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SUSTAINABLE NUTRIENT MANAGEMENT PACKAGE FOR COST-EFFECTIVE BIOENERGY BIOMASS PRODUCTION

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□ *Commercial fertilizer (particularly nitrogen) costs account for a substantial portion of the total production costs of cellulosic biomass and can be a major obstacle to biofuel production. In a series of greenhouse studies, we evaluated the feasibility of co-applying Gibberellins (GA) and reduced nitrogen (N) rates to produce a bioenergy crop less expensively. In a preliminary study, we determined the minimum combined application rates of GA and N required for efficient biomass (sweet sorghum,* Sorghum bicolor*) production. Co-application of 75 kg ha*$^{-1}$ *(one-half of the recommended N rate for sorghum) and a modest GA rate of 3 g ha*$^{-1}$ *optimized dry matter yield (DMY) and N and phosphorus (P) uptake efficiencies, resulting in a reduction of N and P leaching. Organic nutrient sources such as manures and biosolids can be substituted for commercial N fertilizers (and incidentally supply P) to further reduce the cost of nutrient supply for biomass production. Based on the results of the preliminary study, we conducted a second greenhouse study using sweet sorghum as a test bioenergy crop. We co-applied organic sources of N (manure and biosolids) at 75 and 150 kg PAN ha*$^{-1}$ *(representing 50 and 100% N rate respectively) with 3 g GA ha*$^{-1}$*. In each batch of experiment, the crop was grown for 8 wk on Immokalee fine sand of minimal native fertility. After harvest, sufficient water was applied to soil in each pot to yield ~1.5 L (~0.75 pore volume) of leachate, and analyzed for total N and soluble reactive P (SRP). The reduced (50%) N application rate, together with GA, optimized biomass production. Application of GA at 3 g ha*$^{-1}$*, and the organic sources of N at 50% of the recommended N rate, decreased nutrient cost of producing the bioenergy biomass by ~\$375 ha*$^{-1}$ *(> 90% of total nutrient cost), and could reduce offsite N and P losses from vulnerable soils.*

Keywords: gibberellins, manure, biosolids, N and P uptake, leaching losses, dry matter yield, sweet sorghum

INTRODUCTION

Growing environmental and economic concerns over the use of fossil fuels have prompted the search for alternative fuels, such as biofuels. A

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major obstacle to increased production of biofuels, including ethanol, is the relatively high production cost, with a substantial portion of the cost tied to biomass production. Currently, the total cost of commercial fertilizer to meet the nutrient requirements of commonly grown bioenergy biomass range between 600 and \$1100 ha^{-1} yr^{-1}, depending on the nutrient requirement of the bioenergy crop (Bennett and Anex, 2008).

Producing cellulosic biomass cheaply should reduce the expense associated ethanol production for vehicular engines. Few studies have evaluated substitution of the inorganic fertilizers with cheap organic nutrient sources such as manure and biosolids to reduce the cost of producing bioenergy biomass. In a field study, Castillo et al. (2010) demonstrated that dry matter yields of elephantgrass produced with commercial fertilizers were similar to yields obtained when municipal biosolids were incorporated into soil as the sole nitrogen (N) and phosphorus (P) sources for the crop. Substituting biosolids for commercial fertilizers reduced the cost of nutrient supply from \$800 ha^{-1} yr^{-1}, to \$150 ha yr^{-1} when biosolids were applied at a rate based on N requirement of the grass [and P was incidentally supplied, leaving only potassium (K) fertilizer cost].

However, applying manure or biosolids based on crop N needs (150–350 kg N ha^{-1} yr^{-1}, Mylavarapu et al., 2007) simultaneously supplies excess P to an ecosystem. In the field study of Castillo et al. (2010), applying the biosolids based on the N requirements of the crop resulted in 130 kg P ha^{-1} yr^{-1} addition to the soil. However, only a maximum of 43 kg P ha^{-1} yr^{-1} was removed by the crop, leaving excess P in the soil. Excess P can cause undesirable environmental effects, threatening surface or ground waters with eutrophication when the receiving waters are P-limited. If nutrient uptake and use efficiencies can be improved, less N and P could be applied to produce optimum yields, thereby reducing offsite nutrient losses to the environment. One way of improving nutrient uptake and use efficiencies could be the application of growth stimulating hormones to the bioenergy crops to enhance efficient biomass production.

