CHARACTERIZING THE LONG-TERM LABILITY OF BIOSOLIDS-PHOSPHORUS

By

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To my Dad whom GOD gave a steadfast commitment to family, abundant patience, and most importantly, a sense of humor so that he could endure my time in school.

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LIST OF ABBREVIATIONS

Al	Aluminum
AN	Ammonium nitrate
Biosolids-P	Biosolids-phosphorus
BPR	Biological phosphorus removal
Ca	Calcium
CRD	Completely randomized design
EC	Electrical conductivity
EPA	Environmental Protection Agency
FDEP	Florida Department of Environmental Protection
Fe	Iron
GLM	General linear model
GRU	Gainesville Regional Utility
K	Potassium
Mg	Magnesium
MCP	Monocalcium phosphate
N	Nitrogen
NRCS	National Resource Conservation Service
OCUD S	Orange County south
P	Phosphorus
PAN	Plant available nitrogen
PSI	Phosphorus saturation index
PSR	Phosphorus saturation ratio

PWEP	Percent water-extractable phosphorus
QA/QC	Quality assurance/quality control
RPA	Relative phosphorus adsorption
RPP	Relative phosphorus phytoavailability
RPL	Relative phosphorus overall lability
SAS	Statistical analysis software
SRP	Soluble reactive phosphorus
S	Sulfur
TC	Total carbon
TN	Total nitrogen
TP	Total phosphorus
TSP	Triple super phosphate
USEPA	United States Environmental Protection Agency
WEP	Water-extractable phosphorus

Abstract of Thesis Presented to the Graduate School
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Land-application is a critical disposal route for the biosolids generated by many municipalities and provides farmers with numerous agronomic benefits. However, heightened concern that biosolids land-application can potentially accelerate cultural eutrophication of surface waters threatens to limit land-based biosolids recycling programs. Previous short-term studies successfully distinguished the phosphorus (P) release characteristics of biosolids from highly soluble inorganic fertilizer sources, and illustrated how various biosolids have vastly different P release characteristics. However, the studies did not address the long-term lability of biosolids-phosphorus (biosolids-P). A prolonged (16-month) greenhouse study using large columns was conducted to characterize the long-term phytoavailability and environmental lability of biosolids-P (collectively referred to as overall P lability) and to provide understanding of the ultimate fate of biosolids-P. Seven biosolids and triple super phosphate (TSP) were used as P-sources. The biosolids selected represent a wide-range of P-solubility—percent water extractable-P (PWEP) values ranging from 0.58 to 47%.

Sources of P were applied to a P-deficient, low P retention capacity Immokalee fine sand at three application rates: 56 kg P ha^{-1} (P-based rate), 112 kg P ha^{-1}, and 224 kg P ha^{-1} (N-based rate). Bahiagrass (*Paspalum notatum* Flugge) grew continuously for 498 days after planting.

Bahiagrass tissue was harvested every 4-8 weeks to characterize P uptake, and columns were leached immediately after each tissue harvest to characterize environmental P lability. Less soluble-P biosolids (PWEP ≤ 8.4%) were significantly less environmentally labile than biological P removal (BPR) biosolids, BPR-like biosolids, and TSP. The environmental lability of BPR and BPR-like biosolids-P was similar to TSP-P. The relative P phytoavailability (RPP) of less soluble-P biosolids was ~40-80% that of TSP, but BPR and BPR-like biosolids were 98-131% as phytoavailable as TSP. Less soluble-P biosolids pose significantly less environmental P risk than BPR and BPR-like biosolids, and TSP. Biosolids application rates should account for the relative P phytoavailability of less soluble-P biosolids, but no P-based application rate adjustment is warranted for BPR and BPR-like biosolids. Data from the greenhouse study suggest that the ultimate lability of less soluble biosolids-P is less than TSP-P, but BPR and BPR-like biosolids-P is ultimately as labile as TSP-P. A correlation of overall P lability to the labile P load (biosolids P saturation index x total-P load) suggests that biosolids P saturation index (PSI) is a useful *a priori* indicator of overall biosolids-P lability. Biosolids PSI can also provide a useful indication of the relative phytoavailability (RPP) of biosolids-P.

CHAPTER 1
INTRODUCTION

Land-based biosolids recycling programs are critical to municipalities. Wastewater treatment produces 6.5 x 10^6 dry Mg of biosolids annually in the US, and production is expected to increase to 8.2 x 10^6 Mg by 2010 (USEPA, 1999; Epstein, 2003). Traditionally, municipalities relied on three main biosolids disposal options: land application, landfill disposal, and incineration (Elliott et al., 2007). Curtailing of biosolids landfill disposal—mostly due to high costs and limited available landfill capacity—is occurring in many states (Epstein, 2003), although there appears to be a resurgence in land-filling in other states (Elliott et al., 2007). New Jersey currently allows biosolids landfill disposal only under emergency conditions (Elliott et al., 2007). Incineration is constrained by high operating and capital costs, and air quality concerns. The limitations of land-fill disposal and incineration are prohibitive to many municipalities, and emphasize the importance of maintaining viable land-based recycling programs. Converting municipal wastes into biofuels is an increasingly attractive disposal option (Champagne, 2007). Municipalities are often attracted to processes used to convert biosolids into biofuels because of advantages unrelated to energy production. A deep-well biosolids injection project in Los Angeles, California reduced political opposition from communities that might receive biosolids because municipal wastes are treated and managed onsite (Attai et al., 2008).

Land application is the most common biosolids disposal route utilized by municipalities. Fifty to sixty percent of biosolids produced annually in the US are land applied (Epstein, 2003). Florida land applies 66% of the more than 3.0 x 10^5 dry Mg of biosolids produced annually (FDEP, 2007). Land application is federally regulated under the EPA Title 40 CFR Part 503 rule, and extensive risk assessment documents the safety of biosolids land application practices conducted in accordance with existing regulation (USEPA, 1993a; USEPA, 1995; NRC, 2002;

Moss et al., 2002). Land applying biosolids provides agronomic benefits, making land application programs mutually beneficial to farmers and municipalities. The organic matter in biosolids (most biosolids are ~50-80% organic matter) increases soil cation exchange capacity, and beneficially affects soil physical properties such water retention, porosity, and bulk density (Moss et al., 2002). Biosolids contain macronutrients, micronutrients, and trace elements, and are useful as low grade fertilizers (Elliott and O'Connor, 2007). Low costs and agronomic benefits make biosolids an economically favorable alternative to inorganic fertilizers.

Nutrients are the major benefit to farmers involved in land-based recycling programs, yet paradoxically, nutrient concerns are the primary threat to biosolids land application (Shober and Sims, 2003; Brandt et al., 2004; Elliott and O'Connor, 2007). Biosolids are generally applied to meet crop nitrogen (N) requirements in accordance with the EPA part 503 rule (USEPA, 1993a), which restricts application rates exceeding agronomic N demands. Applying biosolids at N-based rates generally exceeds the rate of crop phosphorus (P) removal, resulting in an accumulation of soil-P (O'Connor et al., 2004). Excess soil P is not harmful to crops (Peterson et al., 1994); however, accelerated eutrophication of most freshwaters is limited by P inputs, and high-P soils represent an increased risk for non-point pollution of surface waters (Sharpley et al., 1994; Sharpley et al., 1996).

Increasingly stringent effluent P regulations are exacerbating soil-P accumulation problems in biosolids amended soils (Elliott et al., 2002). Decreased effluent P concentrations result in more P partitioning to biosolids, decreasing the N:P ratio of the materials (Stehouwer et al., 2000). Decreasing N:P ratios in biosolids increases P loading and soil-P accumulation when biosolids are applied at N-based rates. The wastewater treatment method used to meet the increasingly stringent effluent P concentration affects the P-release characteristics of the biosolids (Maguire et al., 2001; Brandt et al., 2004).

Heightened concern that agricultural P losses are adversely affecting surface waters is prompting regulators to utilize a variety of P management tools to reduce non-point source P pollution. The P index is a P management tool developed by the U.S. Department of Agriculture Natural Resource Conservation Service (NRCS) to assess the potential for P loss from agricultural fields (Lemunyon and Gilbert, 1993). The P index uses a field-scale scoring matrix to identify sites vulnerable to P-loss. Most states, including Florida, chose to adopt a phosphorus indexing approach to environmental P management (Elliott and O'Connor, 2007).

Biosolids Land Application under P-based Management

The P index dictates application rates based on crop P demands (P-based rates) on soils with high P loss risks so that P loading is reduced on soils particularly susceptible to P losses. Accurate determinations of biosolids relative P phytoavailability—the plant availability of biosolids-phosphorus (biosolids-P) compared to fertilizer-P—is necessary if the P index forces land-based recycling programs to apply biosolids at P-based rates. Accounting for relative P phytoavailability (RPP) determinations assures that biosolids-P is applied at rates agronomically equivalent to fertilizer-P recommendations (O'Connor et al., 2004). Sarkar and O'Connor (2004) found that a high native-P Millhopper sand with high P-sorption capacity masked the effects of P-source additions; however, P-source effects were significant in the moderate P-sorption capacity P Candler sand, and clearly expressed in a low P-sorption capacity Immokalee fine sand. The relative efficacy of P-sources is of particular interest in Florida where many sandy soils possess little P retention capacity to mask P release differences among P-sources.

The USEPA (1995) assigns a 50% agronomic “relative effectiveness” value to biosolids-P compared to fertilizer-P. Wen et al. (1997) found sewage sludge-P was less available than manure-P to crops, and hypothesized the decreased phytoavailability was due to the greater Fe and Al content in biosolids. O’Connor et al. (2004) suggested grouping biosolids into three categories of phytoavailability relative to the inorganic fertilizer triple super phosphate (TSP): low (<25% of TSP), moderate (25-75% of TSP), and high (>75% of TSP). A four-month greenhouse study identified most biosolids as being in the moderate category, BPR biosolids as being in the high category, and some biosolids with uniquely high total Fe+Al concentrations as being in the low category. A two-year field study validated the short-term RPP differences suggested by the greenhouse study (O’Connor and Elliott, 2006). Chinault (2007) further verified short-term RPP differences among additional P-sources in a four-month greenhouse study: the relative P phytoavailability of BPR and BPR-like materials was similar to TSP, and conventional biosolids-P was less phytoavailable than TSP-P. Results of the studies suggest increasing application rates of non-BPR biosolids are necessary to supply plant available P in quantities equivalent to TSP. The long-term availability of biosolids-P, however, is incompletely characterized.

The P index considers both P transport mechanisms and P source characteristics in evaluating environmental risks, and maintains that significant P loss only occurs where a P transport pathway and a labile P source coexist (Sharpley et al., 2003). Most states utilizing the P-index have soils with appreciable P sorbing capacity and P leaching is not considered an important P loss mechanism (Peterson, 1994). Elliott et al. (2002) showed that even soils with only moderate P sorbing capacity can limit P leaching. Florida is dominated by sandy soils with low native P-sorbing capacity, and P-leaching is a critical P loss mechanism (He et al., 1999).

Phosphorus can migrate through sandy Florida soils to shallow groundwater tables that are hydrologically linked to surface water bodies (Harris et al., 1996; Sims et al., 1998; Lu and O'Connor, 2001; Elliott et al., 2002). The potential for P-leaching results in high P transport risks in the soils; thus, the environmental lability of P-sources is a critical factor controlling P loss.

Studies that account for P-leaching losses and that utilize soils with minimal P-retention capacity (to reduce the soil's ability to mask P-source effects) are the most relevant for accurately distinguishing environmental and agronomic P lability differences among P-sources. Column P-leaching from an Immokalee fine sand (sandy, siliceous, hyperthermic Arenic Alaquods) amended with six conventional biosolids at N-based rates was less than 1% of P-applied and not statistically different from controls. In contrast, 21% of the P-applied leached in columns amended with TSP (Elliott et al., 2002). Chinault (2007) conducted a laboratory incubation study to assess the ultimate lability of biosolids-P in a worse-case scenario; the study soil had minimal P-retention capacity, and no crops were grown to remove labile P. Leaching continued until there was no further change in drainage-P concentration, suggesting "ultimate" release. Results suggested that conventional biosolids are ultimately less labile than TSP, and ultimate P-release from BPR and BPR-like biosolids is similar to TSP.

The unique P-release characteristics of biosolids is not explicitly acknowledged by the P indices used by most states, despite abundant evidence suggesting P lability depends critically on whether P originates from inorganic fertilizers, manures, or biosolids (Elliott et al., 2002; O'Connor and Elliott, 2006; Oladeji, 2006). Two clear challenges remain to the inclusion of factors accounting for the P-release characteristics of biosolids in P indices. The first challenge is that limited long-term data exist to validate short-term lability differences among P-sources.

Concerns remain that all biosolids-P will ultimately become plant available, and that regulatory distinctions permitting some biosolids to be applied at rates in excess of crop P demands are not justified. McLaughlin and Champion (1987) found the relative efficacy of sewage sludge P increased over time (8 croppings over 209 days) from 44 to 90% of monocalcium phosphate (MCP) and from 64 to over 100% of MCP in two Oxisols. A long-term study is necessary to distinguish ultimate P-lability differences among P-sources and to address concerns that biosolids-P is ultimately bioavailable.

The second challenge is determining a useful *a priori* measure of ultimate biosolids-P lability. Empirically determining long-term lability differences among all biosolids is impractical; thus, an *a priori* measure of ultimate biosolids-P lability is a necessary tool to distinguish P-release differences among P-sources. Studies show water extractable P (WEP) is highly correlated to runoff and leachate P in manures and manure-amended soils, and could provide a useful indication of P loss potential in waste-amended soils (Kleinman et al. 2002; Sharpley and Moyer, 2000). Biosolids typically have lower percent water extractable phosphorus (PWEP) values than manures and TSP, suggesting biosolids represent smaller P loss risk than highly soluble inorganic fertilizers or typical animal manures (Brandt et al., 2004). Biosolids PWEP values were strongly correlated to runoff and leaching P loss in two rainfall simulation studies (Oladeji, 2006; Agyin-Birikorang, 2008). Elliott et al. (2002) found biosolids phosphorus saturation index (PSI)—the ratio of oxalate extractable P to oxalate extractable iron and aluminum—is a useful gauge of biosolids P-leaching potential in low P-sorbing soils. A lab incubation study suggested that PSI and PWEP were good qualitative indicators of ultimate biosolids-P lability, but established no clear quantitative relationship (Chinault, 2007).

Moreover, no *a priori* measure exists to determine the relative phytoavailability of biosolids-P. Citric acid-extractable P, used to characterize "plant available P" in most states, is a poor indicator of biosolids RPP (O'Connor et al., 2004). O'Connor and Elliott (2006) found estimates of RPP tracked well with P-source WEP values in short-term greenhouse study. Yet, a two-year field study identified total-P as the most important variable in accounting for differences in RPP (Oladeji, 2006). An accurate *a priori* measure of biosolids RPP would provide a useful tool to adjust biosolids application rates so that the quantity of biosolids-P applied is equivalent to recommended fertilizer-P rates.

Hypotheses and Research Objectives

Previous studies successfully characterized the short-term P-release characteristics of different biosolids. However, characterizing the long-term phytoavailability and environmental lability of biosolids-P is necessary to validate P-release differences suggested by short-term studies, and to determine the ultimate lability of biosolids-P

- **Hypothesis 1:** The long-term phytoavailability and environmental lability of conventional biosolids-P is less than fertilizer-P (TSP-P). The long-term phytoavailability and environmental lability of BPR and BPR-like biosolids-P is similar to TSP-P.

- **Hypothesis 2:** Short-term greenhouse and lab incubation studies adequately approximate the relative phytoavailability and environmental lability of biosolids-P.

- **Hypothesis 3:** Some measure of biosolids-P is a useful indicator of ultimate biosolids-P lability and biosolids-P phytoavailability.

- **Objective 1:** Characterize the long-term phytoavailability and environmental lability of biosolids-P.

- **Objective 2:** Compare long-term characterizations of biosolids-P release to previous short-term characterizations.

- **Objective 3:** Correlate various measures of biosolids-P to ultimate biosolids-P lability, and select the best *a priori* measure of ultimate biosolids-P release.

Study Approach

The phytoavailability and environmental lability of eight P-sources (7 biosolids and TSP) were evaluated. The selected biosolids represent a wide-range of P solubility: low soluble P (high Fe+Al materials) to high soluble P (BPR and BPR-like biosolids). Bahiagrass (*Paspalum notatum* Flugge) was grown in columns containing Immokalee fine sand (low native P-fertility and P-sorbing capacity) for 16 months. Bahiagrass tissue was harvested every 4 to 8 weeks, and P-uptake was determined to characterize P-source phytoavailability. Environmental lability was characterized by P leaching after each harvest. Overall biosolids-P lability is represented by cumulative mass of P removed from the column in bahiagrass tissue and column leachate. Previously determined characterizations of biosolids-P were correlated to P-uptake and overall P lability.

CHAPTER 2
MATERIALS AND METHODS

Greenhouse Study Design

A greenhouse experiment was utilized to characterize the environmental lability and phytoavailability of biosolids-P. The greenhouse study was established as an 8 x 3 factorial experiment in a complete randomized block design. The study was arranged in 4 blocks; each block contained 1 treatment replicate. A control column, which received no P-source application, was placed in each of the four blocks.

Seven biosolids were selected as P-sources. The biosolids selected represent a wide range of wastewater treatment processes and P solubility (Tables 2-1, 2-2). The Gainesville Regional Utility (GRU), Boca Raton, Lakeland NS, and Orange County South (OCUD S) biosolids possess PWEP values greater than 15% and are herein considered as more soluble-P biosolids. The Boca Raton biosolids is a BPR biosolids, and the GRU, Lakeland NS, and OCUD S biosolids are BPR-like biosolids (Chinault, 2007). Milorganite and Greenedge are commercial, thermally dried biosolids produced from conventional wastewater treatment processes. Milorganite and Greenedge biosolids possess PWEP values less than 1.1% and are herein considered as less soluble-P biosolids. The Disney is a composted biosolids with a PWEP value of 8.4% and is also herein considered as a less soluble-P material. Phosphorus saturation index (PSI) values also vary greatly among the biosolids selected, ranging from 0.64 to 2.9. Triple super phosphate (TSP) was also selected as P-source to compare the lability of biosolids-P to highly soluble (PWEP = 85%) inorganic fertilizer-P.

The P-sources were mixed with 4 kg of the A horizon of the Immokalee fine sand. The Immokalee fine sand was selected to represent a typical sandy Florida soil with minimal P content and minimal P-sorption capacity (Chinault, 2007). A base sand commonly used to topdress turfgrass greens was selected to represent an minimally P-reactive E horizon underlying the Immokalee fine sand A horizon. The Immokalee A horizon and the base sand contained very low quantities of native-P, and possessed little P sorption capacity (Table 2-3). Utilizing low P retention capacity, low native-P soils was important for characterizing ultimate P lability differences among P-sources (de Haan, 1980; Sarkar and O'Connor, 2006). The sandy, low P sorption capacity soils were also chosen to maximize environmental P leaching risks.

Sources of P were applied to the Immokalee A horizon at three rates, equivalent to 56 kg P ha^{-1}, 112 kg P ha^{-1}, and 224 kg P ha^{-1}. The 56 kg P ha^{-1} and 224 kg P ha^{-1} application rates represent P-based and N-based rates, respectively. The 112 kg P ha^{-1} rate was included to better define the biosolids relative P phytoavailability and lability response curves. The biosolids-amended Immokalee A horizon was wetted to approximately field capacity, and allowed to equilibrate in zip-lock bags for 2 weeks prior to use in the greenhouse study.

Large columns were utilized as soil containers for the greenhouse study. The columns consisted of 45 cm long, 15 cm diameter polyvinyl chloride (PVC) pipes with a screen at the bottom to support overlying soil. Columns were fitted with PVC caps that contained tubes at the bottom to direct leachate into collection bottles. Columns were filled with 30 cm (~ 8 kg) of base sand to simulate a native E horizon (bulk density ≈ 1.5 g cm^{-3}) and to allow adequate rooting depth for the pasture grass. The base sand was saturated and allowed to drain to remove readily soluble constituents. The 4 kg of incubated, P-source-amended Immokalee A horizon (bulk density ≈ 1.5 g cm^{-3}) was placed on top of the 30 cm of base sand in each column. Soil columns were seeded with 5 grams of bahiagrass seed (~ 29 Mg seed ha^{-1} equivalent). The seeding rate exceeded the 11 Mg seed ha^{-1} field rate recommended by Chambliss et al. (2001) to ensure thorough bahiagrass coverage of the soil and to maximize P-uptake potential. Seed was covered with a thin layer of soil, and misted about every 3-4 hours until germination occurred. Following germination, the grass was watered using daily to semi-daily applications of pH 5.0 tap water. The tap water was analyzed prior to use in the greenhouse study, and the soluble P concentration in the tap water was below the detection limit of 0.01 mg P L^{-1} (Chinault, 2007). Soil moisture content was maintained at ~80% of column pot-holding capacity mass throughout the study by bi-weekly weighing of the columns and adding water as necessary. Excessive watering was carefully avoided to prevent accidental leaching events. Columns were rotated one position within their respective blocks every week to minimize potential greenhouse positioning effects.

