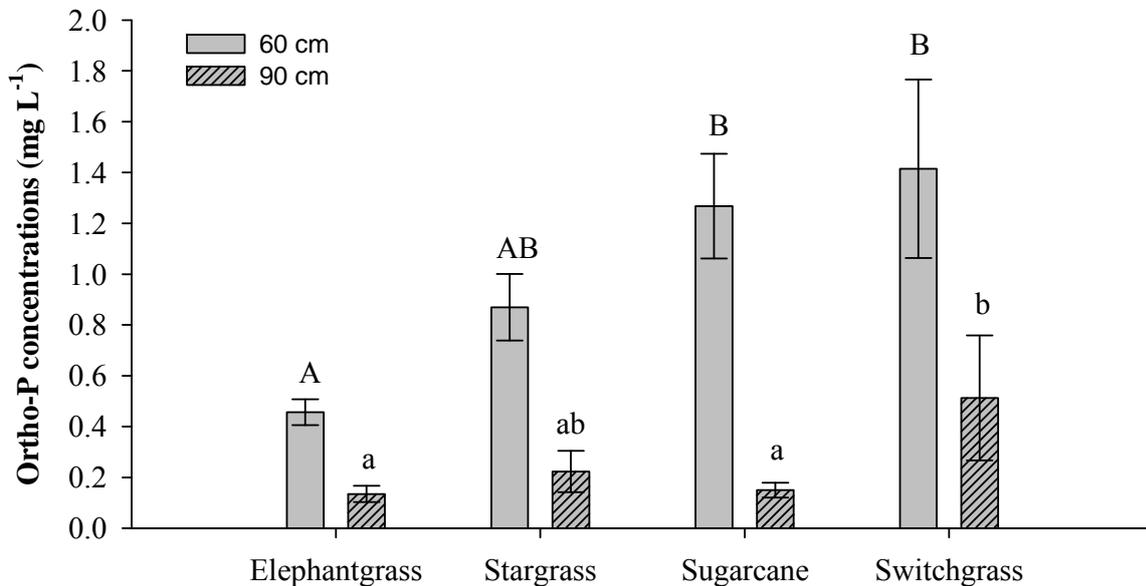


Figure 3-17. Average leachate ortho-P concentrations at the 60- and 90-cm depths for different treatments in the large plot study. Bars represent the mean ortho-P concentrations of all sampling dates for each treatment. Means followed by same letter are not significantly different in each depth ($P > 0.05$). Error bars indicate one standard error of the mean within the group.