Pioneering studies demonstrated that various plant growth processes are regulated by hormones that move from one part of the plant to another (Yabuta and Hayasi, 1940; Santner et al., 2009). In general plant hormones are present at very low concentrations and act either locally, at or near the site of synthesis, or in distant tissues. Over the years, the database of plant hormones has grown and now includes (but is not limited to) abscisic acid (ABA), indole-3-acetic acid (IAA or auxin), brassinosteroids (BRs), cytokinin, gibberellins (GAs), ethylene, jasmonic acid (JA) and salicylic acid (Santner et al., 2009). Collectively, plant hormones regulate every aspect of plant life, from pattern formation during development to responses to biotic and abiotic stress (Santner et al., 2009).

The GAs are a large family of tetracyclic, diterpenoid growth regulators essential for normal growth, and affect a wide variety of plant developmental processes (Richards et al., 2001). Gibberellin was originally isolated in 1938 as a metabolite from the rice fungal pathogen *Gibberella fujikuroi* (Schwechheimer and Willige 2009). Several studies have shown that GAs play a major role in diverse growth processes including promotion of cell division, organ elongation, the control of flowering time, and seed development (Croker et al., 1990; Richards et al., 2001).

Gibberellins have been used to accelerate growth of pastures through reserve mobilization, leaf and stem elongation, and promotion of flowering, and have been used as a pasture production stimulant since its isolation (Croker et al., 1990; Hedden and Phillips, 2000). Gibberellic acid is now commercially available, and application rates as low as 5–10 g ai ha^{-1} have been shown to produce cost-effective responses. Accordingly, there is renewed interest in GAs use to increase pasture production or to manipulate seasonality of production (Richards et al., 2001).

We hypothesized that co-application of growth stimulating hormones and N fertilizers at optimum rates will enhance efficient production of bioenergy crops, and that growth stimulating hormones will increase nutrient uptake and thereby reduce nutrient losses to leachate and runoff from soils receiving fertilization. The overall goal of the study is to identify ways of producing bioenergy biomass cheaply. Specific objectives include: (i) identifying the minimum combined application rates of N and GA that optimize biomass yield of a bioenergy crop, and (ii) evaluating cheap sources of N that could be used in combination of the GAs to optimize biomass yield of a bioenergy at a reduced cost with minimal negative environmental impact.

MATERIALS AND METHODS

The studies were conducted in a greenhouse at University of Florida campus, Gainesville. Greenhouse temperature was maintained at 27°C (day) and 17°C (night).

Soil and Nitrogen Sources Used

A typical Florida Spodosol (Immokalee fine sand, sandy, siliceous, hyperthermic Arenic Alaquods) was utilized for the study. Native Immokalee sand, not contaminated by manure depositions and having "very low" native fertility, was collected from the University of Florida Research and Education Center in Immokalee, FL. Multiple random bulk samples were collected from the A horizon (0–15 cm) and thoroughly mixed to yield a composite sample. Selected physicochemical characteristics of the soil are presented in Table 1.