Biosolids-N concentrations varied among biosolids; thus, applying biosolids at uniform P-based rates resulted in varying applications of plant available nitrogen (PAN). A 40% biosolids-N mineralization rate was assumed for all biosolids (O'Connor and Sarkar, 1999). Ammonium nitrate (AN) was used to supplement biosolids-N supply so that a uniform N application rate of 300 kg N ha^{-1} was supplied to all columns per cropping (4 harvests). Total N additions were substantially greater than the 179 kg N ha^{-1} rate recommended by Kidder et al. (1998) for bahiagrass to ensure that PAN supply was uniform among all treatments and that N supply would not limit P uptake. Ammonium nitrate was applied in split-application; 75 kg N ha^{-1} was applied each harvest (300 kg N ha^{-1} was applied per cropping). A fertilizer known as "sul-po-mag" (22% S, 18% K, and 11% Mg) was also added to columns at the equivalent rate of 444 kg ha^{-1} after each harvest to supply bahiagrass with ample and uniform amounts of sulfur, potassium, and magnesium.

Leachate and Bahaigrass Tissue Analyses

Bahiagrass was planted June 12, 2006 and the grass grew continuously for 498 days until the study was terminated on October 18, 2007. Bahiagrass tissue harvests occurred every 4-8 weeks. Bahiagrass tissue was cut to a height of 3.8 cm above the soil surface for each harvest. Harvested tissue was placed in pre-weighed paper bags and dried at 68^{o} C to constant weight as a measure of grass yield Immediately following each tissue harvest, columns were leached with sufficient pH 5.0 water to yield approximately 500 mL (¼ pore volume) of drainage (leachate), collected in 1 L collection bottles beneath the columns. The bottles were weighed to determine the exact volume of leachate. A ~250 mL aliquot of leachate was used in laboratory analyses.

Soluble reactive P (SRP), electrical conductivity (EC), and pH were determined for all leachate samples within 48 hours of leachate collection. The mass of P leached was determined as the product of SRP in the leachate and leachate volume. The concentration of SRP was determined using the ascorbic acid method (Murphy and Riley, 1962). Total P (USEPA, 1993b) was also determined on darkly colored leachate samples to confirm that SRP constituted the vast majority of total leachate-P.

Plant uptake of P was determined as the product of plant tissue P concentration and dry tissue yield. Dried plant tissue was ground in a Wiley mill to pass through a #20 (0.85 mm) sieve, ashed, and the ash digested following Andersen (1976). Total P was determined in the digest using the ascorbic acid method (Murphy and Riley, 1962). Total-N concentrations were determined on the harvested bahiagrass tissue from the N-based treatments and controls. The ground bahiagrass tissue was ball-milled to a fine powder, and N concentrations determined by combustion at 1010^{o}C, according to Nelson and Sommers (1996), using a Carlo Erba TC/TN analyzer (NA-1500 CNS, Carlo Erba, Milan, Italy).

Soil Analysis

Three 2.5 cm diameter soil cores were taken from each column following the last harvest. The first soil core was taken from 0-45 cm and represented the entire soil profile. The 0-45 cm soil core was utilized for a mass balance analysis. The second soil core was taken from ~0-15 cm to characterize the Immokalee A horizon. The third soil core was taken from the base sand layer at a depth interval of ~15-45 cm. A stark contrast existed between the Immokalee A horizon and the base sand, and great care was taken not to mix the Immokalee A horizon and base sand during sampling. The Immokalee A horizon and base sand were analyzed separately to determine the quantity of P retained in the base sand layer, and to characterize the quantity of P that moved out of the Immokalee A horizon initially amended with P-sources.

Wet soil samples were stirred regularly and allowed to air-dry for 3 weeks. Following air-drying, the 0-45 cm soil samples were sieved (0.85 mm) to remove plant roots. Plant roots were ground using a household coffee bean grinder, and then thoroughly mixed back into the soil samples. Roots were included in the 0-45 cm samples so that the mass balance analysis would represent the total-P remaining in the column soil profile. Roots were generally present in clumps, and obtaining a representative sample of the soil/root mixture was impossible if roots clumps remained intact. Roots were ground so that an even mixing of soil and roots would occur and a representative sample could be obtained. Plants roots present in the 0-15 cm and 15-45 cm samples were removed mostly by sieving so that essentially, only soil total-P was determined. Soil samples were ashed and digested according to Andersen (1976), and P was determined using the ascorbic acid method (Murphy and Riley, 1962).

Root Mass Determinations

Column root masses were quantified following soil sampling to determine if P-application rate and/or P-source lability affected root proliferation within the columns. The bahiagrass roots mats were sufficiently thick to allow the root mats to be carefully pulled from the columns. The columns were wetted thoroughly to promote separating the roots mats from the columns. The roots mats were placed on a #10 (2.00 mm) sieve and soil was washed from the roots using a garden hose. The roots mats were washed over a large plastic wash tub to collect material passing through the sieve. The roots were repeatedly washed until a visual assessment indicated the soil was removed. Fine roots that washed through the sieve floated on the surface of the water in the large plastic wash tub. The wash tub water was passed through a #20 sieve to collect fine roots. The coarse and fine roots were placed into pre-weighed bags, and dried at 68^{o}C to constant weight.

Quality Assurance/Quality Control

All data were obtained in strict accordance to standard quality assurance/quality control (QA/QC) protocol (Kennedy et al., 1994). Total-P recoveries from soil and tissue digestions were determined using standard reference materials (Standard reference materials 1547 and 2709, National Institute of Standards and Technology). Total-P recoveries from the standard reference materials were 85% to 101% of the certified value. Blanks and replicates were also incorporated into soil and tissue digestions according to standard QA/QC protocol. All calibration curves for colorimetric P determinations achieved an $r^2 \geq .9999$. Method reagent blanks, spikes, replications, and certified standards were included in colorimetric P determinations. Recoveries from certified standards and spikes were within 5% of the expected value. The relative standard deviations of replicated samples were less than 5%. Analyses that did not satisfy standard QA/QC protocol were repeated until all QA/QC criteria were satisfied.

Statistical Analysis

Differences among treatments were statistically analyzed as a factorial experiment with a completely randomized design (CRD), using the general linear model (GLM) of the SAS software (SAS Institute, 2007). The means of the various treatments were separated using Tukey's mean separation procedure (Tukey, 1949) at a significance (α) level of 0.05 The data were tested for the normal distribution and constant variance assumptions of analysis of variance using the normal probability plots and the residual plots, respectively (SAS Institute, 2007). Data that did not conform to the normality and homogeneity assumptions were appropriately transformed based on the results of the Box-Cox transformation procedure (Box and Cox, 1964) before statistical analysis.

Regression analyses were performed using the PROC REG procedure of the SAS software (SAS Institute, 2007). Prior to the regression analyses, stepwise regressions were utilized to determine best predictor(s) of biosolids-P uptake and lability using the procedure described by Hocking (1976).

Time series analysis was conducted using the PROC TSCSREG procedure of the SAS software (SAS Institute, 2007).Time series analysis contrasts were performed at harvests 4, 8, 11 for the P-based rate treatments and 4, 8, and 12 for the N-based rate treatments. Contrasts among all harvests yielded little useful information. Consequently, sub-divisions of the greenhouse study were necessary to practically assess changes over time. Traditional greenhouse studies are often a single growing season in length and composed of four tissue harvests; thus, every fourth harvest (except for harvest 11 for the P-based treatments) was selected to perform time series analysis contrasts for the greenhouse study.

Table 2-1. Treatment processes used to produce the biosolids selected for use in the greenhouse study.

Material	Class[a]	Digestion	BPR[b] (Yes/No)	Final treatment
Milorganite	AA	Aerobic	No	Thermally dried
Greenedge	AA	Anaerobic	No	Thermally dried
Disney	AA	Composted	Yes	Composted material
GRU	B	Aerobic	No	Thickened
Boca Raton	B	Anaerobic	Yes	Thickened, dewatered
Lakeland Northside	AA	Aerobic	No	Thickened, dewatered, ATAD system
OCUD S Cake	B	Anaerobic	No	Thickened, dewatered, bio-N removal

[a] Determined by the biosolids quality standards established by the 40 CFR Part 503 rule (USEPA, 1993a). Class AA biosolids are considered exceptional quality biosolids, while Class B biosolids meet less stringent standards for pathogens.

[b] Biological phosphorus removal

Table 2-2. Various measures of P for the P-sources utilized in the greenhouse study (Chinault, 2007).

	-----------Total P----------		Fe-strip			----Oxalate extractable----			
P Source	Determined	Producer	P	WEP	PWEP	P	Al	Fe	PSI
	--------------------g kg^{-1}---------------------				---%---	------------g kg^{-1}------------			
Milorganite	21	23	0.08	0.12	0.58	16	1.2	25	1.0
GreenEdge	17	19	0.12	0.19	1.1	13	5.0	13	1.0
Disney Compost	11	27	0.95	1.2	8.4	11	6.6	20	0.6
GRU	31	48	1.7	7.9	26	21	6.4	3.7	2.1
Boca Raton	26	39	0.02	3.9	15	33	14	7.3	2.1
Lakeland NS	29	29	0.35	14	47	22	3.2	8.3	2.0
Orange County South	23	30	0.14	4.8	21	23	5.0	4.4	2.9
Triple Super Phosphate (TSP)	190	210	ND[a]	170	85	186	11	6.8	NA[b]

[a] Not determined

[b] Not applicable

Table 2-3. Selected chemical properties of the Immokalee A horizon and base sand utilized in the greenhouse study (Chinault, 2007).

Parameter	Units	Immokalee A horizon	Base sand
Sand	%	95	100[a]
pH		4.8	5.1
Organic matter	g kg^{-1}	7.0	ND[b]
Total P	mg kg^{-1}	15.5	12.0
Mehlich-1 extractable P	mg kg^{-1}	1.5	ND[b]
Water extractable P	mg kg^{-1}	1.1	0.2
Oxalate extractable P	mg kg^{-1}	13.1	3.7
Oxalate extractable Al	mg kg^{-1}	40.1	17.6
Oxalate extractable Fe	mg kg^{-1}	85.6	10.0
Phosphorus Saturation Ratio[c]		0.1	0.1
Soil Phosphorus Storage Capacity[d]	mg kg^{-1}	0.8	0.2
Relative Phosphorus Adsorption (RPA)[e]	%	2.0	8.6

[a] Estimated from field characterization

[b] Not determined

[c] The molar ratio of oxalate extractable-P to oxalate extractable Al+Fe in soils (Maguire and Sims, 2002; Nair and Harris, 2004)

[d] A measure of the amount of P that can be added to the soil before the soil becomes an environmental concern (Nair and Harris, 2004)

[e] The relative amount of P sorbed from a 400 mg P kg^{-1} load (Harris et al., 1996)

CHAPTER 3
RESULTS AND DISCUSSION

The greenhouse study characterized the fate of biosolids-P over more than 16 months. The residual value of biosolids-P was characterized for more than one year following the initial 4 month short-term characterization conducted by Chinault (2007). Twelve tissue harvest and column leaching events occurred for the 112 kg P ha^{-1} and 224 kg P ha^{-1} amended columns, and 11 events occurred for the 56 kg P ha^{-1} amended columns. An implicit study objective was to determine the ultimate fate of biosolids-P, and no predetermined study end-point existed at the beginning of the study. Bahiagrass is commonly found in grazed pasture systems of Florida, and consideration was given to maintaining tissue nutrient contents that are sufficient for grazing cattle. The study continued until bahiagrass tissue N and P concentrations declined below suggested minimum concentrations (described more thoroughly in later discussions). We regarded the characterization of biosolids-P lability from the 16-month greenhouse study as a long-term biosolids-P lability characterization because the supply of labile biosolids-P sufficient for most agronomic conditions was exhausted and additional P fertilization would be necessary.

A mass balance analysis was conducted to confirm that tissue harvests and column leaching characterized all P losses from the study columns, and that no egregious P losses or additions occurred during the study. The average P recovery for all columns was 92%. Triple super phosphate (TSP) is a highly soluble inorganic fertilizer, and near complete dissolution of TSP pellets is expected after 14-16 months of water additions. Mass balance for TSP treatments was expected to be the least affected by column sampling error due to high degree of expected TSP dissolution, and a mass balance analysis for TSP-amended columns was expected approach 100% recovery. The average recovery for TSP-amended columns was 97%, indicating the study successfully quantified column-P losses.

Tissue Yield

Significant differences in cumulative tissue yield existed among P-sources across the rates of P application (Fig. 3-1). Apparent yield differences among P-sources early in the study raised concerns that the nutrient supply was not uniform among P-sources (Chinault, 2007). Biosolids-N mineralization was assumed to be 40% of total biosolids-N for all biosolids, and ammonium nitrate supplemented biosolids-N so that PAN supply was uniform among treatments. If biosolids-N mineralization was incorrectly estimated, PAN supply differences are likely most apparent at the N-based application rate where biosolids-N represented the greatest proportion of calculated PAN supply. Thus, tissue-N concentrations were measured for columns amended at the N-based application rate to guage if PAN supply was sufficient and uniform across P-sources.

Tissue-N concentrations for harvests 1-4 suggested some differences in PAN supply among P-sources (Fig. 3-2). Further, the data suggested the biosolids PAN supply was underestimated for harvest 1, and overestimated for harvests 2-4 (Chinault, 2007). Tissue N concentrations following harvest 4 were near the 11 g N kg^{-1} regarded as minimally sufficient for grazing beef cattle (NRC, 1996). This initial tissue N concentration analysis increased concern that PAN supply was not adequate and uniform across P-sources, and suggested a change to the N fertilization regime was necessary.

Following harvest 5, 300 kg PAN ha^{-1} of ammonium nitrate, applied in 4 split applications was supplied to columns to ensure PAN supply was adequate, and to compensate for PAN supply differences that existed among P-sources. Supplying the additional N increased tissue N concentrations, and tissue N concentrations exceeded the suggested minimally sufficient tissue concentration of 11 g N kg^{-1} for harvests 6-8 (Fig. 3-3). Tissue N concentrations became increasingly difficult to maintain above 11 g N kg^{-1} after harvest 8 (despite additional N-fertilizer additions), and only harvest 11 exceeded the suggested minimum concentration. Tissue N concentrations in bahiagrass are affected by photoperiod (Sinclair et al., 2003), and decreased tissue N concentrations during longer day length months—the months corresponding to harvests 8, 9, 10, and 11—was expected. The seasonal pattern in tissue N concentrations reported by Sinclair et al. (2003) suggested that tissue N concentrations should increase from harvest 11 to harvest 12 because the photoperiod decreased; however, tissue N concentrations declined appreciably from harvest 11 to harvest 12. The persistent tissue N concentrations below 11 g N kg^{-1}—in spite of abundant PAN supply—suggested that the bahiagrass could no longer maintain sufficient N uptake. Concern that insufficient N uptake could confound P uptake interpretations resulted in the decision to terminate the study following harvest 12.

Yield-weighted tissue N concentrations were calculated using equation 3-1 to determine if overall nitrogen uptake was uniform among P-sources, and bahaigrass tissue N concentrations were sufficient for grazing beef cattle.

where for any given P-source:
Y_i = yield at ith harvest
N_i = tissue N concentration at ith harvest

$$\frac{(Y_1 * N_1) + (Y_2 * N_2) \ldots (Y_{12} * N_{12})}{Y_1 + Y_2 \ldots Y_{12}} = \text{Yield-weighted tissue N concentration} \qquad (3\text{-}1)$$

No significant differences in yield-weighted N concentrations existed among P-sources (Fig. 3-4), and yield-weighted tissue N concentrations exceeded the suggested minimum of 11 g N kg^{-1}. The data suggest the greenhouse study fertilization regime adequately controlled PAN supply, and that potentially confounding effects of N insufficiency on P uptake were minimized, overall. Nevertheless, incorrect estimations of biosolids-N mineralization in early harvests and decreased

N uptake in later harvests illustrate that interactions among plant nutrients in prolonged greenhouse studies are difficult to control.

The relatively uniform yield-weighted tissue N concentrations also suggest that the overall differences in cumulative tissue yield among P-sources were not due to differences in N-uptake. Poor tissue growth occurred in 3 of 4 columns amended with Lakeland NS biosolids at the N-based rate, suggesting some property of the material negatively affected the bahiagrass at high application rates. However, the differences in cumulative tissue yields for other sources are likely due to differences in long-term P-lability. McLaughlin and Champion (1987) noted ryegrass (*Lolium multiflorum*) yields were greater for sewage sludge amended sesquioxic soils than for soils amended with monocalcium phosphate, and attributed the increased yields to a prolonged supply of phytoavailable P from sewage sludge.

Tissue P Concentrations

Bahiagrass tissue P concentrations are useful indicators of when the residual value of P-sources is no longer sufficient to meet agronomic demands, and when additional P fertilization is necessary. Oladeji (2006) proposed an agronomic threshold for bahiagrass of 2.0 g P kg^{-1}. Adjei et al. (2000) suggested bahiagrass tissue P concentrations of 1.6 to 1.7 g P kg^{-1} are agronomically limiting. Critical P tissue concentrations are also determined by considering the P nutritional requirements of grazing beef cattle. If the current live weight of a grazing beef steer is just maintained and bahiagrass tissue is the only P-source, a minimum tissue P concentration of 0.9 to 1.1 g P kg^{-1} is suggested by Ternouth et al. (1996). The suggested tissue concentration can increase considerably to values beyond 2.0 g P kg^{-1} if live weight gains or milk production are desired. O'Connor and Elliott (2006) suggested a critical bahiagrass tissue P concentration of

1.0 g P kg^{-1}. The 1.0 g P kg^{-1} value represents a conservative estimation of the critical P tissue concentration of bahiagrass and is used as the indicator value for this study.

The residual-P value of all P-sources applied at N-based rates maintained tissue P concentrations above 1.0 g P kg^{-1} for more than 16 months—12 harvests—of continuous bahiagrass growth (Table 3-1). Although the tissue P concentrations exceed the critical value, the tissue P concentrations are below most agronomic standards (Adjei et al., 2000; Oladeji, 2006) and are likely agronomically limiting. Plants translocate stored P from older tissues to younger leaves under P-deficient conditions (Schachtman et al., 1998), suggesting tissue P concentrations could remain elevated some time after the plant is subjected to P limited conditions. The low tissue P concentrations at even the N-based application rates following 12 harvests suggest the ultimate agronomic value of biosolids-P was sufficiently characterized.

The time series analysis suggests the P-release characteristics of Milorganite is unique from other biosolids and TSP at N-based application rates (Table 3-1). Tissue P concentrations are not significantly different at harvest 4, 8, and 12. The relatively constant tissue P concentrations for the N-based rate of Milorganite is consistent with the slow P release characteristics previously shown for heat-dried, high Fe+Al biosolids such as Milorganite (O'Connor et al., 2004; Chinault, 2007). Tissue P concentrations decline significantly from harvest 4 to 8 for other P-sources (Table 3-1).

Tissue P concentrations for the P-based rate treatments did not change significantly following harvest 8 (Table 3-2). Tissue concentrations are ≤ 1.0 g P kg^{-1} for harvests 8 through 11, suggesting that P-sources applied at P-based rates have little residual agronomic value beyond 8 harvests. The persistently low tissue P concentrations following harvest 8 suggested the ultimate phytoavailability of P-sources amended at P-based rates was characterized, and columns amended at the 56 kg P ha^{-1} rate were terminated after the 11th harvest.

Yield-weighted tissue P concentrations were determined for the P-based rate treatments and compared to the critical P concentration of 1.0 g P kg^{-1} to assess if the overall P-supply was sufficient to exceed the minimum agronomic P demand. Yield-weighted tissue P concentrations for Milorganite and Greenedge biosolids—heat-dried, high Fe+Al biosolids—are approximately 1.0 g P kg^{-1} (Fig. 3-5), suggesting minimally sufficient P supply to meet the 14 month agronomic P demand. An additional P-based application of P within 14 months of the initial application is likely necessary to assure sufficient P supply to grazing beef cattle.

Abundant P supply is associated with increased root growth (Havlin et al., 1999), and the differences in P-supply among treatments were expected to affect below-ground biomass. Bahiagrass root masses were quantified for the P-based and N-based treatments following study termination to gauge the hypothesized effect of P-supply differences on below-ground biomass. No significant differences in bahiagrass root mass existed among P-sources at either rate, and only the root mass for the N-based Boca Raton treatment was significantly different from the controls (Fig. 3-6). Average N-based treatment root masses tended to be greater than the average root mass of P-based treatments; however, only the controls were significantly different from N-based rate treatments. Quantifying root masses following the study's termination makes interpreting P-supply effects on root masses difficult. The P-based treatment tissue P concentrations were deficient for some time (Table 3-2) before root masses were quantified, and plants can increase root length and root hair numbers in response to P-deficiency (Vance et al., 2003). Plant physiological responses in P deficient conditions could confound root mass interpretations.

Environmental P Lability

Eleven leaching events occurred for P-based rate treatments, resulting in 2.75 pore volumes of leachate collected. Phosphorus leaching from any P-sources applied at the P-based rate was minimal—less than 1% of the P applied. Nevertheless, environmental P lability differences existed among P-sources at the P-based application rate (Fig. 3-7A). Milorganite, a high Fe+Al biosolids, leached significantly less P than BPR and BPR-like biosolids, and TSP. Notably, the mass of P leached from the less soluble-P biosolids—Milorganite, Greenedge, and Disney—treatments was not significantly different than the mass of P-leached from the controls. More soluble-P biosolids (BPR and BPR-like biosolids) and TSP leached significantly more P than the controls. Although significant differences in environmental P lability exist among P-sources, the cumulative mass of P leached ($\leq$ 0.27 mg P) suggests P-sources applied at P-based application rates pose minimal environmental risk.