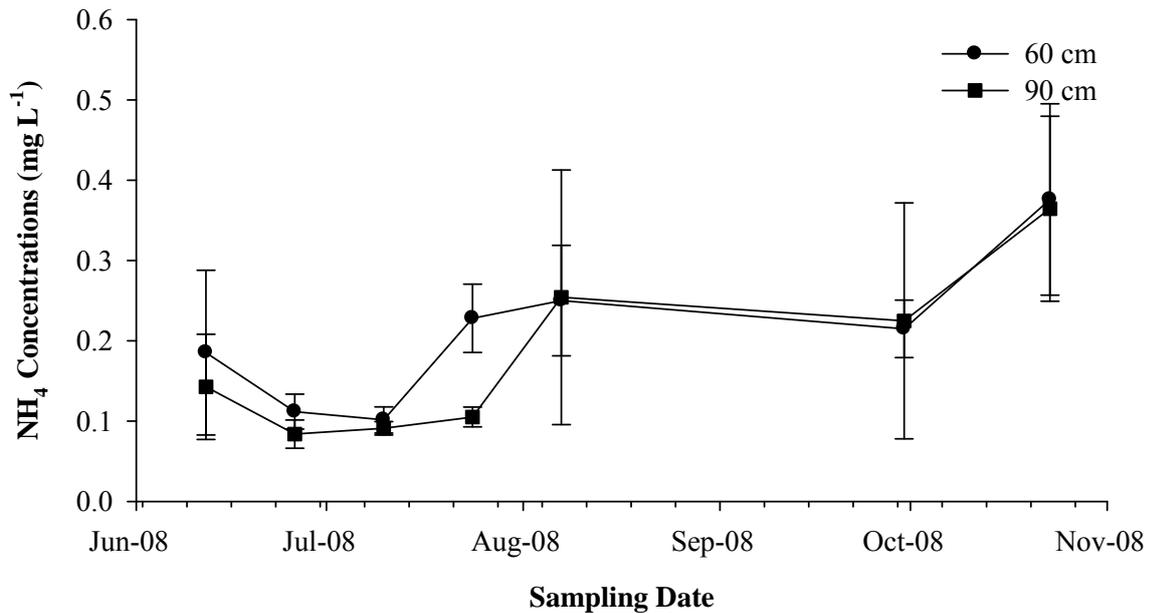


Figure 3-18. Average leachate ammonium concentrations at two depths collected across the growing season in the large plot study. Each data points represents 16 samples collected in every sampling event. Error bars indicate standard error of the mean within the group. Differences among sampling events are not significant for either depth ($P > 0.05$).

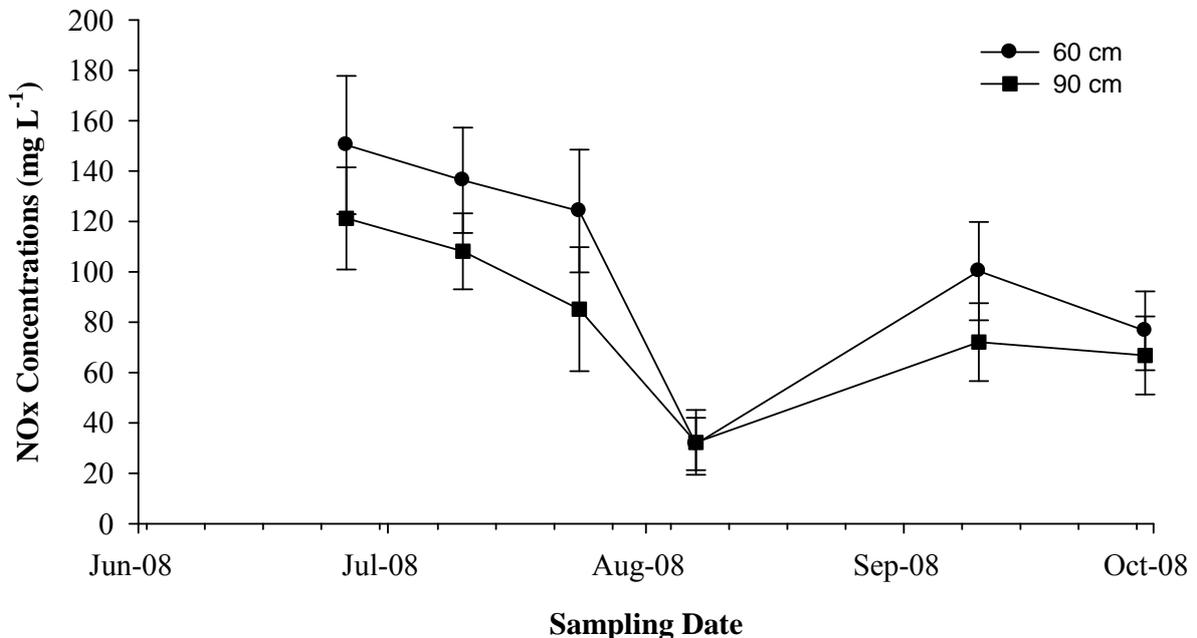


Figure 3-19. Average leachate nitrate concentrations at two depths collected across the growing season. Each data points represents 16 samples collect in every sampling event. Error bars indicate standard error of the mean within the group. Differences among sampling events are not significant for both depths ($P > 0.05$).