Two biosolids [Gainesville Regional Utility (GRU) and Milorganite biosolids], poultry manure, and one mineral fertilizer (ammonium nitrate) were used in the study. Selected physicochemical characteristics of the

TABLE 1 Selected physicochemical properties of the Immokalee fine sand utilized for the study. Numbers are mean values of six replicates ± one standard deviation

Property	Value
pH	5.62 ± 0.34
Sand (g kg^{-1})	976 ± 58.9
Silt (g kg^{-1})	9.96 ± 1.13
Clay (g kg^{-1})	14.2 ± 1.26
Total carbon (g kg^{-1})	8.84 ± 1.58
Total nitrogen (mg kg^{-1})	1.47 ± 0.16
Nitrate-nitrogen (mg kg^{-1})	0.76 ± 0.08
Ammonium-nitrogen (mg kg^{-1})	0.42 ± 0.07
Total phosphorus (mg kg^{-1})	3.22 ± 0.42
Mehlich-3 extractable phosphorus (mg kg^{-1})	2.39 ± 0.21
Water extractable phosphorus (WEP) (mg kg^{-1})	1.16 ± 0.18
Oxalate extractable phosphorus (mg kg^{-1})	2.72 ± 0.26
Oxalate extractable aluminum (mg kg^{-1})	32.1 ± 9.14
Oxalate extractable iron (mg kg^{-1})	54.2 ± 6.26
PSR [a]	0.04 ± 0.01

[a] Phosphorus saturation ratio = oxalate extractable P (mmol)/oxalate extractable Fe + Al (mmol).

biosolids, manure, and the ammonium nitrate are presented in Table 2. The GRU biosolids was obtained from the water reclamation facilities of the Gainesville Regional Utilities (Gainesville, FL) and was produced through aerobic digestion. Milorganite biosolids, obtained from Milwaukee Metropolitan Sewerage District, Milwaukee, WI, was generated from

TABLE 2 Selected chemical properties of the nitrogen sources used for the study. Numbers are mean values of six replicates ± one standard deviation

Property	Milorganite biosolids	GRU biosolids	Manure
pH	6.36 ± 0.21	6.40 ± 0.43	6.82 ± 0.24
Solids (%)	95.4 ± 3.27	5.45 ± 0.92	87.0 ± 4.23
Carbon (g kg^{-1})	360 ± 24.2	400 ± 42.6	320 ± 28.3
Nitrogen (g kg^{-1})	60.3 ± 5.82	62.6 ± 7.13	66.2 ± 5.82
Total iron (g kg^{-1})	41.6 ± 5.28	7.70 ± 0.83	1.53 ± 0.32
Total aluminum (g kg^{-1})	2.72 ± 0.35	5.52 ± 0.46	0.94 ± 0.14
Total P (g kg^{-1})	21.5 ± 2.3.4	26.4 ± 3.62	18.9 ± 3.85
WEP[†] (g kg^{-1})	0.12 ± 0.02	6.91 ± 0.84	1.85 ± 0.12
PWEP[‡] (%)	0.58 ± 0.12	22.2 ± 2.54	9.78 ± 1.01
Oxalate P (g kg^{-1})	16.1 ± 2.13	21.2 ± 2.43	10.4 ± 1.20
Oxalate Fe (g kg^{-1})	1.25 ± 0.32	6.46 ± 0.91	0.82 ± 0.32
Oxalate Al (g kg^{-1})	25.4 ± 3.52	3.73 ± 0.66	0.79 ± 0.14
PSI[††]	0.55 ± 0.02	2.67 ± 0.21	NA[‡‡]

[†] Water extractable phosphorus.

[‡] Percent water extractable phosphorus.

[††] Phosphorus saturation index.

[‡‡] Not applicable.

anaerobically digested material that was heat-dried and pelletized. The Milorganite biosolids was stabilized with iron (Fe) salts to decrease P solubility of the biosolids. The poultry manure utilized for the study was obtained from an egg producing farm in Indiantown, FL.

Characterization of Soil, Manure, and Biosolids Used

Soil samples were air-dried and passed through a 2-mm sieve before analyses. Particle size distribution of the samples was determined using the pipette method (Day, 1965). Soil pH was determined in a 1:2 soil:DDI water ratio using a glass electrode (McLean, 1982). Total carbon (C) and N of soil samples were determined by combustion at 1010°C using a Carlo Erba NA-1500 CNS analyzer (NA-1500 CNS, Carlo Erba, Milan, Italy) as outlined in Nelson and Sommers (1996). Soil test P concentration was determined using the Mehlich 3 extraction protocol (Mehlich, 1984), and water extractable P (WEP) determined following the procedure of Kuo (1996). Soil extracts were filtered (Whatman No 42) and analyzed colorimetrically for P with the Murphy and Riley (1962) method.