Twelve leaching events resulted in 3 pore volumes of leachate from N-based rate amended columns, and much greater P leaching occurred (Fig. 3-7B). Significantly more P leached from BPR biosolids, BPR-like biosolids, and TSP than from the less soluble-P biosolids at the N-based rate. The mass of P leached from the more soluble-P biosolids and TSP treatments represented more than 12% of the total P applied. Remarkably, the mass of P leached from Milorganite N-based rate treatments was not significantly different than the controls, and represented < 0.1% of the total P applied. The leaching data suggest that Milorganite poses minimal P leaching risk, even at application rates approximately four times plant P requirements. The time series analysis suggests P-release kinetics also differed among P-sources (Table 3-1). An appreciable portion of the P leaching occurred in the first 4 harvests for the more soluble-P biosolids and TSP, whereas P-leaching is more gradual for less soluble-P biosolids.

Overall differences in environmental P lability exist among P-sources across application rates (Fig. 3-8). The environmental lability of heat-dried, high Fe+Al Milorganite and Greenedge biosolids, and the composted Disney material is significantly less than BPR biosolids, BPR-like biosolids, and TSP. Fertilizer-P (TSP) leached the greatest mass of P; however, the environmental lability of BPR and BPR-like biosolids-P is not statistically different from TSP-P, across application rates.

Relative environmental P lability differences among P-sources are well documented (Elliott et al., 2002; Oladeji, 2006; Chinault, 2007). Elliott et al. (2002) determined environmental P lability differences among biosolids by quantifying P leaching in a short-term greenhouse study. Yet, concern remained that short-term environmental lability differences are not necessarily indicative of long-term environmental P lability differences. The relative differences in environmental P lability among biosolids determined in this 16-month greenhouse study are similar to the short-term (4-month) Elliott et al. (2002) study—less soluble biosolids-P is significantly less environmentally labile than BPR biosolids-P and TSP-P.

Despite the similar determinations of relative biosolids-P leaching risks, the fraction of P-source total P that is environmentally labile is markedly different between the two greenhouse studies. Columns amended with TSP at the 224 kg P ha^{-1} and 56 kg P ha^{-1} rate leached approximately 21% and 14% of the P-applied, respectively, in the 4 month Elliott et al. (2002) study. Approximately 13% and 0.2% of P-applied leached at the 224 kg P ha^{-1} and 56 kg P ha^{-1} rate, respectively, in the current greenhouse study. The substantially greater mass of TSP-P leached in Elliott et al. (2002) study—despite leaching two times less leachate than the current greenhouse study—raised concern that the base sand in the current greenhouse study retained more P than Elliott's base sand. Columns in both studies were amended at the same N-based rate, and utilized a similar Immokalee fine sand as surface (0-15 cm) soils. However, the base sand underlying the Immokalee A horizon material differs between the studies. The Elliott et al. (2002) study utilized a Myakka (Sandy, siliceous, hyperthermic Aeric Alaquods) E horizon with a low P-sorbing capacity—RPA ≈ 3.2%. The current greenhouse study utilized a base sand capable of sorbing almost three times as much P—RPA ≈ 8.6% (Table 2-3). Although the 8.6% RPA value of the base sand represents a low P sorption capacity, the relatively large mass of base sand in the columns (~8 kg) resulted in an appreciable sink (~275 mg) for labile P, representing 267%, 133%, and 67% of the total-P applied at the 56, 112, and 224 kg P ha^{-1} treatments, respectively. Thus, the base sand used in the current study would be expected to retard P-leaching to a greater extent than the base sand used by Elliott et al. (2002), and explains the differences in P leachate losses.

Biosolids-P Phytoavailability

Eleven harvests, over 14 months of continuous bahiagrass growth, characterized P phytoavailability in the P-based rate treatments. Uptake of P from all P-sources was significantly greater than control P uptake (Fig. 3-9A). Uptake of P from only two biosolids treatments was significantly different than TSP at the P-based application rate; Milorganite-P uptake was significantly less than TSP-P uptake, and OCUD S-P uptake was significantly greater than TSP-P uptake.

Twelve harvests, over 16 months of continuous bahiagrass growth, characterized P phytoavailability in the N-based treatments. Uptake of P from almost all BPR and BPR-like biosolids was not statistically different than TSP-P uptake (Fig. 3-9B); P uptake from OCUD S biosolids treatments was significantly greater than TSP treatments. Uptake of P from the less soluble-P biosolids was significantly less than P uptake from the more soluble-P biosolids and

TSP treatments—notably, P uptake from Milorganite treatments was significantly less than P uptake from all other P-source treatments.

Differences in P uptake across rates of application were analyzed to determine overall P-source phytoavailability differences (Fig. 3-10). Phytoavailability trends present in individual P-based and N-based rate analyses were also distinguished in the overall P-source phytoavailability analysis. Uptake of P from BPR and BPR-like biosolids was similar to, or greater than, P uptake from TSP across application rates. Uptake of P from OCUD S biosolids treatments was significantly greater than from TSP treatments across application rates. Uptake from Milorganite and Greenedge treatments was significantly less than P uptake from more soluble-P biosolids and TSP—bahaigrass P uptake from Milorganite treatments was significantly less than uptake for all other P-sources. The environmental lability of Disney biosolids-P was distinguished from more soluble biosolids-P and TSP-P (Fig. 3-8), but P uptake from Disney treatments was not significantly different from TSP and GRU biosolids treatments.

O'Connor et al. (2004) and Chinault (2007) utilized a slope-ratio approach to estimate the relative P phytoavailability (RPP) of biosolids compared to TSP by regressing P uptake as a function of application rate. The linear regression lines were forced through a y-intercept equal to the average P uptake of the control treatment. The ratio of the slope of biosolids-P uptake regression lines to the TSP-P uptake regression line reflected the relative P phytoavailability of the biosolids. Chinault (2007) also utilized point estimates of RPP to determine the RPP of biosolids where a linear regression poorly described the relationship between biosolids-P uptake and application rate for some treatments.

A similar slope-ratio approach was utilized here to determine the long-term RPP of biosolids; however, the O'Connor et al. (2004) and Chinault (2007) slope-ratio method was modified to distinguish treatment effects on P uptake and to account for the difference in the number of harvests (11 for the 56 kg P ha^{-1} rate and 12 the 112 and 224 kg P ha^{-1} rates). Cumulative P uptake from the control treatment was subtracted from cumulative P uptake for the various P-source treatments to isolate the contribution of P-sources (Fig. 3-11). The origin in a plot of P uptake versus P-source application rate represents zero P-source uptake when no P-source is applied, and linear regressions of P uptake from P-sources versus P application rate were forced through the origin. One study objective was to determine the long-term phytoavailability of biosolids-P, and characterizing the ultimate agronomic value of biosolids-P is implicit to achieving the objective. Tissue P concentrations suggested 11 harvests were sufficient to characterize the ultimate agronomic value in the P-based treatments, but an additional harvest was deemed necessary to more fully characterize the residual value of biosolids-P applied at N-based rates. Data for P uptake from the additional 12th harvest could not be utilized if the regression was forced through a y-intercept equal to the average cumulative control P uptake because cumulative control P uptake differs between harvest 11 and 12. Utilizing only harvest 11 data for the 112 and 224 kg P ha^{-1} rates would ignore almost two months of additional P uptake data. Accounting for the cumulative control P uptake at each rate, and forcing the regression through the origin, permits the utilization of harvest 12 P uptake data without compromising the determination of relative differences among P-sources.

The slope-ratio estimates of long-term RPP suggest less soluble biosolids-P is ultimately less phytoavailable than TSP-P (Table 3-3). Milorganite-P is ultimately less than half as phytoavailable as TSP-P. The RPP values of BPR and BPR-like biosolids-P are similar to, or greater than, values for TSP-P. Notably, the slope-ratio RPP estimates suggest OCUD S biosolids-P is approximately 130% as phytoavailable as TSP-P. The PWEP value for TSP is ~4x the PWEP value for OCUD S biosolids; yet the percent of total P that is oxalate extractable is 98% and 100% for TSP and OCUD S, respectively (Table 2-2). The water and oxalate extractions suggest that while only about 20% of OCUD S total P is water soluble, almost all of the total-P is potentially accessible to plant roots, which can exude organic acids to extract labile P (Vance et al., 2003). Recall that P leaching was greatest in TSP treatments and that most of the P leaching occurred in the first leaching event (Tables 3-1, 3-2). Thus, an appreciable portion of the labile pool of TSP likely leached out of the root zone before plant uptake could occur. Conversely, less P leached in OCUD S biosolids amended columns, and the leaching occurred less rapidly. Much (~80%) of the OCUD S biosolids-P labile pool is not water soluble and likely remained in the root zone for plant uptake. Moreover, OCUD S biosolids treatment tissue yields were significantly greater than all other P-sources across application rates (Fig. 3-1)—despite similar yield-weighted tissue N concentrations among P-sources (Fig. 3-4). Apparently, some additional nutritive quality of the OCUD S biosolids improved bahiagrass yields, and thus, increased P uptake.

Point RPP estimates were also utilized to estimate biosolids RPP values (Table 3-4). Point estimates were determined by averaging P uptake at each rate of P-source applied, and dividing by the average P uptake of TSP treatments at each rate. Similar to slope-ratio RPP estimates, control P uptake values were subtracted from P-source cumulative P uptake values to isolate the contribution of P-sources to P uptake. The point RPP estimates were averaged across rates and compared to the slope-ratio RPP estimates. Strong agreement exists between slope-ratio and point estimate methods of RPP calculation; RPP point and slope-ratio estimates differed less than 10% for all biosolids, and the average difference was less than 4%. Empirical P uptake data also supports the slope-ratio and point estimates of biosolids RPP. The RPP value of Milorganite is about 50%, suggesting Milorganite-P uptake will be similar to TSP-P uptake if twice as much Milorganite-P is applied as TSP-P. Indeed, TSP-P uptake at the 56 kg P ha^{-1} application rate is equivalent to Milorganite-P uptake at the 112 kg P ha^{-1} application (P uptake is ~79 mg for both treatments).

Biosolids RPP estimates increased appreciably from harvest 4 to harvest 12 (Tables 3-3, 3-4); however, relative differences among biosolids remained similar. The greatest increase in RPP values occurred for less soluble-P biosolids. Milorganite and Greenedge biosolids maintained an overall moderate RPP classification, but the RPP estimate increased about 20% for both biosolids from harvest 4 to harvest 12. The RPP estimate for the Disney biosolids increased > 30%, and the long-term RPP estimate suggests the relative phytoavailability of Disney-P changes from moderate to high. Modest increases in RPP estimates occurred for BPR and BPR-like biosolids following the additional year of plant uptake. Comparison of short-term versus long-term RPP estimates suggests that short-term RPP values successfully distinguish relative differences among biosolids, but long-term RPP estimates are necessary to fully characterize the agronomic value of biosolids-P relative to TSP. Nevertheless, the categorization (low, moderate, high) of biosolids RPP based on a short-term (4 month) characterization was appropriate in all but one case.

McLaughlin and Champion (1987) noted that relative efficiency of sewage sludge-P compared to monocalcium phosphate (MCP) increased over time, and that the relative efficiency of sewage sludge P was ultimately similar to or greater than MCP. The current greenhouse study confirms that biosolids RPP estimates tend to increase with time; however, less soluble biosolids-P ultimately remained less phytoavailable than TSP-P. McLaughlin and Champion (1987) regarded sewage sludge as a slow-release P fertilizer. The appreciable increase in RPP with time for Milorganite, Greenedge, and Disney biosolids indicates the slow-release description is appropriate for the less soluble-P biosolids, and that a 20-50% increase in less soluble-P biosolids application rate is necessary to supply P at rates agronomically equivalent to TSP. The RPP estimates of BPR and BPR-like biosolids-P were similar to TSP throughout the study, and the slow-release description is not appropriate for such biosolids. No agronomic P application rate adjustment is justified for BPR and BPR-like biosolids.

No *a priori* measure of biosolids-P phytoavailability exists to guide agronomic biosolids application rates, and empirical evidence is necessary to justify application rate adjustment. Chinault (2007) characterized the total-P, PWEP, oxalate extractable P, iron strip-P, and PSI of the biosolids utilized in the greenhouse study. Biosolids-P uptake was correlated to the various measures of biosolids-P to identify a potentially useful measure of biosolids-P phytoavailability. The load of total-P, oxalate extractable-P, and iron strip-P that was applied in each treatment was utilized in the regression analysis to account for rate effects. Biosolids PWEP and PSI values were multiplied by the total-P load to account for rate effects. The product of PWEP and total-P load was termed the "environmentally effective P load" (Agyin-Birikorang, 2008), and biosolids PSI multiplied by total-P load is henceforth designated the "labile P load".

The labile P load was the best predictor of biosolids-P uptake, and explained 85% of the variability in biosolids-P uptake (Fig. 3-12). The strong, linear relationship between biosolids-P uptake and the labile P load suggests biosolids PSI is a potentially useful *a priori* measure of biosolids-P phytoavailability. Indeed, a good linear relationship ($r^2 = .76$) exists between biosolids RPP and biosolids PSI (Fig. 3-13), which suggests that *a priori* determinations of biosolids PSI could be used to make application rate adjustments for agronomic consideration. Oladeji (2006) determined long-term RPP values for two biosolids in a two-year field study, and in a greenhouse pot study utilizing two croppings of bahiagrass and one cropping of ryegrass (*Lolium perenne* L.). The RPP estimates and PSI values of the two biosolids utilized in the Oladeji (2006) studies were added to the dataset from the current greenhouse study to confirm that biosolids PSI is a good *a priori* indicator of long-term biosolids RPP values. A strong linear relationship (r^2 = .82) exists between the RPP estimates and PSI values determined in the current greenhouse study and in the Oladeji (2006) studies (Fig. B-1).

Additional datasets were available to validate that biosolids PSI is a useful *a priori* indicator of biosolids-P phytoavailability. O'Connor et al. (2004) and Chinault (2007) conducted short-term (4-month) greenhouse studies that utilized methods identical to the current greenhouse study to characterize biosolids-P phytoavailability: bahiagrass was grown in columns that contained ~30 cm of base sand which underlaid ~15 cm of P-source amended Immokalee A horizon. A strong relationship (r^2 = .82) existed between cumulative biosolids-P uptake and the labile P load in the two short-term greenhouse studies (Fig. 3-14). The estimates of biosolids RPP determined in the two short-term greenhouse studies also correlated well (r^2 = .81) with biosolids PSI (Fig. B-2). O'Connor et al. (2004) also quantified P uptake utilizing a P-source amended Candler A horizon (RPA ≈ 15%). The relationship between cumulative biosolids-P uptake and the labile P load was less strong on the amended Candler A horizon (r^2 = .62, Fig. B-3) than the amended Immokalee A horizon (r^2 = .82, Fig. 3-14). The RPA value of Candler A horizon is nearly three times the RPA value of the Immokalee A horizon, which suggests that even modest increases in P retention capacity can decrease the ability of an *a priori* measure of biosolids-P to predict biosolids-P phytoavailability once the biosolids are applied to soils.

A single, large dataset that described the relationship between cumulative biosolids-P uptake and the labile P load was desired; however, combining cumulative P uptake data from the short-term greenhouse studies with the prolonged (16-month) greenhouse cumulative P uptake data was not appropriate because cumulative P uptake values differed appreciably between the studies. Cumulative P uptake was determined over 4 harvests for the O'Connor et al. (2004) and Chinault (2007) studies, whereas cumulative P uptake was determined over 12 harvests in the current greenhouse study. However, relative estimates of biosolids-P phytoavailability in the short-term and long-term studies could be combined into a single dataset. Short-term estimates of biosolids RPP (O'Connor et al., 2004; Chinault, 2007) were combined with long-term estimates of biosolids RPP (Oladeji, 2006; the current greenhouse study) were combined into a single dataset to determine an overall relationship between short and long-term estimates of biosolids RPP and biosolids PSI values. A good relationship (r^2 = .70) exists between the short-term and long-term estimates of biosolids RPP and biosolids PSI values (Fig. 3-15). These data suggest that, although long-term estimates of biosolids RPP are greater than short-term RPP estimates (Table 3-3), biosolids PSI is a useful *a priori* indicator of biosolids-P relative phytoavailability regardless of whether the RPP values are long-term or short-term estimates.

O'Connor and Elliott (2006) suggested PWEP is a potentially useful indicator of biosolids RPP. The PWEP value is likely a good indicator of the immediately available P pool, but does not sufficiently quantify the entire labile P pool ultimately available to plants. Biosolids PSI better describes the ultimately phytoavailable P pool by utilizing an oxalate extraction to quantify labile P and the reactive Fe and Al capable of retaining potentially phytoavailable P. Thus, biosolids with the greatest ratio of oxalate extractable-P to oxalate extractable-Fe+Al (greatest PSI values) represent the most phytoavailable biosolids-P sources. Notably, the PSI concept is not applicable to all biosolids. Alkaline stabilized biosolids (and the chemistry of P contained therein) are dominated by Ca, and the PSI concept is not applicable to these materials (Elliott et al., 2002).

Overall P Lability

Previous greenhouse studies often considered the environmental lability and phytoavailability of biosolids-P separately and did not address differences in overall (agronomic and environmental) lability. Combining cumulative P leached with cumulative P uptake characterizes the overall lability of P-sources. Leaching of P contributed little to the overall P lability at the P-based application rates, and P-source lability differences resembled P-uptake differences. The overall P lability values of more soluble-P biosolids (BPR and BPR-like biosolids) were similar to values for TSP, and Milorganite-P was significantly less labile than the more soluble-P biosolids and TSP-P (Fig. 3-16A).

Leaching of P represented an appreciable portion of the overall P lability for the more soluble-P biosolids and TSP applied at the N-based rate. The overall P lability of more soluble-P biosolids was statistically similar to TSP. At the N-based application rate, Milorganite-P was significantly less labile than all other P-sources, and all less soluble-P biosolids were significantly less labile than more soluble-P biosolids, and TSP (Fig. 3-16B). Moreover, the clear difference in overall P lability between less soluble-P biosolids and BPR biosolids, BPR-like biosolids, and TSP was distinguished by overall P lability comparisons across rates (Fig. 3-17).

The RPP value describes the relative agronomic effectiveness of biosolids-P and only considers P uptake. Incorporating P leaching with P uptake to quantify overall P lability characterizes the overall relative P lability (RPL) of the biosolids. Biosolids RPL estimates provided a better indication of ultimate P release differences between biosolids and TSP than biosolids RPP estimates because the RPL considers P lost to leaching. The slope-ratio method used to determine biosolids RPP values was similarly utilized to estimate the RPL of biosolids (Fig. 3-18). Point estimates of biosolids RPL were also utilized to validate slope-ratio RPL estimates.

Slope-ratio RPL estimates suggest less soluble-P biosolids are 37-64% as overall labile as TSP, and that BPR and BPR-like biosolids-P overall lability is approximately equal to TSP-P (Table 3-5). The greatest mass of P leached in TSP treatments; thus, incorporating P leaching decreased biosolids RPL estimates compared to RPP estimates. Estimates of RPL were appreciably less than RPP estimates for less soluble-P biosolids because these P-sources leached relatively little P.

Point RPL estimates suggest that BPR and BPR-like biosolids are about 90-120% as labile as TSP and that less soluble-P biosolids are roughly 50-75% as labile as TSP-P (Table 3-6). Point estimates of biosolids RPL values are similar to slope-ratio estimates of biosolids RPL, although point RPL estimates are approximately 10% greater than slope-ratio estimates of biosolids RPL for all biosolids except GRU. Point RPL estimates at each rate suggest appreciable changes in RPL values occur from P-based to N-based application rates. Relatively little P leaching occurred at the 56 and 112 kg P ha^{-1} application rates, and RPL estimates are similar to RPP estimates. The greatest relative P leaching difference between biosolids and TSP occurred at the 224 kg P ha^{-1} application rate, resulting in smaller RPL estimates at the N-based application rate than the 56 and 112 kg P ha^{-1} application rates.

Slope-ratio and point RPL estimates increased approximately 20% from the 4th harvest to the 12th harvest. Short-term RPL estimations tended to underestimate the long-term overall relative lability of biosolids-P; however, four harvests were sufficient to distinguish the relative differences among P-sources and determine the general category of biosolids RPL. Despite the increase in RPL estimates from harvest 4 to 12, less soluble biosolids-P remained only about one-half to three-fourths as labile as TSP-P. The long-term overall lability of BPR and BPR-like biosolids-P is similar to TSP-P, and application rates of these more soluble-P biosolids should not exceed agronomic or environmental fertilizer-P recommendations.

A stepwise regression was utilized to determine a useful *a priori* measure of ultimate biosolids-P lability. The same method utilized to correlate cumulative biosolids-P uptake to the measures of biosolids-P determined by Chinault (2007) was used to correlate overall biosolids-P lability to the total-P, PWEP, oxalate extractable P, iron strip-P, and PSI values of the biosolids. Labile P load (biosolids PSI*total-P load) was the best predictor of overall biosolids-P lability, and explained 90% of the variability in overall biosolids-P lability (Fig. 3-19). Biosolids RPL values were also correlated to biosolids PSI values to validate biosolids PSI as a useful *a priori* indicator of biosolids-P lability. A good linear relationship (r^2=.79) exists between biosolids RPL values and biosolids PSI values (Fig. 3-20). Recall the labile P load explained 85% of the variability in cumulative biosolids-P uptake (Fig. 3-12). Biosolids PSI was also a useful indicator of biosolids-P uptake because P uptake was the predominant mechanism of P removal. The labile P load correlated with overall P lability better than cumulative P uptake, which suggests that the biosolids PSI most accurately predicts ultimately labile biosolids-P—soluble P lost to leaching and P ultimately available to plant roots.