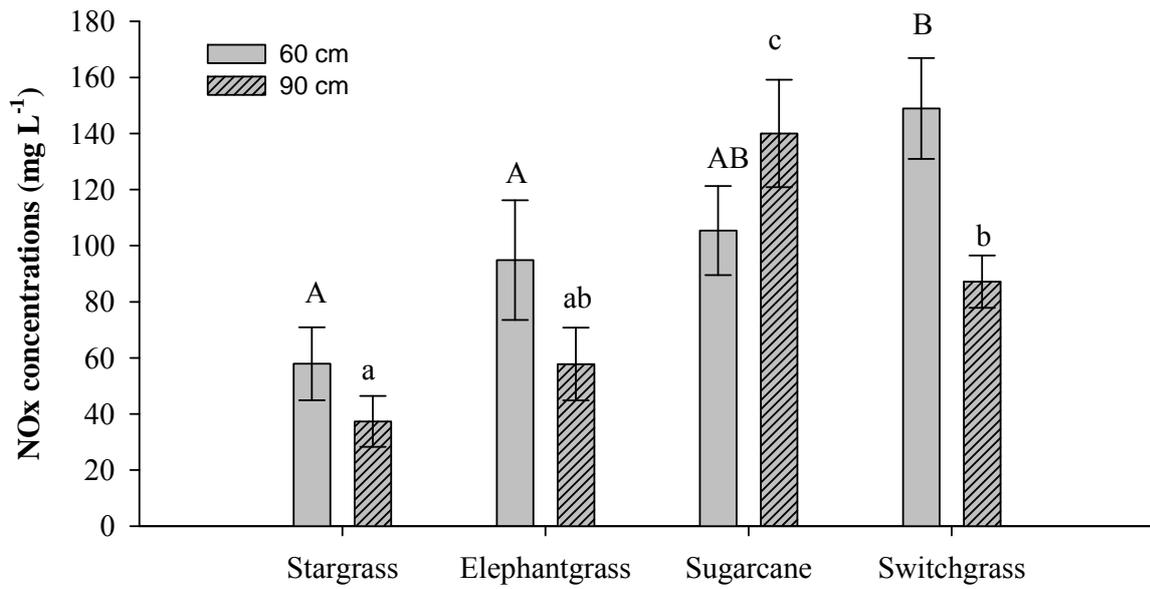


Figure 3-20. Average leachate nitrate concentrations at 60- and 90-cm depths for different treatment in the large plot study. Bars represent the mean ortho-P concentrations of all sampling dates for each treatment. Means followed by same letter are not significantly different at each depth ($P > 0.05$). Error bars indicate one standard error of the mean within the group.

CHAPTER 4 DISCUSSION

Soil-Phosphorus Decrease, Phosphorus Uptake and Phosphorus Leaching

Results of the project indicated that P uptake only accounts for part of the soil-test-P decrease (Table 3-2). Poor mass balance was observed between crop P uptake and soil P decrease (15 - 79% of P uptake relative to decrease of soil test P). Similar results have been reported in the literature. For instance, Brown (2006) studied the winter forage-corn silage system and found that P uptake only accounted for half of the soil P decrease. The authors suggested that the natural P-sorption processes also contributed to reduction in soil-test-P concentrations. Similarly, McDowell and Sharpley (2001) studied the loss of P in subsurface flow from three cultivated soils [Alvira (*Aeric Fragiaquult*), Berks (*Typic Dystrudept*) and Watson (*Typic Fragiudult*) channery silt loams]. They suggested that poor P balance could be induced due to the large amount of P lost by leaching. Nair et al. (1995) studied soil P fractions of dairy systems in South Florida's Lake Okeechobee watershed. They that no major shifts in labile P (defined as 1 M NH₄Cl-extractable P) to more stable soil P forms were observed even after the manure-impacted soil had been abandoned for 12 yr. Therefore, given the soil and environmental conditions where our study was conducted, it is reasonable to believe that P leaching rather than shifts in P forms in the soil is main reason for the poor mass balance observed in our study.