Biosolids and the manure were analyzed for percentage total solids, total N and C, WEP, and total P (TP) concentrations. Percentage solids were determined by drying materials at 105°C (Gardner, 1986), and pH measurements were performed on the materials as described by Thomas (1996). Total C and N concentrations were determined in the biosolids by combustion at 1010°C as described above for the soil samples. For WEP analysis, 100 mL of distilled/deionized (DDI) water was added to a 0.5 g (oven dry equivalent) of N-source to give a 1:200 solid/solution ratio. High solid/water ratios reportedly relate better to runoff potential than lower solid/water ratios (Brandt et al., 2004; O'Connor and Elliott, 2006; Sharpley and Moyer, 2000). Samples were shaken on a reciprocating shaker at a rate of 100 strokes^{-1} for 1 h at room temperature ($24 \pm 2°C$). The suspensions were then centrifuged at a relative centrifugal force of 800× g for 15 min, and the supernatant filtered through 0.45-um filter paper. Phosphorus concentrations in the extracts were determined by the Murphy and Riley (1962) colorimetric method. Five grams each (oven-dry equivalent) of the manure and biosolids were analyzed for total P, Fe, and aluminum (Al) by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (PerkinElmer Plasma 3200, PerkinElmer, Wellesley, MA, USA) following acid-peroxide digestion according to the United States Environmental Protection Agency (USEPA) Method 3050A (USEPA, 1986). The percentage of total P that is water-extractable (PWEP) was then calculated. Oxalate (200 mM) extractable P, Fe, and Al were determined by ICP-AES after extraction at a 1:60 solid/solution ratio, following the procedures of Schoumans (2009). The P saturation index ($PSI = [P]/[Al + Fe]$) was calculated from the molar concentrations of oxalate-extractable P (P_{ox}), Al (Al_{ox}), and Fe (Fe_{ox}) in the biosolids (Elliott et al., 2002). The PSI of biosolids is a measure of P retention/release potential from a particular biosolids. Since only Fe_{ox} and Al_{ox} together with P_{ox}, are considered in the calculation of PSI, the index is not applicable for calcium (Ca)-dominated amendments, such as poultry manure.

Greenhouse Experiments

Preliminary Study

In a preliminary greenhouse study, we determined the minimum combined application rates of N and GAs that optimized dry matter yield and N uptake of the bioenergy crop. Five rates of GAs, in the form of gibberellic acid (GA) (99% purity; Fisher Scientific, Asheville, NC), (0, 2, 3, 4, and 5 g GA ha^{-1}) were combined with five N rates (0, 37.5, 75, 112.5, and 150 kg N ha^{-1}) representing 0, 0.25, 0.5, 0.75, and 1X the recommended N rate for sorghum production (Mylavarapu et al., 2007). Ammonium nitrate fertilizer (34-0-0) was used as the sole N source. Each of the 25 GA × N application rates combinations was randomly assigned to a pot containing 4 kg of soil. The pots were arranged in a randomized complete block design (RCBD) in the greenhouse with three replicates for each treatment, yielding a total of 75 experimental units. The soil in each pot was wetted to, and maintained at ~80% of the water holding capacity and allowed to equilibrate for 1 wk before planting.