Further validation of biosolids PSI value as a useful *a priori* indicator of ultimate biosolids-P lability was sought. O'Connor et al. (2002) and Chinault (2007) conducted short-term (4-month) greenhouse studies that utilized methods identical to the current greenhouse study to characterize P uptake and P leaching from a P-source amended Immokalee A horizon. A strong, linear relationship ($r^2 = .80$) existed between overall P lability and the labile P load in the two short-term studies (Fig. 3-21), suggesting that the labile P load is also a useful *a priori* indicator of short-term biosolids-P lability.

Chinault (2007) conducted a 5.5 month laboratory incubation study using a bare Immokalee soil to characterize ultimate P environmental lability (release and leaching) differences among biosolids. A clear, quantitative relationship existed between cumulative P leaching and the labile P load; however, the labile P load only explained 73% of the variability in the cumulative biosolids-P leaching (Fig. 3-22). Chinault (2007) utilized a bare soil, whereas biosolids were subjected to plant root interactions for more than 16 months in the current greenhouse study. Plant roots can exude organic acids to increase P availability (Vance et al., 2003), and P leaching from a bare soil likely does not sufficiently characterize biosolids-P that is ultimately labile in a grassed pasture system. Thus, cumulative biosolids-P leaching correlated more poorly with the labile P load than overall biosolids-P lability (P uptake and leaching).

Agyin-Birikorang (2008) showed that the environmentally effective P load (PWEP*total-P load) an excellent predictor of P loss in rainfall simulation studies utilizing bare soils. However, the environmentally effective P load underestimated biosolids-P lability in the current greenhouse study (Table A-1). Nevertheless, both the labile P load and the environmentally effective P load are useful indicators of biosolids-P release, provided the indicators are correctly applied to the appropriate P loss mechanisms. The environmentally effective P load best indicates the readily soluble P that is potentially lost to runoff and leaching during rainfall events, but the labile P load is a better predictor of ultimately labile biosolids-P, especially when plants are grown.

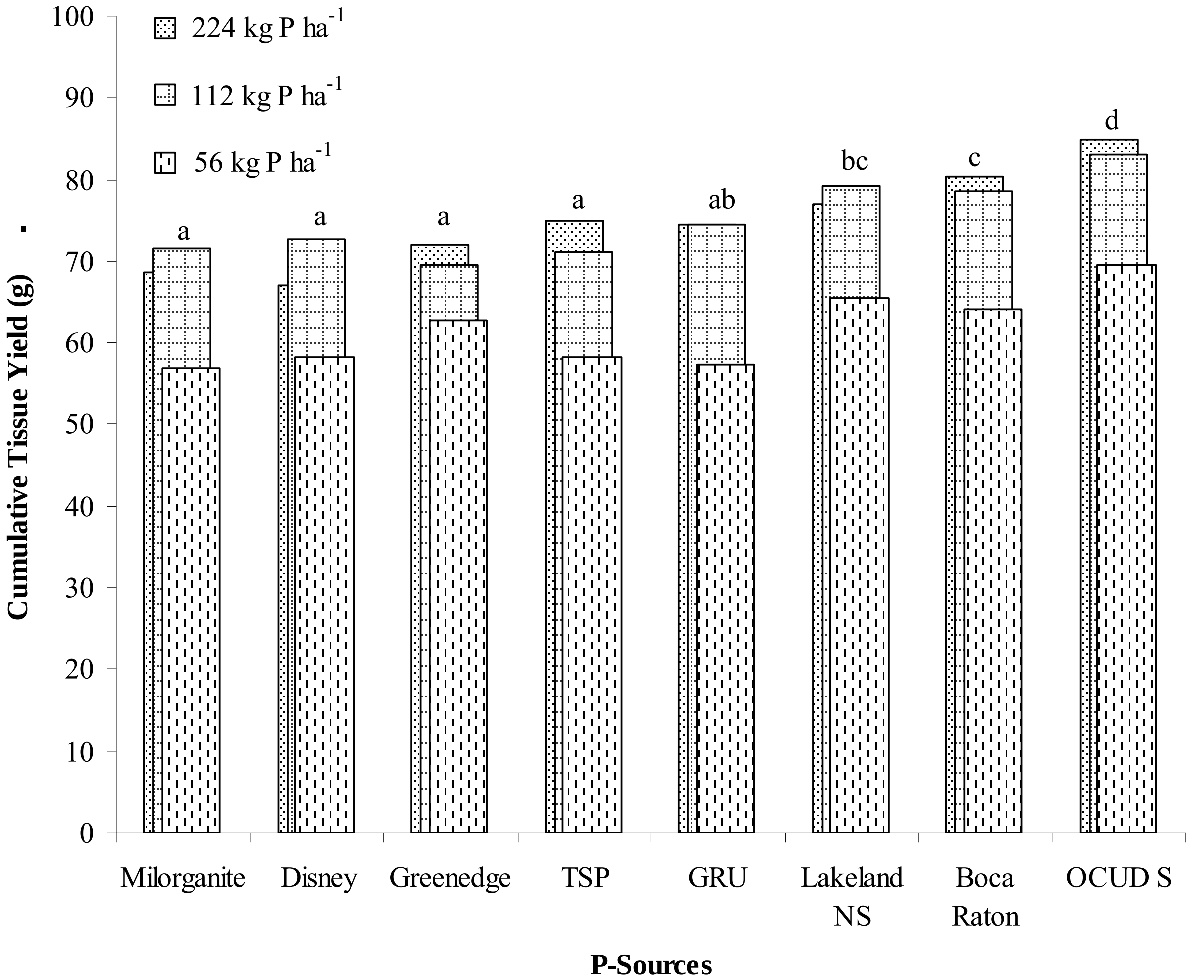


Figure 3-1. Cumulative bahiagrass tissue yields following the 16-month greenhouse study. Cumulative tissue yields were determined after 12 harvests for the 112 and 224 kg P ha^{-1} rates, and 11 harvests for the 56 kg P ha^{-1} rate. Letter designations indicate statistical differences among P-sources across application rates.

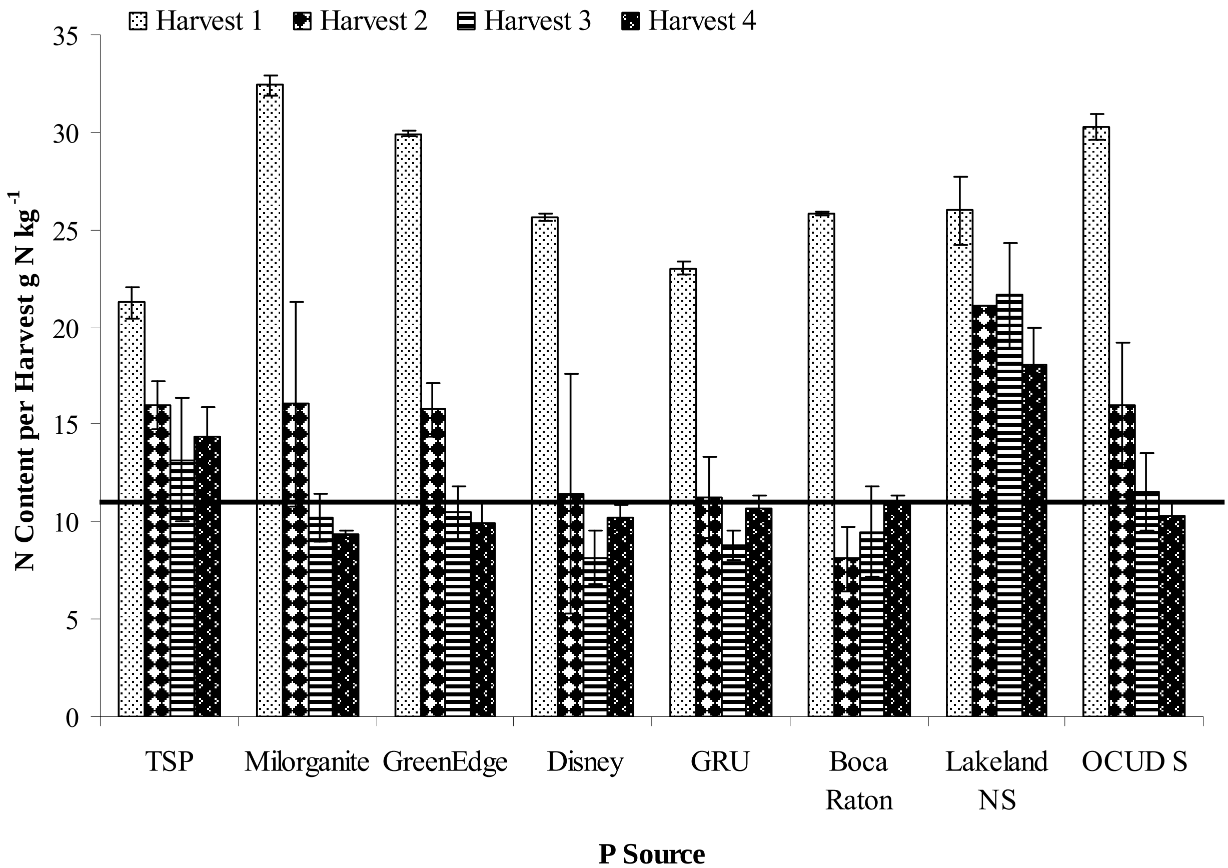


Figure 3-2. Bahiagrass tissue N contents for harvests 1-4. The solid horizontal line represents a tissue N concentration of 11 g N kg^{-1}, regarded as minimally sufficient for grazing beef cattle (NRC, 1996).

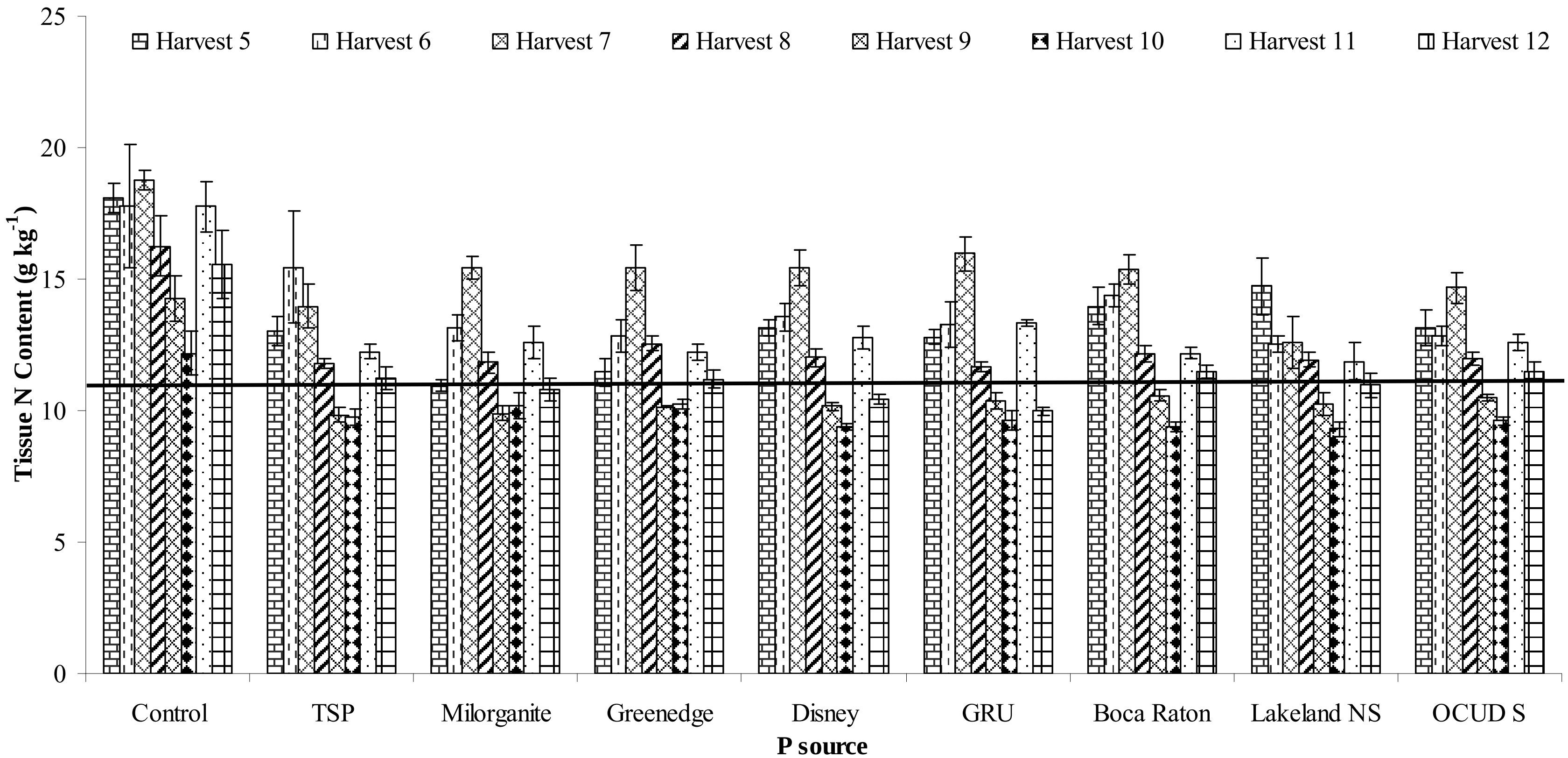


Figure 3-3. Bahiagrass tissue N concentrations for harvests 5-12. The solid horizontal line represents a tissue N concentration of 11 g N kg^{-1}, regarded as minimally sufficient for grazing beef cattle (NRC, 1996). The nitrogen fertilization regime was changed following harvest 5 to supply more PAN as ammonium nitrate.

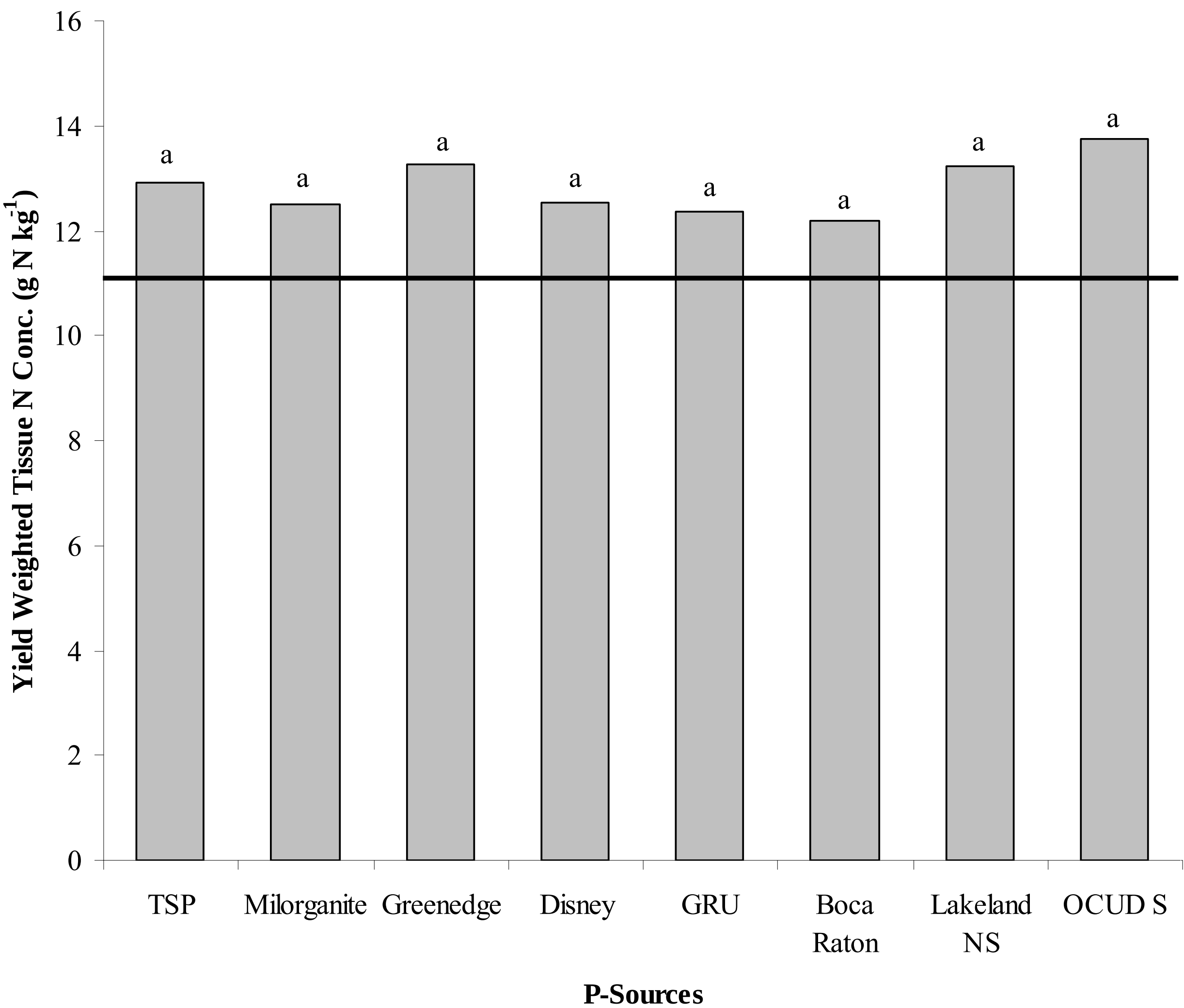


Figure 3-4. Yield-weighted tissue N concentrations for N-based rate treatments determined over 16 months of bahiagrass growth. The solid horizontal line represents a tissue N concentration of 11 g N kg^{-1}, regarded as minimally sufficient for grazing beef cattle (NRC, 1996). The letter designations indicate that no significant differences among P-sources existed.

Table 3-1. Time series analysis of agronomic and environmental P lability for the N-based application rate.

P-Source	Tissue P concentration ----Harvest---- 4	8	11	Time series*	Cumulative P uptake ---Harvest--- 4	8	11	Time series*	Cumulative P leached ----Harvest---- 4	8	11	Time series*	Cumulative P lability ----Harvest---- 4	8	11	Time series*
	-----g P kg^{-1} -----				-------mg------				--------mg-------				--------mg------			
TSP	2.37	1.40	1.05	a→b→b	106	142	177	a→b→c	33.6	45.0	51.7	a→a→a	139	187	228	a→b→c
Milorganite	1.22	1.21	1.42	a→a→a	31	53	89	a→b→c	0.1	0.1	0.2	a→ab→b	31	54	89	a→b→c
Greenedge	2.10	1.65	1.50	a→b→b	54	82	126	a→b→c	0.1	0.2	0.4	a→a→b	54	82	126	a→b→c
Disney	2.46	1.97	1.59	a→b→c	58	93	140	a→b→c	0.1	0.7	1.2	a→b→b	59	94	141	a→b→c
GRU	2.60	1.71	1.36	a→b→c	99	137	175	a→b→c	13.6	25.3	32.5	a→a→a	113	162	208	a→b→c
Boca Raton	2.79	2.05	1.71	a→b→b	95	144	200	a→b→c	5.1	14.5	20.8	a→ab→b	100	159	220	a→b→c
Lakeland NS	3.44	1.74	1.58	a→b→b	58	114	168	a→b→c	6.4	23.6	34.1	a→a→a	65	138	202	a→b→c
OCUD S	2.74	2.10	1.73	a→b→c	115	172	226	a→b→c	5.2	14.6	21.3	a→b→b	120	186	248	a→b→c

* Letters indicate statistical differences among values determined at the 4th, 8th, and 12th harvests.

Table 3-2. Time series analysis of agronomic and environmental P lability for the P-based application rate.

P-Source	Tissue P concentration				Cumulative P uptake				Cumulative P leached				Cumulative P lability			
	----Harvest----			Time	--Harvest--			Time	---Harvest---			Time	--Harvest--			Time
	4	8	11	series*	4	8	11	series*	4	8	11	series*	4	8	11	series*
	-----g P kg^{-1} -----				------m-----				------mg------				-----mg-----			
TSP	1.16	0.72	0.76	a→b→b	49	67	79	a→b→b	0.0	0.1	0.2	a→b→c	49	67	79	a→b→b
Milorganite	1.07	0.79	0.87	a→b→b	24	42	55	a→b→c	0.1	0.1	0.1	a→a→a	24	43	55	a→b→c
Greenedge	1.13	0.88	0.93	a→ab→b	30	51	66	a→b→b	0.0	0.1	0.2	a→ab→b	31	51	66	a→b→b
Disney	1.16	0.80	0.85	a→b→b	37	57	70	a→b→b	0.0	0.1	0.2	a→ab→b	37	57	70	a→b→b
GRU	1.34	0.74	0.98	a→b→b	45	63	75	a→b→b	0.1	0.2	0.2	a→b→b	45	63	75	a→b→b
Boca Raton	1.28	0.96	1.04	a→b→b	45	67	85	a→b→b	0.1	0.1	0.2	a→b→c	45	67	85	a→b→b
Lakeland NS	1.35	0.80	0.95	a→b→b	53	76	89	a→b→c	0.1	0.2	0.3	a→b→c	53	76	89	a→b→c
OCUD S	1.24	0.91	0.88	a→b→b	53	78	95	a→b→c	0.1	0.2	0.2	a→b→b	53	78	95	a→b→c

* Letters indicate statistical differences among values determined at the 4th, 8th, and 11th harvests.