The simultaneous decrease of WEP in Ap horizons and increase of WEP in Bh horizons in the large plots further supported the hypothesis that significant amounts of P were leached to deeper soil depths. The leaching of labile P from P-impacted surface soil has been well documented in the literature. Phosphorus leaching through sandy soils receiving excessive amounts of manure was also observed by (Novaket al., 2000). Heckrath et al. (1995) found that P leached increased rapidly when soil test P exceeded a certain point (60 mg Olsen P kg⁻¹ in their study), which they called the “change point”. Their model suggested that as

soil-test-P concentrations increase, high-energy sorption sites become increasingly saturated, reducing a soil's overall P-retaining strength and increasing the P-leaching potential.

Although in our project we did not measure the change point, the extremely high soil-test-P values at the research location suggest that P was likely subject to leaching.

Dry Matter, Tissue Phosphorus Concentrations, and Phosphorus Uptake

The effectiveness of phytoremediation depends on the total P removed from the target field, thus it is important to increase the DM yield or the tissue P concentrations or both (Novak and Chan, 2002). Results in the projects show that DM yields, tissue P concentrations and P uptake differed among the forage species. Tall grasses yielded greater DM but had lower tissue P concentrations than short grasses. Thus, elephantgrass took up more P than other forages because of its high DM yield. Phosphorus uptake by sugarcane was similar to that of the short grasses due to the low sugarcane tissue P concentrations. It appeared that switchgrass was not adapted to intermittent high water table conditions commonly found during the summer months in south Florida (Figure 3-15) and was not persistent with weeds.

Other factors not fully tested in this project, such as soil drainage, planting timing, row spacing, weed control, fertilization and harvest interval could also affect potential DM yields of forage crops (Stricker, 2003). For example, wider row spacing would result in lower DM yield of tall grasses and sugarcane, while narrower spacing increase stand density resulting in higher DM yield (Stricker, 2003). Though the study of most of the parameters was out of scope of the project, but fertilization management was considered.

Guevara et al. (2000) studied the effect of N fertilization on forage yields in Midwest Argentina and found that each kilogram of N applied (at rate of 25 kg N ha⁻¹) increased rangeland forage production by 12.4 kg. Andrade et al. (2000) studied the effects of N and K fertilization on elephantgrass (cv. Napier) yields in an incomplete factorial design (N rates of 20, 50, 100, 200, 300, 350 and 380 kg ha⁻¹ x K rates of 16, 40, 80, 160, 240, 280 and 304 kg

ha⁻¹). Elephantgrass biomass yields increased 86% in response to N and K fertilizers. Because DM yield is critical for phytoremediation and bioenergy production, continuous N fertilization could be required, which would be expensive and may increase the risk of N leaching into groundwater.

In this project, no hyperaccumulator of P was identified. Short grasses had higher tissue P concentrations (around 4 g P kg⁻¹) than tall grasses (3 g P kg⁻¹ for elephantgrass, 1.6 g P kg⁻¹ for sugarcane). Novak and Chan (2002) suggested that breeding approach could be used to increase the tissue P concentration in DM of plants. For example, Delhaize and Randall (1995) found that a mutant strain of *Arabidopsis thaliana* (L.) could accumulate as high as 14.5 g P kg⁻¹ of DM in leaves, whereas a normal strain accumulated 6.8 g P kg⁻¹. However, no study has been conducted to validate the adaptability of such mutant strains to the field conditions.

Although tissue P concentration is important in terms of phytoremediation, in the two-fold use of forage crops to remediate P-impacted soils and to produce bioenergy, DM yield is more important. The more DM a forage can produce, the more P can be taken up and the more biomass available for energy production. The results this project suggest that elephantgrass can be the most suitable species for both P remediation and bioenergy purposes.

Ground Water Effects

The surface soils of the Lake Okeechobee watershed are mainly sands, with very low nutrient retention capacity. Water table fluctuated between the subsurface horizon and the soil surface for extended periods each year (Graetz and Nair, 1995). Thus, there is significant potential for subsurface nutrient movement when groundwater carries nutrients vertically or laterally.

High N-fertilization rates were used in the project to promote high DM yield. However, results suggested that such high N rates lead to substantial N leaching to the shallow groundwaters. Therefore, further studies are needed to address the impacts of N fertilization on water quality and DM yield.