Sweet sorghum was selected as the test bioenergy crop. Several studies have shown that sweet sorghum is a promising bioenergy crop, well adapted to several agro-ecosystems, and produces high bioenergy yields (Bennett and Anex, 2008; Monti and Venturi, 2003). Seeds of sweet sorghum [*Sorghum bicolor* (L.) Moench], variety CSH-5, were sown in the pots and misted every 3–4 h to facilitate uniform germination. Five days after emergence, the seedlings were thinned to four plants in each pot, and the N rate treatment assigned to each pot was applied. One week after emergence, the plants were sprayed with appropriate concentrations of aqueous mixtures of GA. Phosphorus was applied to each pot at the recommended rate of 54.6 kg P ha^{-1} (125 kg P_2O_5 ha^{-1}; Mylavarapu et al., 2007). A blend of potassium-magnesium sulfate ("sul-po-mag"; 22% S, 18% K, 11% Mg) was added (0.91 g, equivalent to ~444 kg ha^{-1}) to supply adequate and uniform S, K, and Mg. Micronutrients were supplied using soluble trace element mix (The Scotts Company, Marysville, OH, USA) at 13.9 g L^{-1}.

The experiment was blocked to minimize greenhouse positioning effects. The pots were rotated one position within each block weekly to further minimize greenhouse effects on growth of the plants. The pots were weighed daily and the loss in weight was made up by adding distilled water. The aboveground plant material of each pot was harvested 8 wk after emergence.

The fresh plant materials were placed in pre-weighed bags and dried at 68°C to constant weight to represent dry matter yield. The dried tissue was ground to pass a #20 sieve with a Wiley mill, digested (Andersen, 1976) and analyzed for P via the molybdenum blue method (Murphey and Riley, 1962). Tissue N concentration was determined by grinding the tissue to a fine powder, and analyzing for N using a micro-Kjeldahl method, a modification of the aluminum block digestion technique described by Gallaher et al. (1975), followed by automated colorimetry with a Technicon Auto Analyzer. Phosphorus and N uptake were calculated as the product of dry matter yield and tissue P or N concentration. Nitrogen use efficiency (NUE) was determined according to the methods described by Moll et al. (1982) and Eghball and Maranville (1993). Components of NUE were computed as follows:

$$\mathrm{NUE} = \frac{\mathrm{TDM\ \text{-}\ control}}{\mathrm{NS}} \tag{1}$$

where NUE = Nitrogen Use Efficiency (g g^{-1}); TDM = Total above-ground plant dry matter (g $plant^{-1}$); NS = N supplied in the form of NO_3^- and NH_4^+ obtained from fertilizer N added (g $plant^{-1}$).

Nutrient (N or P) uptake efficiency was computed as:

$$\mathrm{NUpE} = \frac{\mathrm{Nt}}{\mathrm{PNS}} \tag{2}$$

where NUpE = Nutrient (N or P) uptake efficiency (g g^{-1}); Nt = total N or P in the plant (g $plant^{-1}$); PNS = plant available nutrient obtained from N or P fertilizer (g $plant^{-1}$).

Following harvest, sufficient tap water (adjusted to pH ~5.0 to mimic rain water) was initially applied to each pot to yield ~500 mL (~0.25 pore volume) of leachate. The soil was allowed to equilibrate for 1 wk and the leaching was repeated; followed by a third leaching 1 wk thereafter. Thus, a total of ~1500 mL (~0.75 pore volume) leachate was collected. After each leaching event, the leachate collected was analyzed for SRP using the molybdenum blue method. Leachate samples were analyzed for N concentration using the USEPA Method 353.2 (USEPA, 1983). The product of the leachate volume and leachate N or P concentration yielded mass of N or P leached. Although leachates from the pots were not highly colored, TP was determined on the leachates to confirm that organic P loss from the material was not significant.

Follow-Up Greenhouse Experiment

The overall goal of the experiment was to assess the possibility of reducing the nutrient cost of producing bioenergy biomass. Following the preliminary study, we evaluated the feasibility of substituting organic sources of N for inorganic N fertilizers. Specifically, we compared the effectiveness of manure and biosolids, co-applied with 3 g GA ha^{-1} (based on the results of the preliminary study).