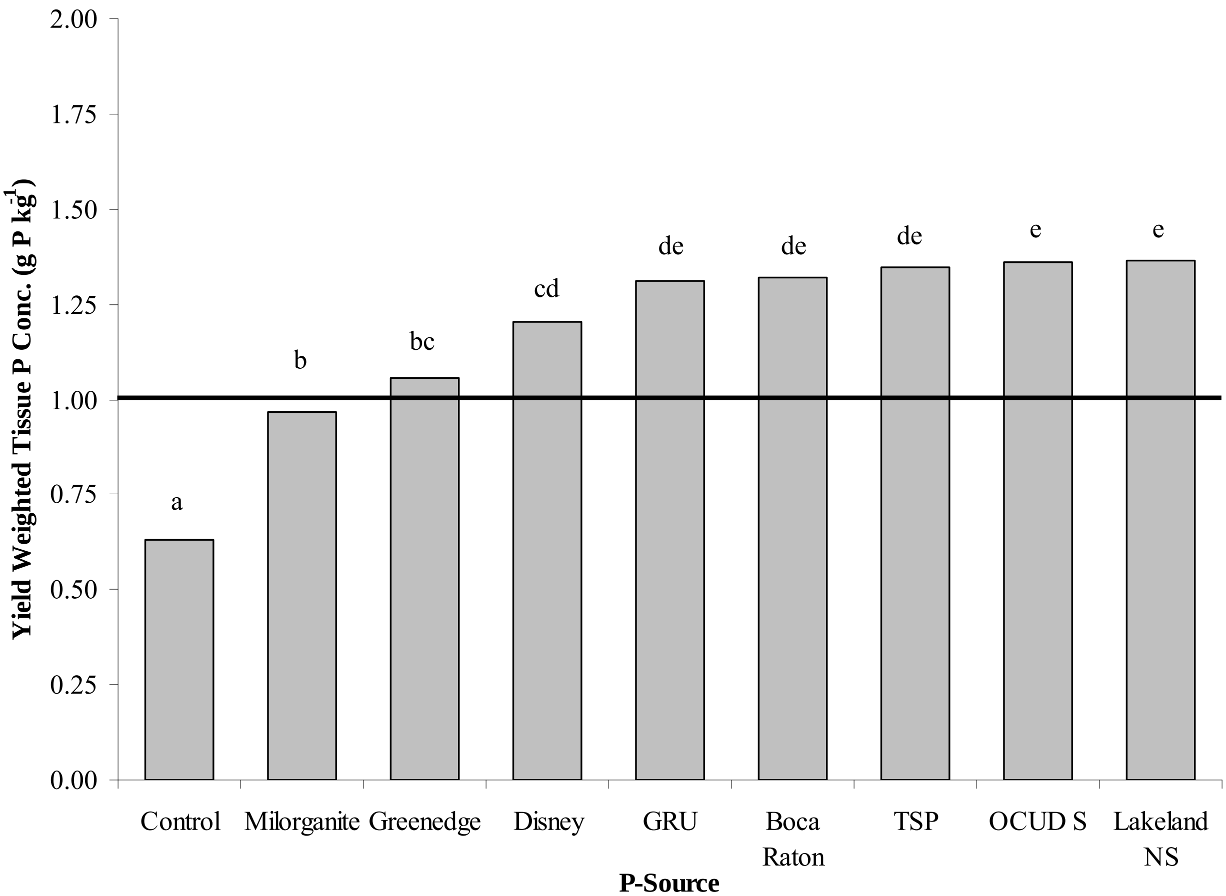


Figure 3-5. Bahiagrass yield-weighted tissue P concentrations (harvests 1-11) for the P-based and control treatments. The horizontal line represents a critical tissue P concentration of 1 g P kg^{-1} (O'Connor and Elliott, 2006).

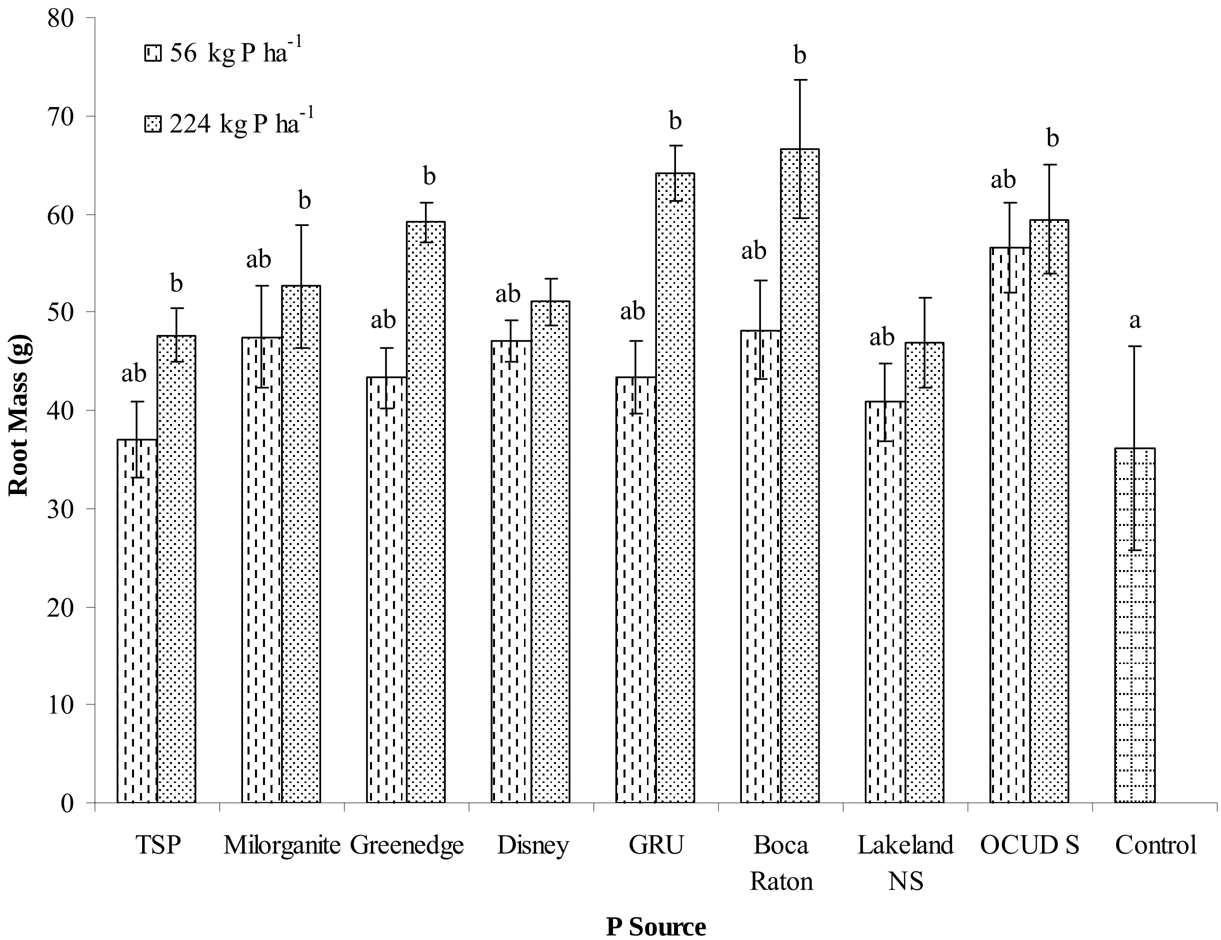
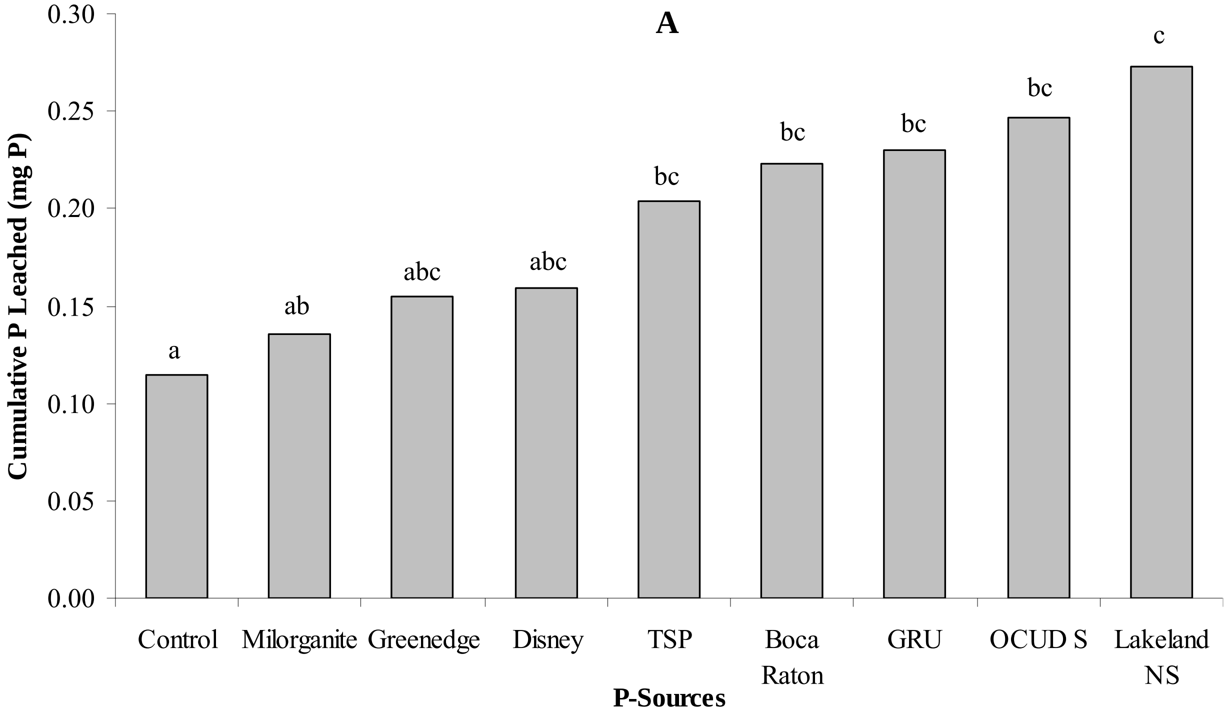


Figure 3-6. Bahiagrass root masses for N-based (224 kg P ha^{-1}) and P-based (56 kg P ha^{-1}) rate treatments. Average N-based rate root masses were significantly different from the control treatment root masses across P-sources.



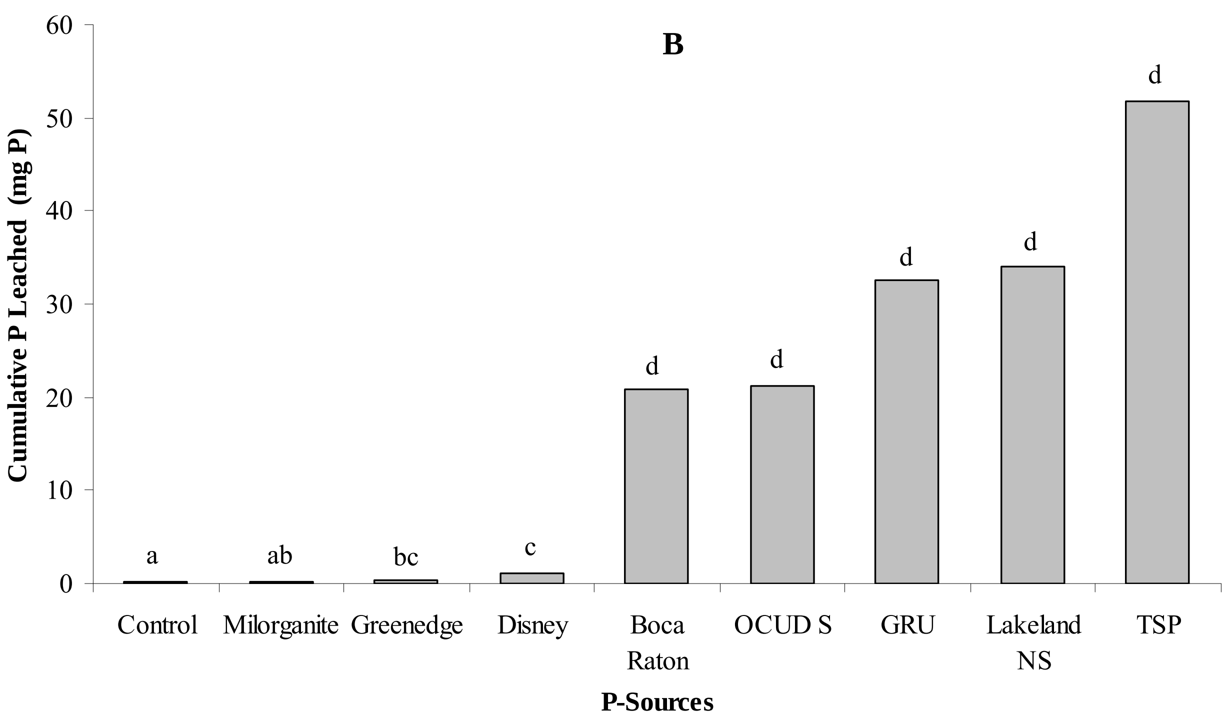


Figure 3-7. Cumulative P leached as a function of P-source for A) P-based rate (56 kg P ha^{-1}) treatments and B) N-based rate (224 kg P ha^{-1}) treatments. Letters indicate significant differences among P-sources. Note the scale differences in the figures.

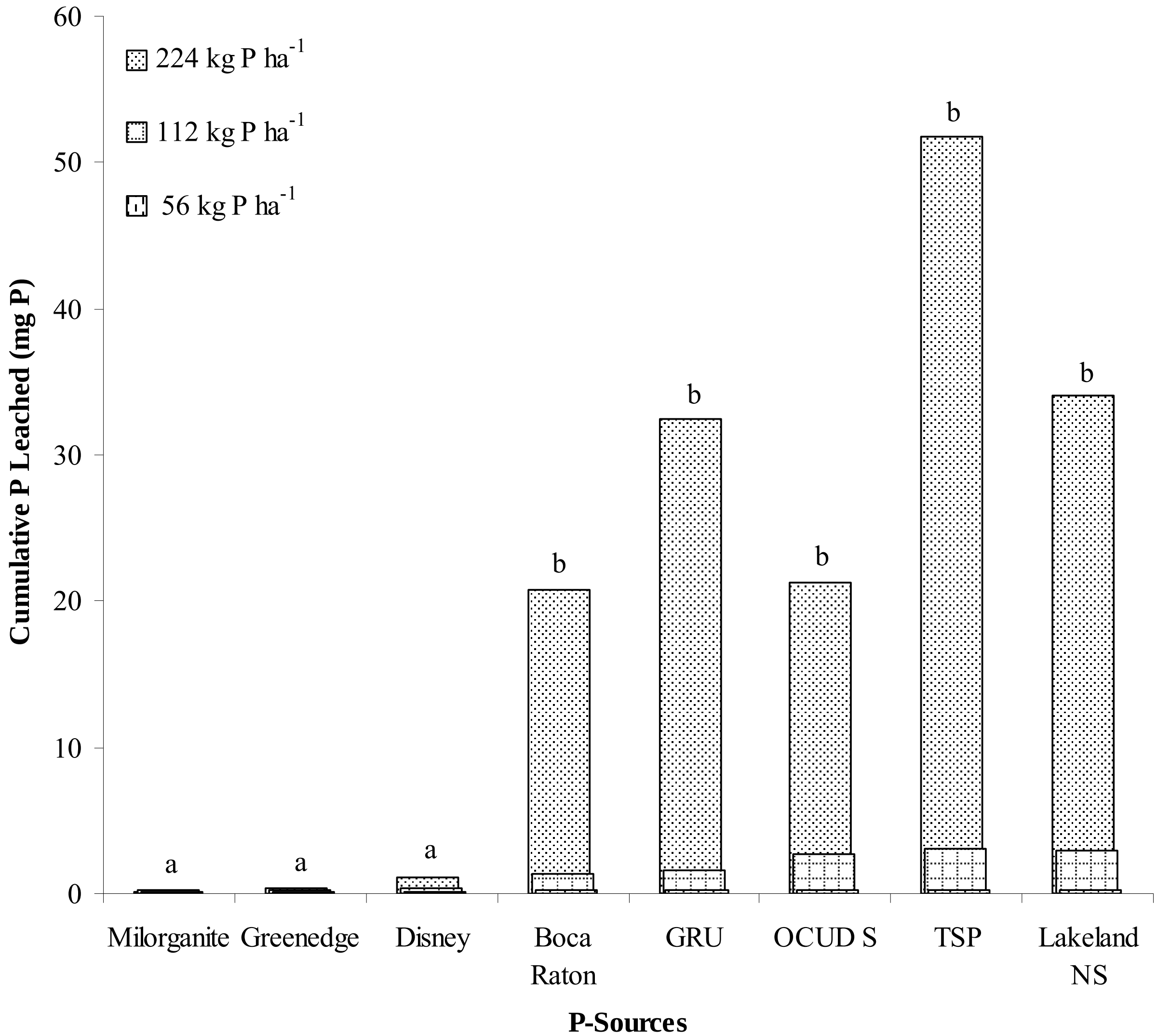
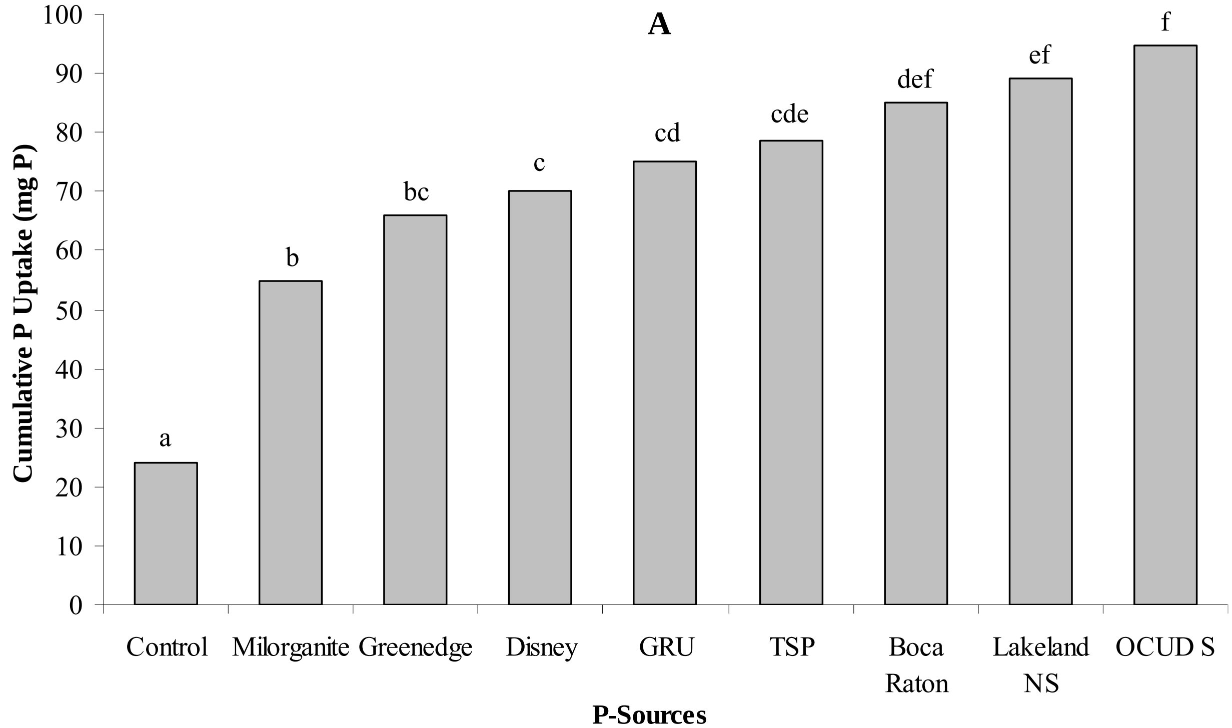


Figure 3-8. Environmental P lability (P leached) differences among P-sources at different rates. Letters indicate statistical differences among P-sources across application rates.



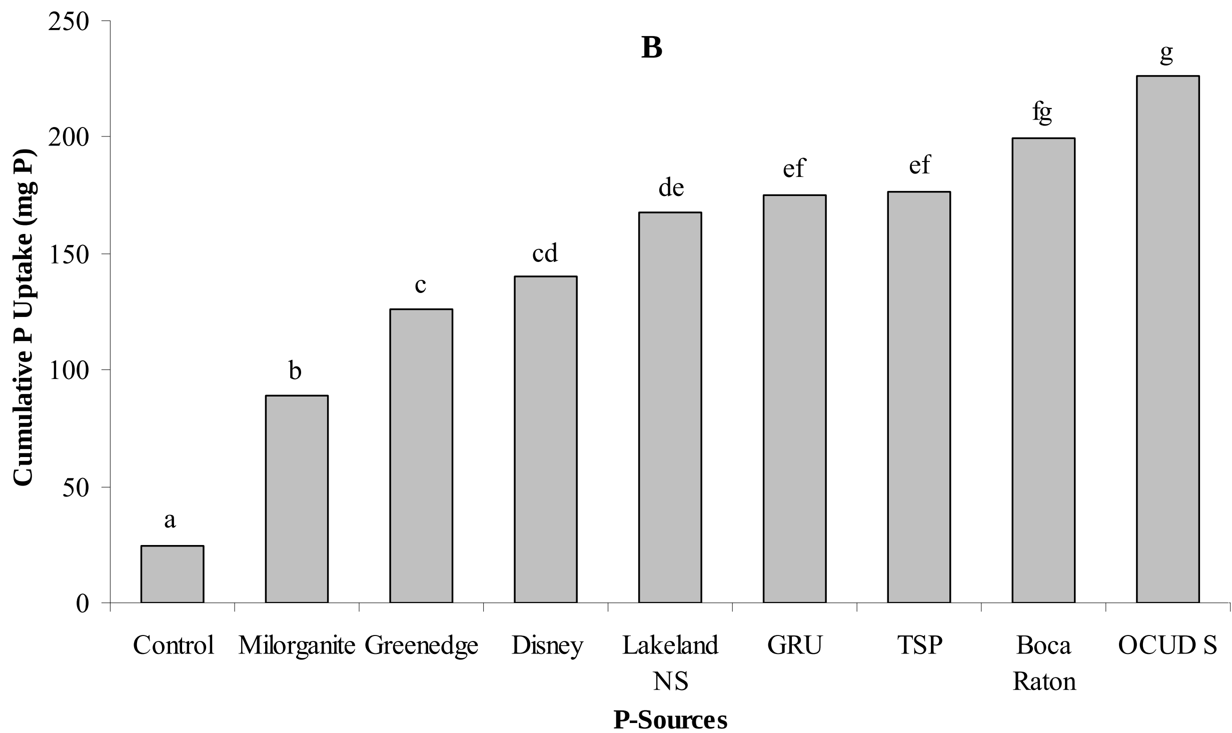


Figure 3-9. Cumulative bahiagrass P uptake as a function of P-source for A) P-based rate (56 kg P ha^{-1}) treatments and B) N-based rate (224 kg P ha^{-1}) treatments. Letters indicate significant differences among P-sources.