Forage species showed different capacities to accumulate nutrients and reducing nutrient leaching. Ortho-P concentrations in the leachates collected at the 60-cm depth appeared to be inversely related to the P uptake of the species. Elephantgrass took up more P than other species and reduced the ortho-P concentrations more than the other species. Species effects were also significant on leachate NO₃ concentrations. Switchgrass plots showed highest leachate NO₃ concentrations, possibly due to low N-uptake by switchgrass. Further studies are needed to determine long-term effects of forage grasses on the groundwater quality.

CHAPTER 5 CONCLUSIONS

The phytoremediation of P-impacted soils may require a long time to reduce soil P concentrations to acceptable levels. Despite the smaller cost associated with establishment and maintenance of phytoremediation schemes compared to other remediation approaches, phytoremediation represents a costly alternative for the majority of the beef cattle producers. Thus, the two fold use of selected forage crops for both the phytoremediation of P-impacted soils and the use of resulting biomass as a renewable energy source represent a potentially feasible alternative.

Results from the 2-yr study showed a decline in Mehlich-1 P in the surface horizon in response to crop uptake. Significant amounts of P were leached from Ap horizon to the subsurface horizon.

Forage species differed in DM yield and P uptake potential. Among the shorter grasses, DM yield ranged from 11 to 28 Mg ha⁻¹ yr⁻¹, and P uptake rate ranged from 48 to 102 kg ha⁻¹ yr⁻¹. Elephantgrass resulted in greater DM yield (49 Mg ha⁻¹ yr⁻¹) and P uptake (139 kg ha⁻¹ yr⁻¹) than the other crops. Although sugarcane DM yields were greater than the shorter grasses, lower tissue P concentrations resulted in overall P uptake (64 kg ha⁻¹ yr⁻¹) that was similar to the shorter grasses.

Forage species significantly affected water ortho-P concentrations at the 60-cm depth. Elephantgrass, due to its high P uptake, resulted in lower water ortho-P concentrations than other species. On the other hand, NO₃ analysis suggested that high N rate of fertilization promoted substantial N leaching into the subsurface horizons, which could cause other issues such as N contamination of the groundwater.

The first hypothesis of this project that soil P concentrations decrease over time in response to P uptake of forage crops was accepted (in the small plot study). The second hypothesis that P removal capacity differs among grass species was also accepted.

Elephantgrass exhibited the greatest annual DM yield and P uptake. Sugarcane produced greater DM yields, yet its low tissue P concentration undermined its P-uptake capacity.

There was no conclusive evidence to support and/or reject the third hypothesis that groundwater quality (in terms of P concentrations) improves in response to P uptake of forage crops. Shallow groundwater P concentrations were less in the elephantgrass plots than other treatments, but water sampling was conducted for only one year. Additional study is necessary to investigate the long-term effects of forage uptake on water quality.

An evaluation of the conversion efficiency of the various forage species into bioenergy production was not part of this study. We assumed that DM yield was a good indicator of the amount of bioenergy producible. Thus, we conclude that elephantgrass is the most appropriate species to phytoremediate P-impacted soils as well as to produce bioenergy. One concern with the growth of elephantgrass is the large amount of N required to maintain adequate DM yields, and possible environmental degradation associated with large N losses. Therefore, long-term studies evaluating the impacts of N fertilization on elephantgrass DM yield, P removal potential, environmental impacts and net energy balance are critically needed.

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BIOGRAPHICAL SKETCH

Xiaolin Sui was born in 1984 a small city in China. Xiaolin received his Bachelor of Science in environmental science in May 2007 at Nankai University of China. After he graduated from college, he decided to dig further in the area of environmental science. In August 2007, Xiaolin joined the Soil and Water Science Department at University of Florida and started his graduate study under the supervision of Dr. Silveira. Most of his research was conducted at Range Cattle Research and Education Center. His master's project focused on the phytoremediation of P-impacted soils. Xiaolin received his Master of Science degree in August 2008.