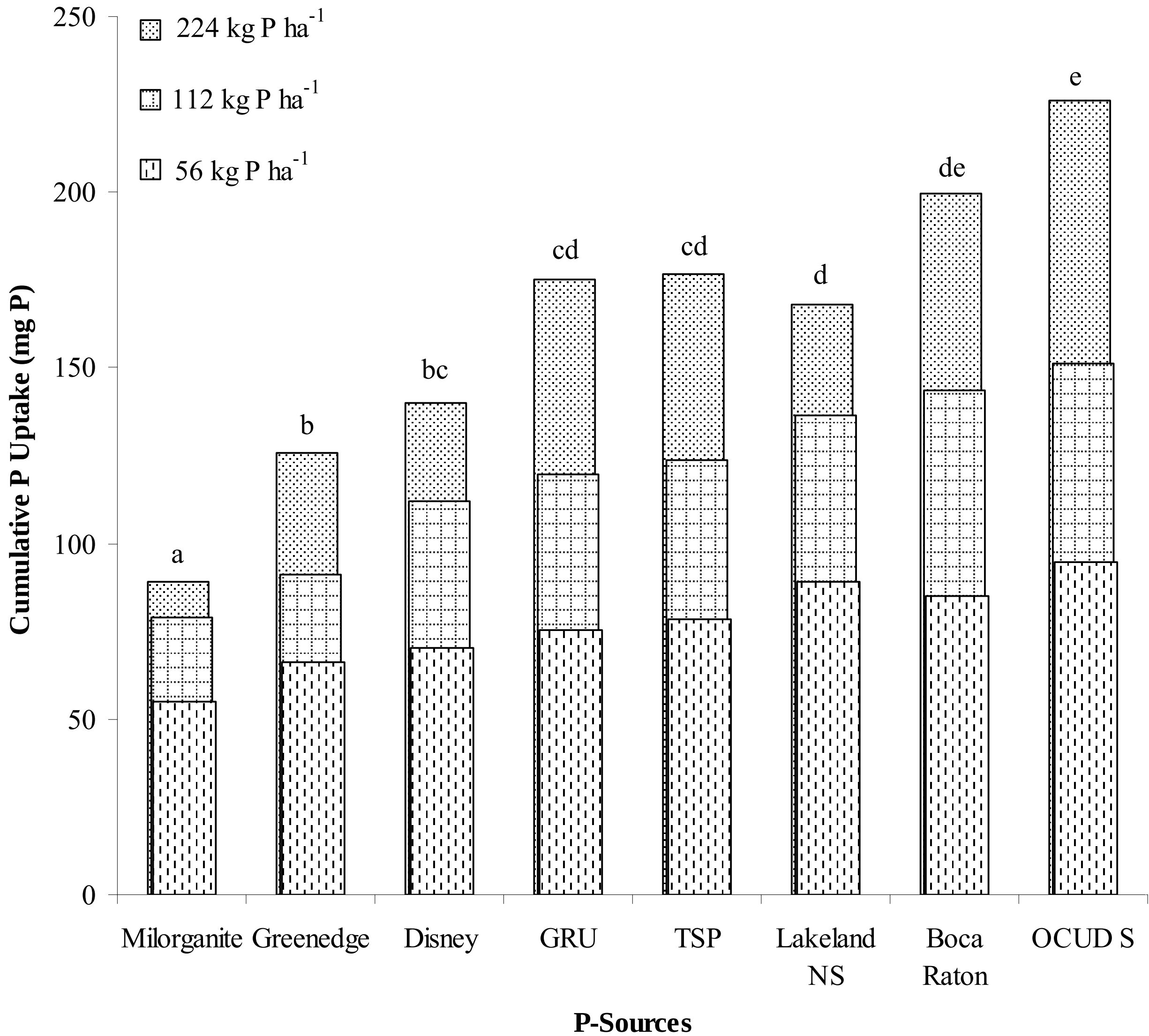


Figure 3-10. Cumulative P uptake differences among P-sources. Letters indicate statistical differences across application rates.

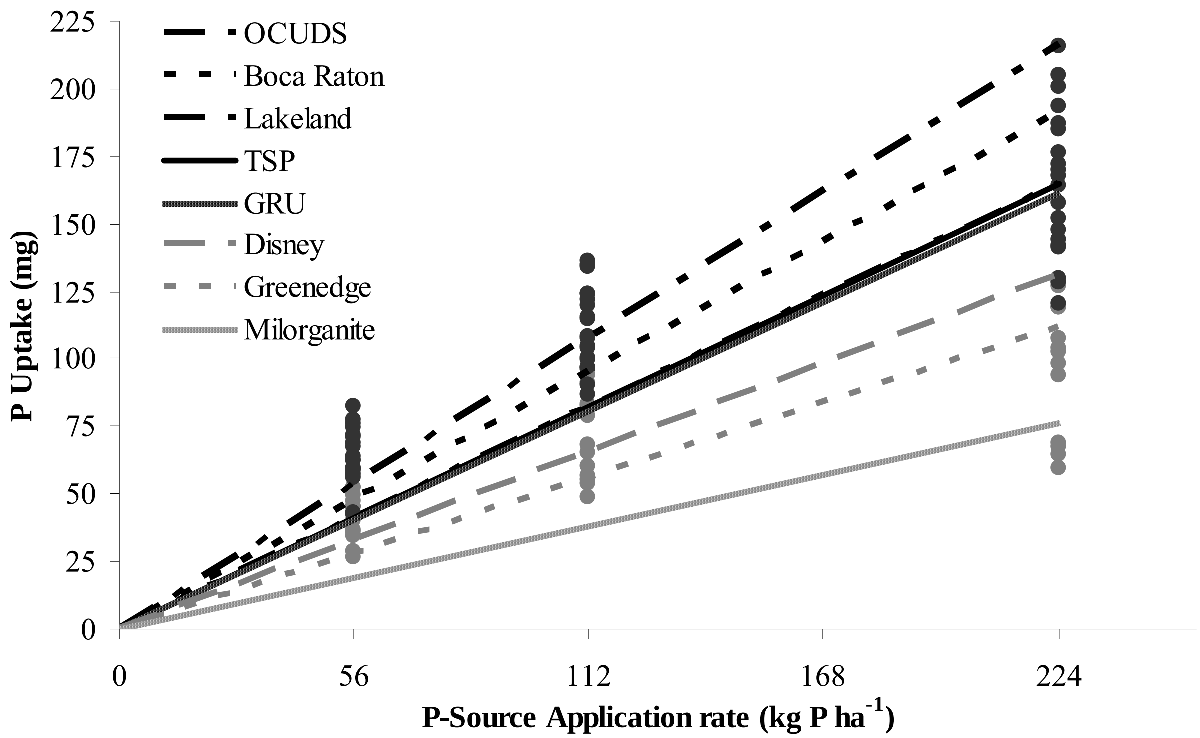


Figure 3-11. Relative P phytoavailability curves for P-sources. Control P uptake was subtracted from treatment cumulative P uptake values, and the regression lines were forced through zero.

Table 3-3. Slope-ratio estimates of biosolids relative P phytoavailability for harvest 4 and harvest 12.

	Determined after 4 harvests				Determined after 12 harvests[Ŧ]			
P Source	r^2	Regress. coeff.	RPP (%)	RPP category	r^2	Regress. coeff.	RPP (%)	RPP category
TSP	0.96	0.472	100	High	0.98	0.736	100	High
Milorganite	0.87	0.122	26	Mod.	0.92	0.342	46	Mod.
Greenedge	0.96	0.222	47	Mod.	0.96	0.498	68	Mod.
Disney	0.91	0.265	56	Mod.	0.95	0.585	79	High
GRU	0.97	0.439	93	High	0.98	0.722	98	High
Boca Raton	0.95	0.429	91	High	0.97	0.854	116	High
Lakeland NS	0.85	0.439	93	High	0.93	0.737	100	High
OCUD S	0.97	0.519	110	High	0.98	0.966	131	High

Ŧ RPP estimates for 56 kg P ha^{-1} rate amended columns were determined after 11 harvests

Table 3-4. Point estimates of biosolids relative P phytoavailability following harvest 4 and harvest 12.

		Determined after 4 harvests			Determined after 12 harvests[Ŧ]		
P Source	Rate	Point RPP	Average	Category	Point RPP	Average	Category
TSP	56	100	100	High	100	100	High
	112	100			100		
	224	100			100		
Milorganite	56	38	31	Moderate	58	52	Moderate
	112	31			55		
	224	23			43		
Greenedge	56	54	49	Moderate	78	71	Moderate
	112	46			67		
	224	47			67		
Disney	56	69	62	Moderate	85	83	High
	112	67			88		
	224	51			76		
GRU	56	90	92	High	94	96	High
	112	94			96		
	224	93			99		
Boca Raton	56	88	92	High	111	115	High
	112	98			120		
	224	89			115		
Lakeland NS	56	109	96	High	119	109	High
	112	106			113		
	224	73			94		
OCUD S	56	108	110	High	128	130	High
	112	113			128		
	224	109			133		

[Ŧ] RPP estimates for 56 kg P ha^{-1} rate amended columns were determined after 11 harvests

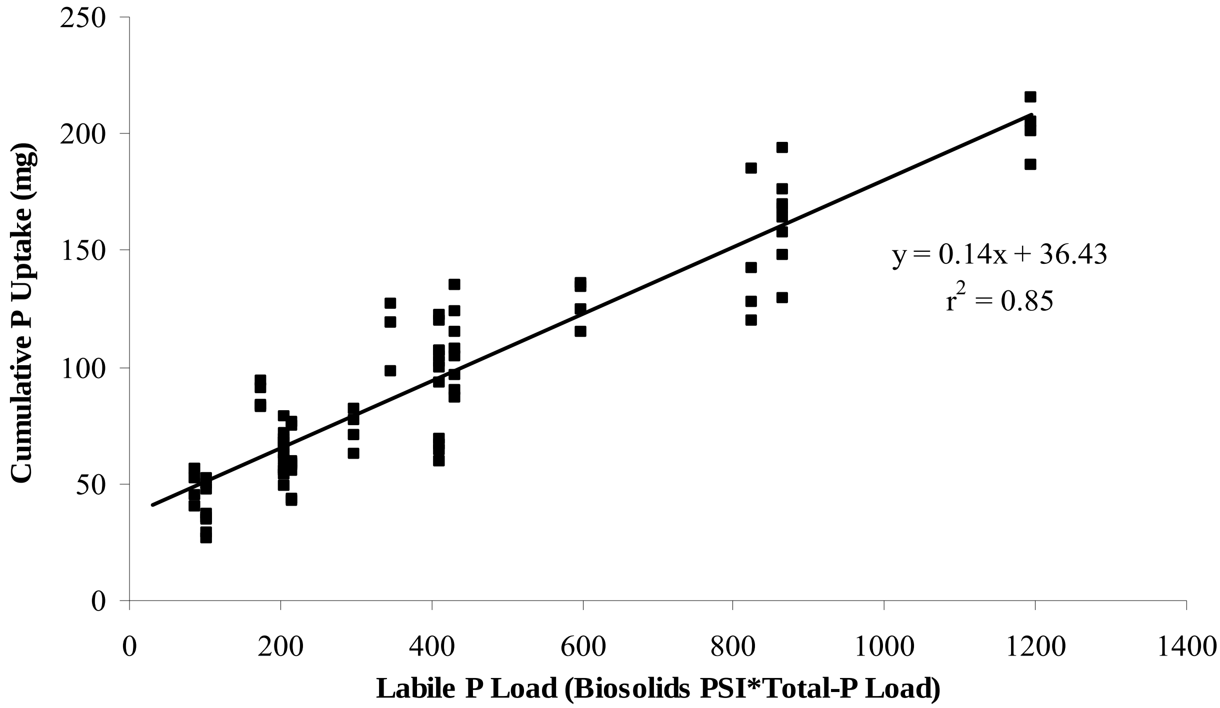


Figure 3-12. Cumulative P uptake plotted as a function of the labile P load (biosolids PSI*total-P load).

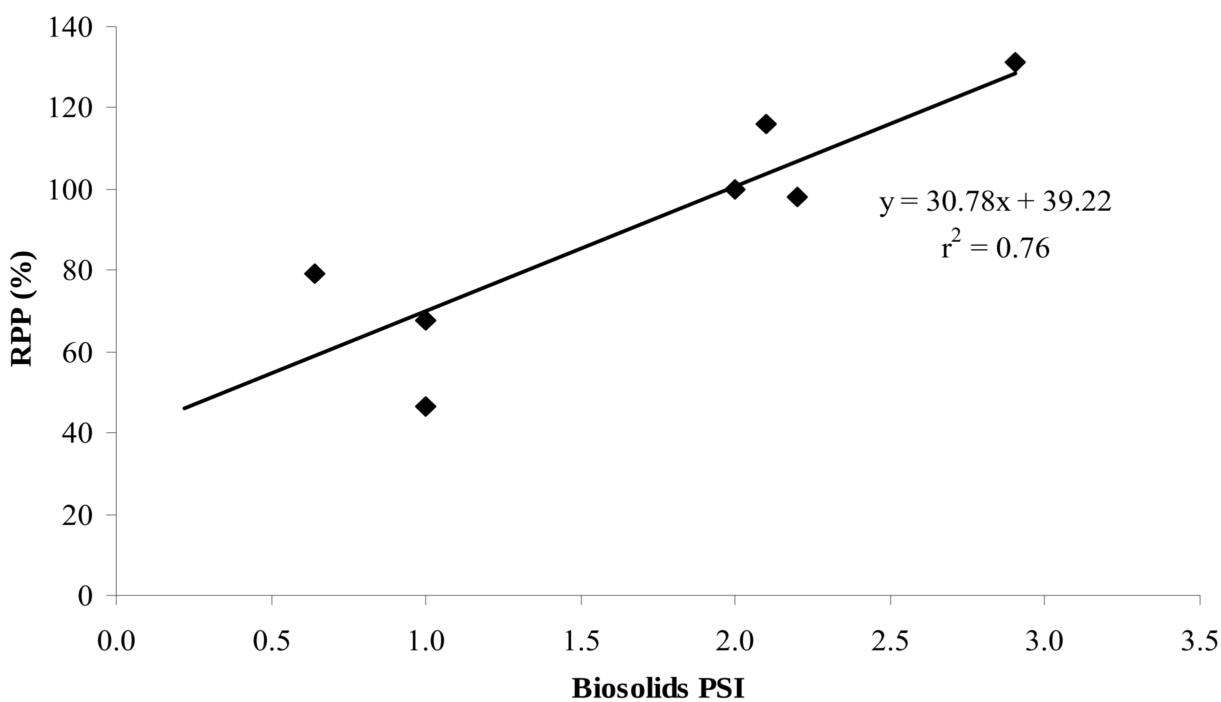


Figure 3-13. Biosolids relative P phytoavailability (RPP) values plotted as a function of biosolids phosphorus saturation index (PSI) values.

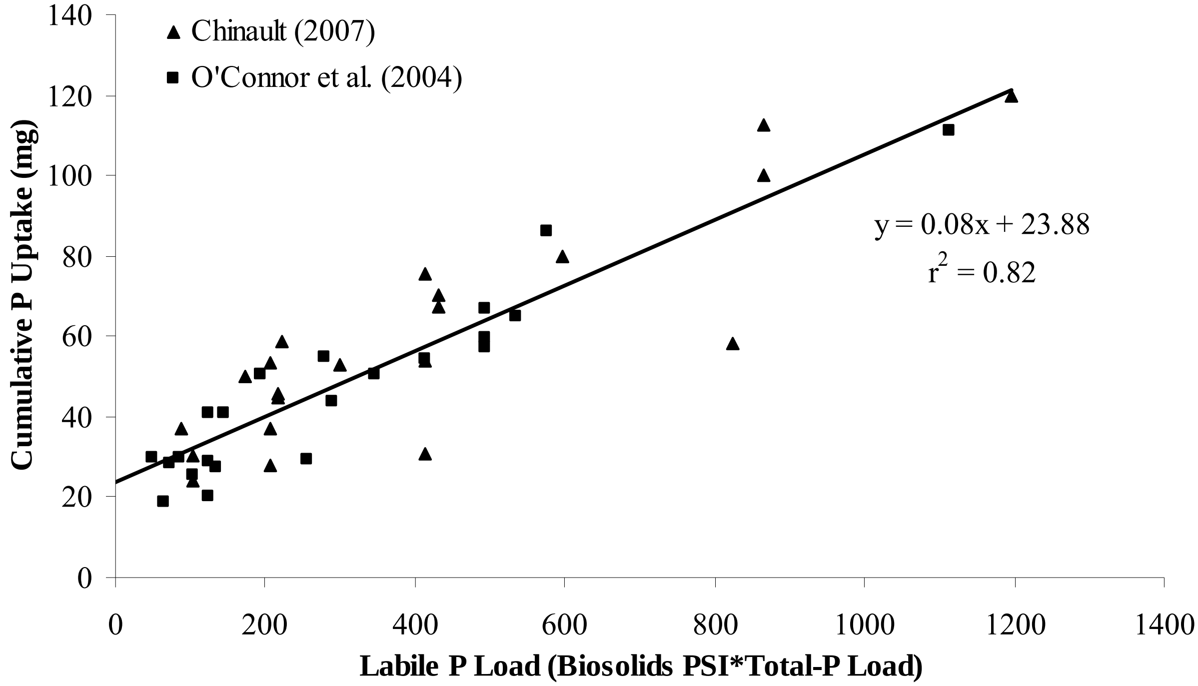


Figure 3-14. Relationship between cumulative P uptake and the labile P load for two previously conducted short-term studies greenhouse studies (O'Connor et al., 2004; Chinault, 2007).

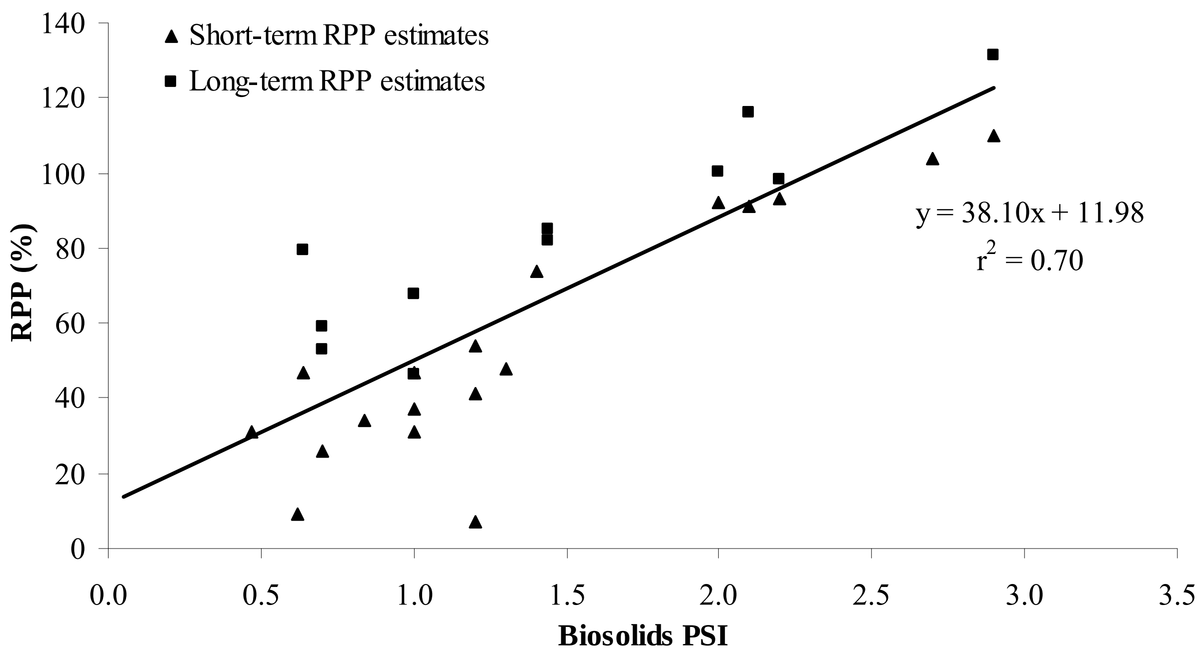
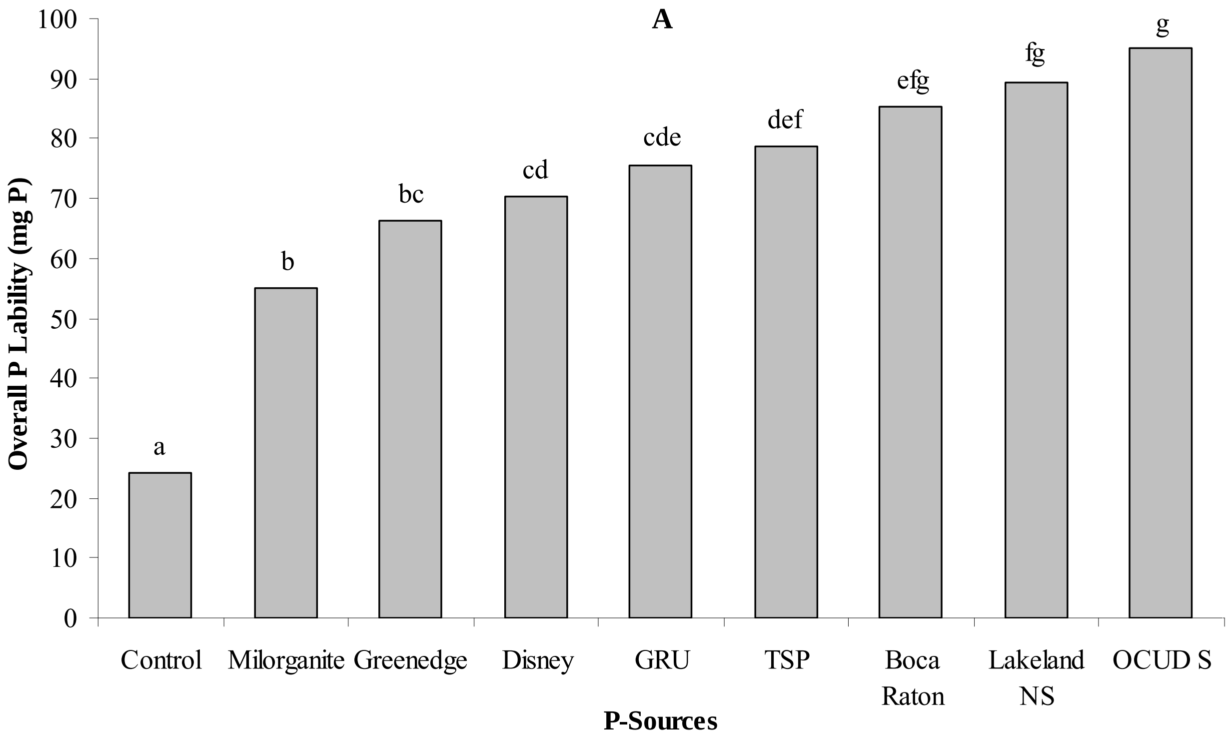


Figure 3-15. Long-term estimates (Oladeji, 2006; current greenhouse study) and short-term estimates (O'Connor et al., 2004; Chinault, 2007) of biosolids relative P phytoavailability (RPP) plotted as a function of biosolids phosphorus saturation index (PSI) values.



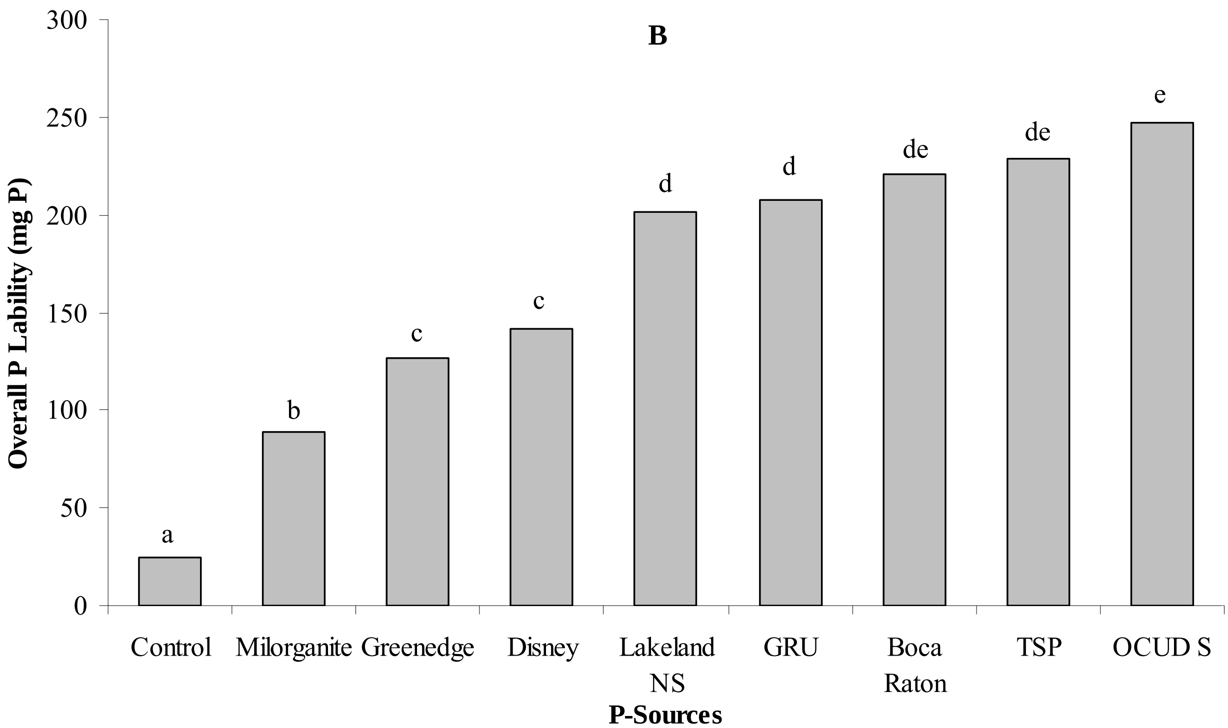


Figure 3-16. Overall P lability (agronomic and environmental) of various P-sources for A) P-based rate (56 kg P ha^{-1}) treatments and B) N-based rate (224 kg P ha^{-1}) treatments. Letters indicate significant differences among P-sources.

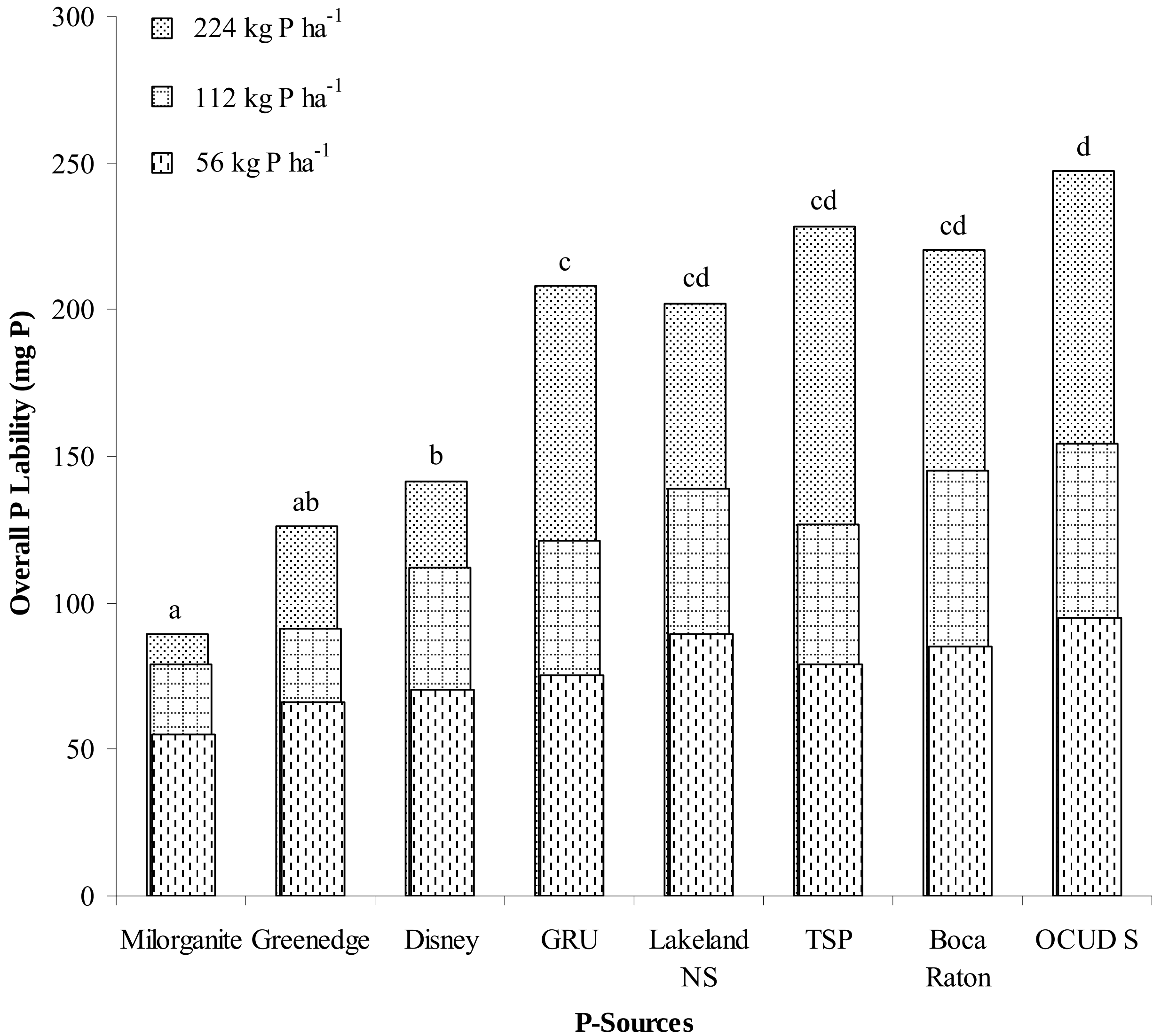


Figure 3-17. Overall P lability (agronomic and environmental) differences among P-sources. Letters indicate statistical differences across application rates.

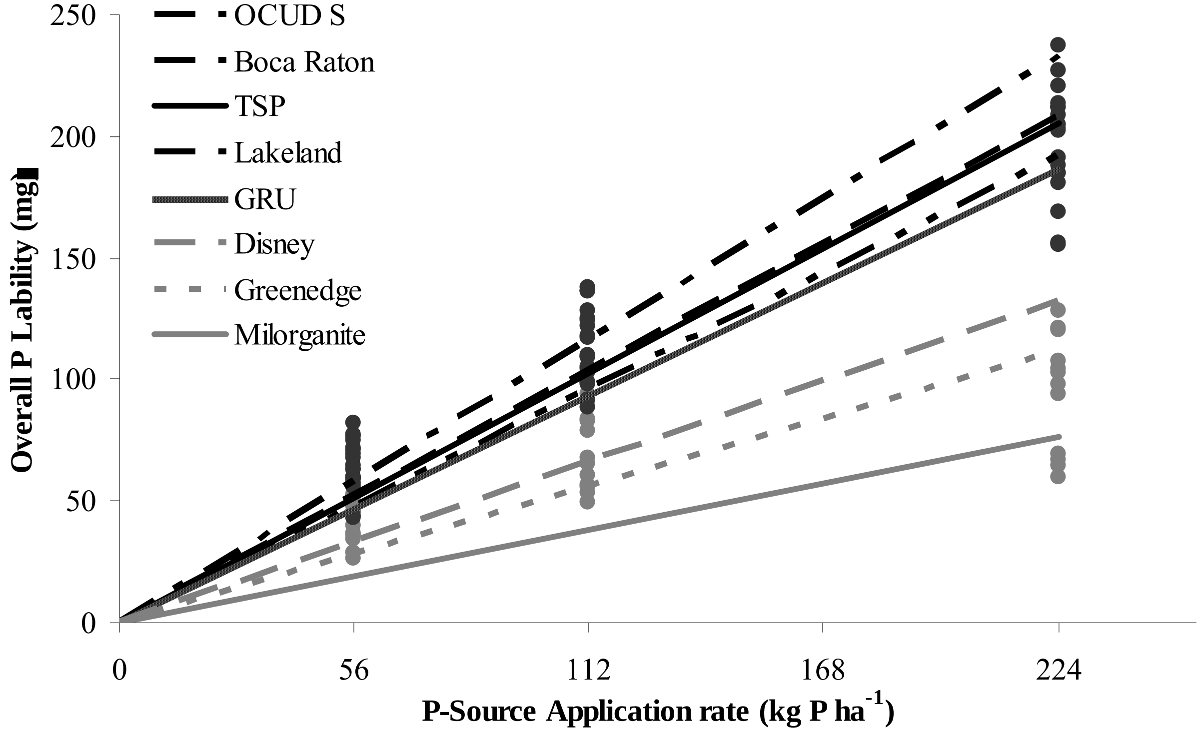


Figure 3-18. Relative P overall lability curves for P-sources. Control P lability was subtracted from treatment overall P lability values, and the regression lines were forced through zero.

Table 3-5. Slope-ratio estimates of biosolids relative P overall lability for harvest 4 and harvest 12.

	Determined after 4 harvests				Determined after 12 harvests[Ŧ]			
P Source	r^2	Regress. coeff.	RPL (%)	RPL category	r^2	Regress. coeff.	RPL (%)	RPL category
TSP	0.99	0.587	100	High	1.00	0.917	100	High
Milorganite	0.87	0.122	21	Low	0.92	0.343	37	Mod.
Greenedge	0.96	0.223	38	Mod.	0.96	0.499	54	Mod.
Disney	0.91	0.265	45	Mod.	0.95	0.589	64	Mod.
GRU	0.98	0.486	83	High	0.99	0.835	91	High
Boca Raton	0.96	0.447	76	High	0.98	0.927	101	High
Lakeland NS	0.87	0.450	77	High	0.97	0.857	94	High
OCUD S	0.97	0.538	92	High	0.99	1.043	114	High

Ŧ RPL estimates for 56 kg P ha^{-1} rate amended columns were determined after 11 harvests

Table 3-6. Point estimates of biosolids relative P overall lability for harvest 4 and harvest 12.

		Determined after 4 harvests			Determined after 12 harvests[Ŧ]		
P Source	Rate	Point RPL	Average	Category	Point RPL	Average	Category
TSP	56	100	100	High	100	100	High
	112	100			100		
	224	100			100		
Milorganite	56	38	28	Moderate	58	48	Moderate
	112	30			54		
	224	17			32		
Greenedge	56	54	45	Moderate	78	65	Moderate
	112	45			65		
	224	35			50		
Disney	56	69	58	Moderate	85	76	High
	112	66			86		
	224	38			57		
GRU	56	90	88	High	94	93	High
	112	93			95		
	224	80			90		
Boca Raton	56	88	85	High	111	108	High
	112	97			118		
	224	70			96		
Lakeland NS	56	110	91	High	119	106	High
	112	106			112		
	224	57			87		
OCUD S	56	109	102	High	128	122	High
	112	113			127		
	224	85			109		

[Ŧ] RPL estimates for 56 kg P ha^{-1} rate amended columns were determined after 11 harvests.

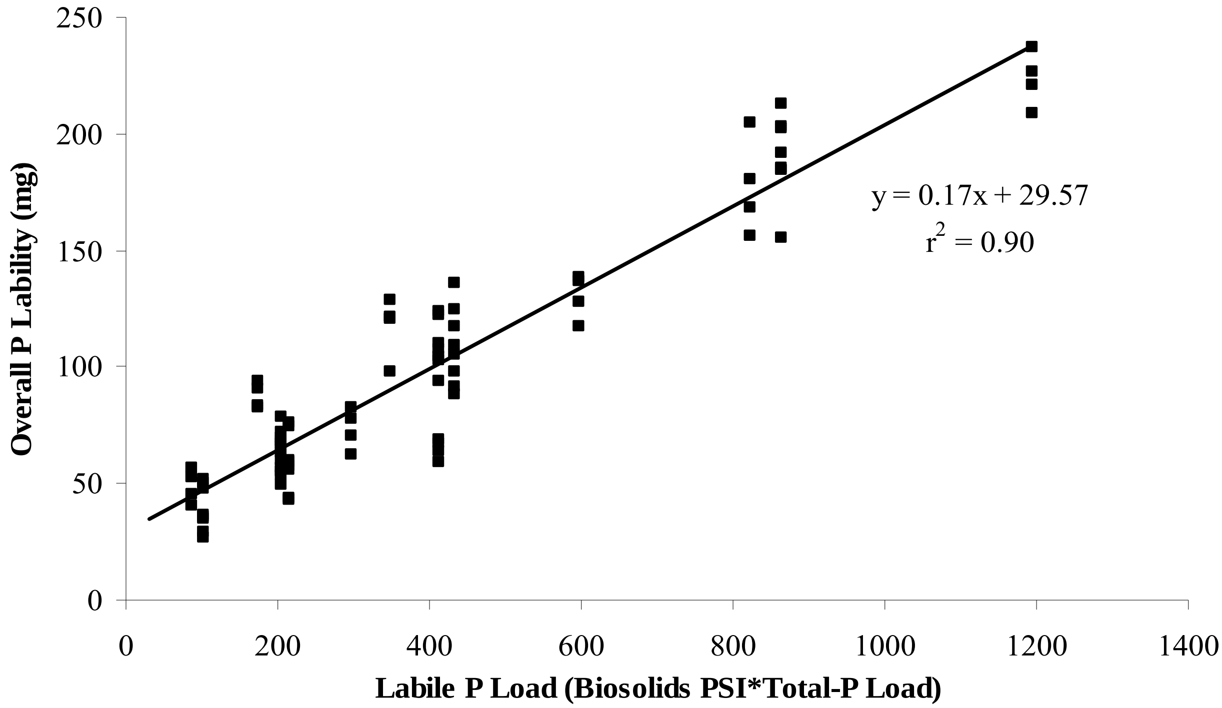


Figure 3-19. Overall P lability plotted as a function of the labile P load.

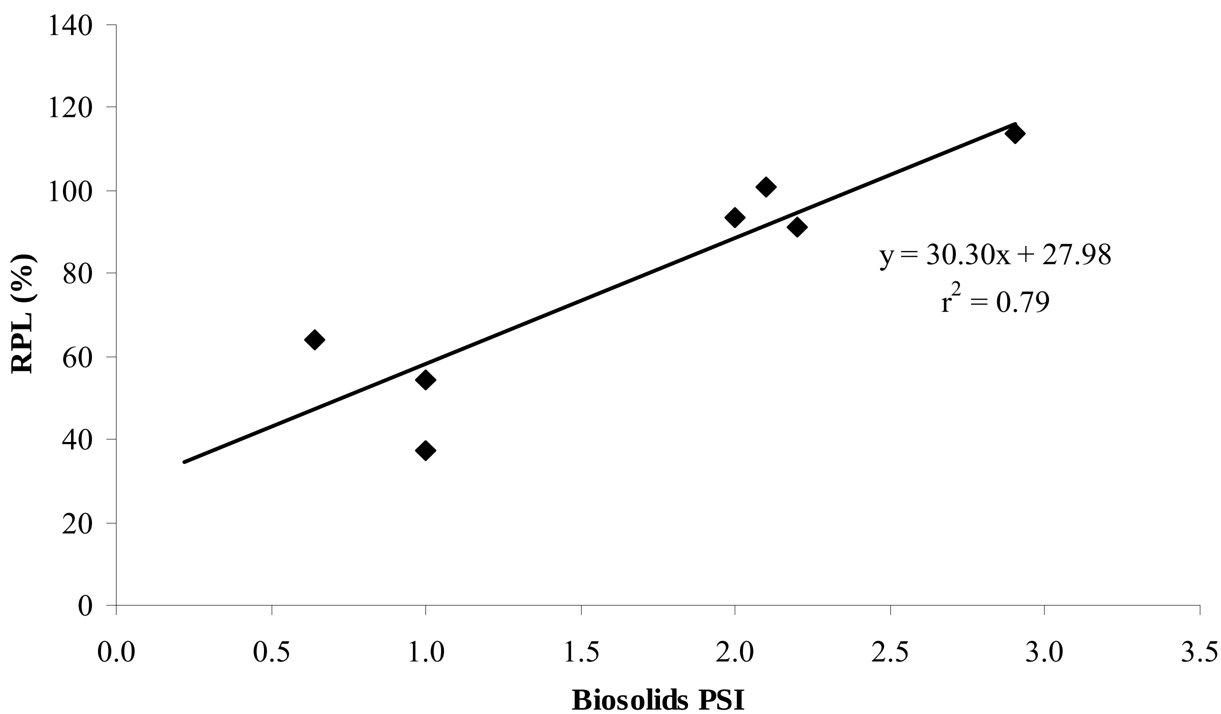


Figure 3-20. Biosolids relative P overall lability (RPL) plotted as a function of biosolids phosphorus saturation index (PSI).

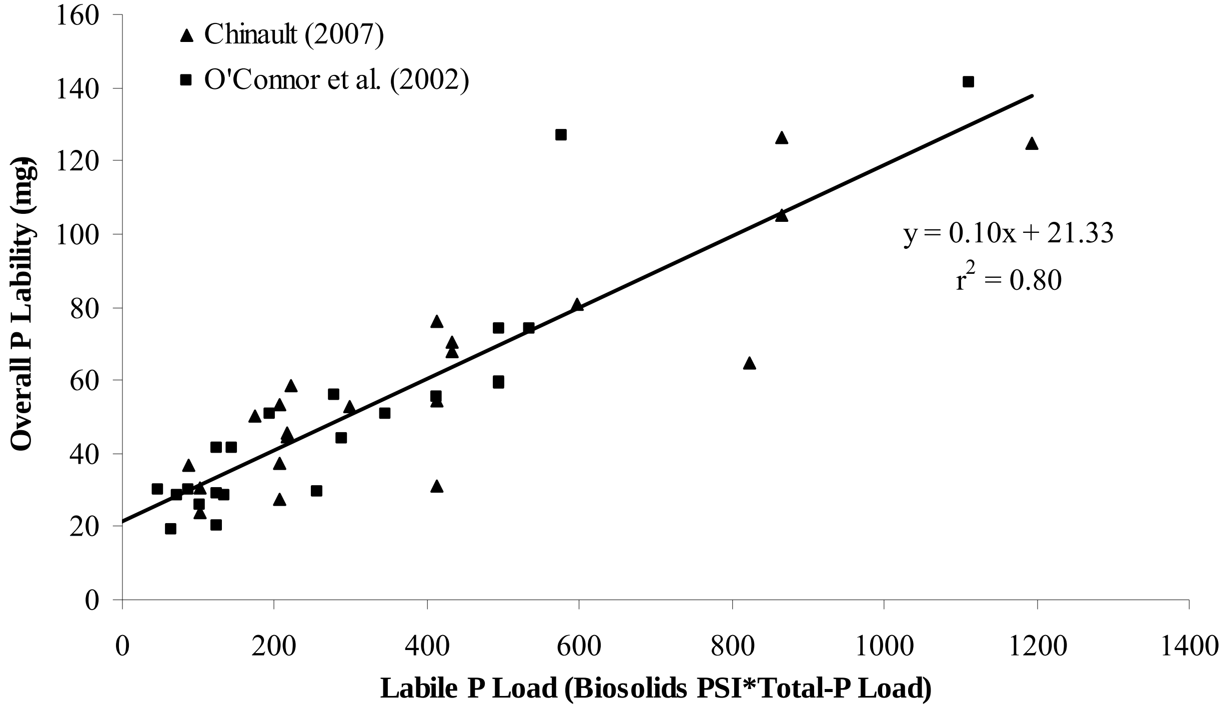


Figure 3-21. Relationship between overall P lability and the labile P load for two previously conducted short-term studies greenhouse studies (O’Connor et al., 2002; Chinault, 2007).

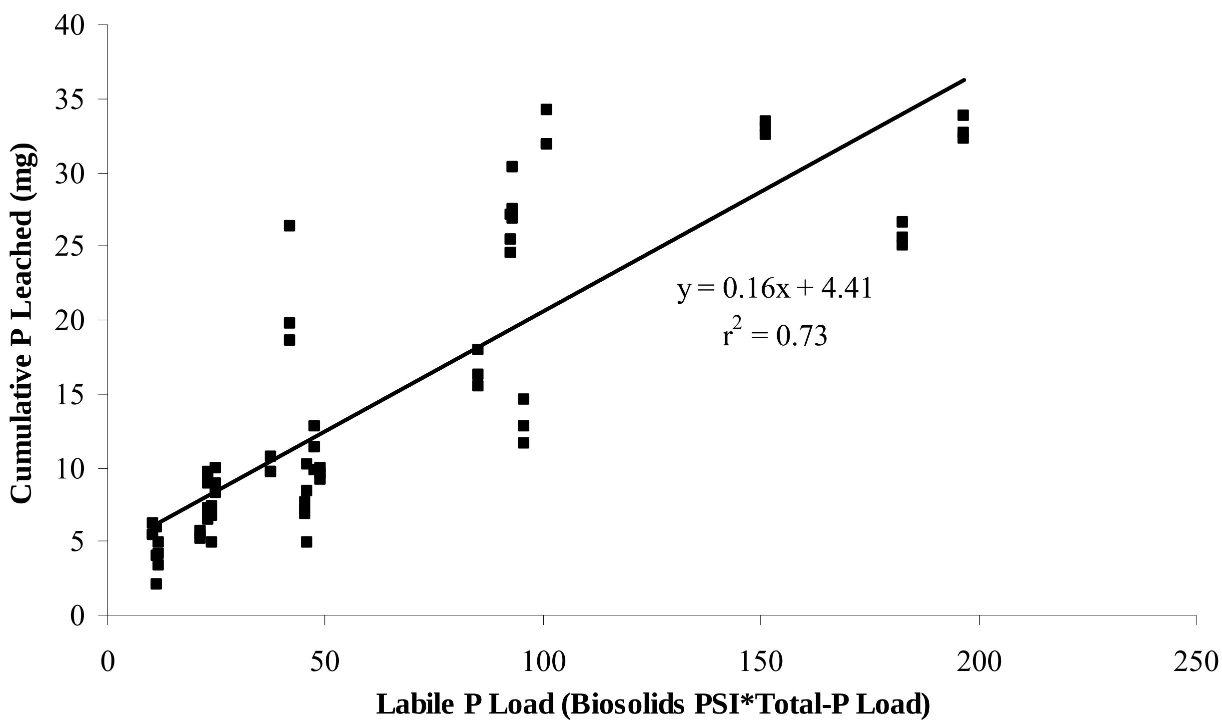


Figure 3-22. Cumulative P leaching from a 5.5 laboratory incubation study plotted as a function of the environmentally effective P load. Data taken from Chinault (2007).

CHAPTER 4
CONCLUSIONS

A major challenge to biosolids land application under P-based management was the absence of a long-term study to validate the P lability characteristics of biosolids suggested by short-term studies. The 16-month greenhouse study described herein represents a substantial effort to characterize the long-term environmental lability and phytoavailability of biosolids-P. Measures of bahiagrass P uptake at the P-based biosolids application rate continued until tissue P concentrations decreased and remained below critical levels. At the N-based application rate, bahiagrass P uptake was quantified until tissue P concentrations were below a conservative critical threshold (1 g P kg^{-1}), and tissue N concentrations declined below the minimum recommended concentration. Eventually, tissue nutrient concentrations at both application rates suggested additional P fertilization was necessary; indicating the 16-month greenhouse study fully characterized the long-term agronomic value of biosolids-P.

Less soluble-P (high Fe+Al, conventionally processed) biosolids clearly pose significantly less environmental P risk than high soluble-P inorganic fertilizers. Most states do not explicitly acknowledge biosolids-P solubility in their P-indices despite the growing number of studies demonstrating the lower lability of conventional biosolids-P compared to inorganic fertilizer-P. Nutrient management plans, including P-Index considerations, could restrict biosolids application rates to P-based fertilizer recommendations and increase land application costs to municipalities. Yet, applying less soluble-P biosolids at rates greater than crop P demands did not appreciably increase the risk of P leaching. Even at the N-based application rate, P leaching from less soluble-P biosolids was $\leq 0.5\%$ of the total-P applied. Data from the greenhouse study suggest that regulatory distinctions in state P-indices are justified for less soluble-P conventional biosolids. However, state P-indices should not utilize a single, "umbrella" factor that suggests all biosolids-P is less environmentally labile. The risk of P leaching is similar for BPR biosolids, BPR-like biosolids, and TSP, and no regulatory distinction is warranted for BPR and BPR-like biosolids. State P-indices should distinguish between more soluble-P biosolids (BPR and BPR-like biosolids), and less soluble-P conventional biosolids. The distinction will become increasing important as more municipalities utilize BPR techniques to meet effluent P standards.

Relative agronomic effectiveness also differs among biosolids. Less soluble biosolids-P is less phytoavailable than TSP-P. Estimates of biosolids RPP suggest application rates of conventional, less soluble-P biosolids should increase 25-50% to supply phytoavailable P in quantities equal to inorganic fertilizer-P. Very low, P-based biosolids application rates are becoming increasingly mandated under environmental P management regulations, and accounting for reduced phytoavailability of less soluble-P biosolids is necessary to ensure that crops receive sufficient P. The long-term phytoavailability of BPR and BPR-like biosolids-P is equal to or greater than inorganic fertilizer, and these more soluble-P biosolids are very effective P fertilizers. No agronomic application rate adjustment is warranted for BPR and BPR-like biosolids.

We hypothesized (hypothesis # 1) that the long-term phytoavailability and environmental lability of conventional biosolids-P is less than fertilizer-P (TSP-P), and that the long-term phytoavailability and environmental lability of BPR and BPR-like biosolids-P is similar to TSP-P. Data from the prolonged (16-month) greenhouse study confirm the expectations, and the first hypothesis is accepted. Combining P uptake and P leaching into an overall P lability characterization provided an indication of the ultimate fate of biosolids-P. Data from the greenhouse study suggest that less soluble biosolids-P is ultimately about 40-65% as labile as

TSP-P, and much of conventional (high total Fe + Al) biosolids-P is ultimately unavailable. Conversely, the ultimate lability of BPR and BPR-like biosolids-P is similar to TSP-P.

The second study hypothesis was that short-term greenhouse and lab incubation studies are good approximations of the relative phytoavailability and environmental lability of biosolids-P. Little change in relative P lability differences among biosolids occurred from harvest 4 to harvest 12, suggesting that short-term studies adequately approximate the relative P lability differences among P-sources. Thus, the second hypothesis is also accepted. However, short-term characterizations tend to underestimate the ultimate relative lability of biosolids-P. Biosolids RPP and RPL estimates increased roughly 10-20% from harvest 4 to harvest 12. Short-term studies underestimated the RPP and RPL values of less soluble-P biosolids in particular, because these materials tend to act as a slow release fertilizer. Ideally, P-source coefficients that are based on long-term relative lability values should be incorporated in state P-indices.

The third study hypothesis was that some measure of biosolids-P is a useful indicator of ultimate biosolids-P lability and biosolids-P phytoavailability. The correlations of overall biosolids-P lability to labile P load (biosolids PSI*total-P load) suggest that biosolids PSI is a useful *a priori* indicator of biosolids-P lability. Biosolids PSI values also correlate well with biosolids RPL values. Values of PWEP are good indicators of biosolids-P runoff and leaching potential, but PWEP values tended to underestimate the quantity of biosolids-P ultimately available to plant roots. The P uptake vs. labile P load correlations suggest that biosolids PSI is also a useful *a priori* indicator of biosolids-P agronomic effectiveness, and adequately predicts biosolids relative P phytoavailability (RPP) values. Thus, the third hypothesis is accepted.

Several caveats to using biosolids PSI as an *a priori* P-lability measure exist. The greenhouse study utilized a P-deficient sandy soil with little P sorption capacity so that the soil would not mask P-source lability differences. An *a priori* P-source P lability measure is especially useful in areas such as Florida where low P sorbing soils dominate and where P lability is expected to be controlled by P-source characteristics. Soils with appreciable P sorption capacity dominate in the majority of the US, and an *a priori* biosolids-P lability measure is of little value because P lability is controlled by the soil. The PSI concept is not applicable to all biosolids. Reactive (oxalate-extractable) Fe+Al is expected to control P lability in most biosolids; however, some municipalities utilize advanced alkaline stabilization techniques and the PSI concept is not applicable for such materials. Lastly, few data exist to show that the PSI concept adequately predicts biosolids-P lability in field conditions. A field study, utilizing biosolids with a wide-range of P solubilities, could further confirm that biosolids PSI is a useful *a priori* measure of biosolids-P lability.

APPENDIX A
R SQUARED VALUES FOR VARIOUS CORRELATIONS

Table A-1. Relationship between various measures of biosolids-P with cumulative P uptake and overall P lability in the 16-month greenhouse study.

Measure of biosolids-P[¥]	Correlation with P uptake	Correlation with overall P lability
	---------------- r^2 value	--------------
Total-P load	0.45	0.47
Iron strip-P load	0.03	0.04
Oxalate extractable-P load	0.64	0.62
Environmentally effective P load[Ŧ]	0.47	0.56
Labile P load[¶]	0.85	0.90

[¥] Determined by Chinault (2007)
[Ŧ] The product of the biosolids percent water extractable-P value (PWEP) and the total-P load
[¶] The product of the biosolids phosphorus saturation index value (PSI) and the total-P load

Table A-2. Relationship between various measures of biosolids-P with estimates of biosolids relative P phytoavailability (RPP) and biosolids relative P lability (RPL) in the 16-month greenhouse study.

Measure of biosolids-P[¥]	Correlation with RPP	Correlation with RPL
	---------------- r^2 value	-------------
Total-P	0.21	0.33
Iron strip-P	0.00	0.01
Oxalate extractable-P	0.50	0.53
Percent water extractable-P (PWEP)	0.34	0.46
Phosphorus saturation index (PSI)[¶]	0.76	0.79

[¥] Determined by Chinault (2007)
[¶] The molar ratio of oxalate extractable-P to oxalate extractable Fe+Al

APPENDIX B
ADDITIONAL BIOSOLIDS PSI CORRELATIONS

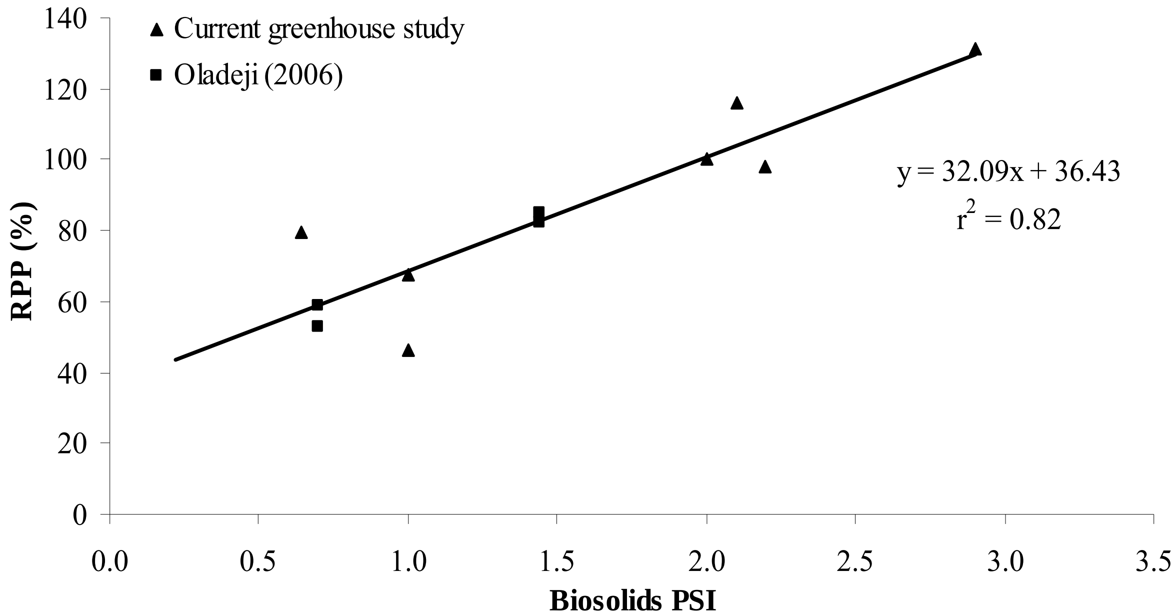


Figure B-1. Long-term estimates of biosolids relative P phytoavailability (RPP) plotted as a function of biosolids PSI values. The long-term RPP estimates are from the current greenhouse study and Oladeji (2006).

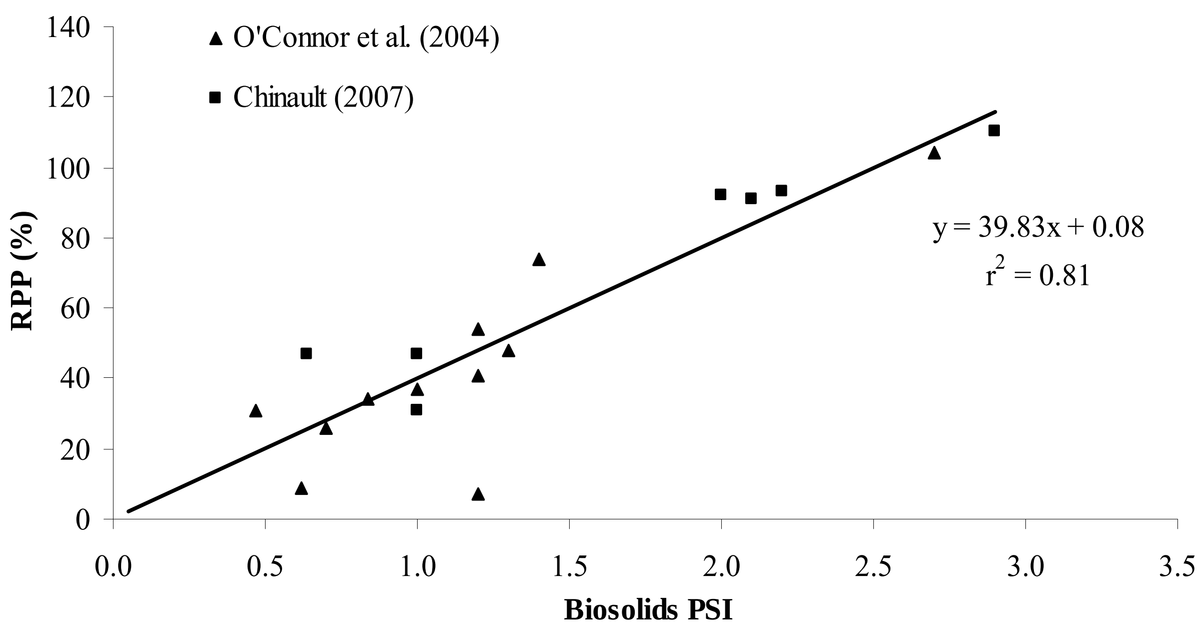


Figure B-2. Short-term estimates of biosolids relative P phytoavailability (RPP) plotted as a function of biosolids PSI values. Short-term RPP values were determined by O’Connor et al. (2004) and Chinault (2007).

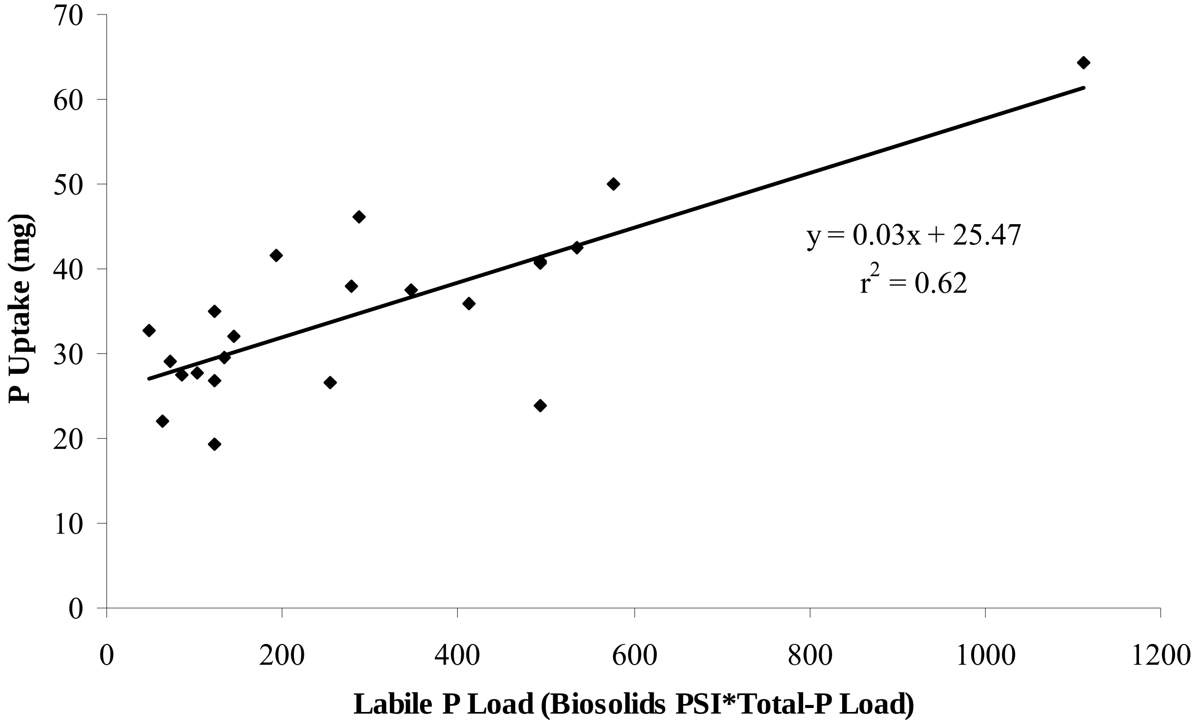


Figure B-3. Cumulative P uptake from a short-term greenhouse study (O'Connor et al., 2004) plotted as a function of the labile P load. The short-term greenhouse study utilized Candler soils with a relative P adsorption value of ~15%.

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BIOGRAPHICAL SKETCH

Matthew (Matt) L. Miller was born in Athens, Georgia, in 1983. Matt is the son of Lane and Sheila Miller and has one older sister, Shannon. Matt spent much of his youth on a small farm in Bishop, Georgia, where he learned to appreciate agriculture and the environment. Matt received a B.S. in forest resources from the University of Georgia in May 2006. He enjoyed the undergraduate research he performed under Dr. Larry Morris at the University of Georgia and chose to attend graduate school. Matt joined the Soil and Water Science Department at the University of Florida and began his graduate study in soil chemistry under the supervision of Dr. George A. O'Connor in August 2006. Matt is scheduled to graduate with his M.S. degree in August 2008.