Two biosolids (GRU and Milorganite biosolids) of different physicochemical properties (Table 2), and poultry manure were selected for the follow-up experiment, which were compared with the inorganic fertilizer (ammonium nitrate) utilized in the preliminary study. The N sources were mixed with 4 kg of A horizon Immokalee soil at 2 rates: 75, and 150 kg PAN ha^{-1}. The 150 kg PAN ha^{-1} represented the recommended N application rate for sweet sorghum,in native Immokalee soil, and the 75 kg N ha^{-1} represented one-half of the recommended N rate (chosen based on the result of the preliminary study). Previous studies (Agyin-Birikorang et al., 2009; Chinault and O'Connor, 2008) suggested ~40% total N mineralization per annum for the manure and biosolids used for the study; therefore, plant available N (PAN) for the manure and biosolids was calculated based on 40% organic N annual mineralization rate. The manure- and biosolids-amended soils were equilibrated (~80% water holding capacity) in zip-lock plastic bags for 2 weeks in the laboratory prior to use in the greenhouse.

The greenhouse experimental layout, design, setup, and data collection followed identical procedures as described above for the preliminary studies.

Statistical Analyses

Differences in N and P uptake among treatments were statistically analyzed as a factorial experiment with a RCBD, using the PROC MIXED procedure of the SAS software (SAS Institute, Cary, NC, USA). The means of the various treatments were separated using the LSMEANS along with the PDIFF option of the analytical procedure (Littell et al., 1996). The variation in dry matter yield caused by the GA application as function of the N applied was determined with a nonlinear regression analysis, using PROC NLIN of the SAS software (SAS Institute,) as:

$$\text{DMY (g)} = a + b\ (1 - e^{-c \text{ x rate}}) \tag{3}$$

where DMY = dry matter yield, a = intercept, representing DMY of the crop in soil applied with or without the various GA additions, but without N application; b = DMY plateau increment above the intercept; c = rate constant for change in DMY for a given change in cumulative amount of N applied; rate = N applied (kg N ha^{-1} soil).

The N and P leaching data were not normally distributed, and were normalized with a square-root transformation, based on the Box-Cox analytical procedure (Box and Cox, 1964). The transformed data were then analyzed using the PROC GLM procedure. Means of the leachate N and P content resulting from the treatment were separated using a single degree of freedom orthogonal contrast on the transformed data.

RESULTS AND DISCUSSION

Properties of Soil, Manure and Biosolids Used

Selected physicochemical characteristics of the soil samples utilized for the study are presented in Table 1. The soil was moderately acidic, but the pH was within the range of pH values (5–7.5; Mylavarapu et al., 2007) suitable for sweet sorghum production. The Mehlich-3 P and total N content of the soil suggest the soil can be characterized as having "very low" native fertility (Mylavarapu et al., 2007), and thus require the maximum recommended rates of N and P to support normal growth and development of crops. The soil contained low total carbon and clay contents, which implied that the soil possessed small capacity to adsorb cations. Although the water extractable P content of the soil is low, it represented ~50% of total P. The high PWEP might be the consequence of the soil's low Al and Fe content, which are major sorbents of P in soils.

Selected properties of the two biosolids, and manure, utilized for the study are presented in Table 2. The two biosolids had total N levels $\geq$60 g kg^{-1}, typical of biosolids produced nationally. Total P values of the biosolids were within the range of values ($\sim$ 8–40 g kg^{-1}; O'Connor and Elliott, 2006; USEPA, 1995) reported for biosolids produced nationally. Total concentrations of major elements (Al, Fe, and Ca) were also representative of biosolids produced nationally, and reflected individual wastewater and sludge treatment processes. Thus, Fe or Al concentrations were generally $\leq$10 g kg^{-1}, unless chemicals were added to the waste stream for P removal (e.g., Milorganite biosolids).

The manure and biosolids varied widely in the amount of labile P estimated as WEP. The Milorganite biosolids, stabilized with Fe salts, had low WEP values. Water-extractable P was shown in previous work (Brandt et al., 2004; Elliott et al., 2005; O'Connor and Elliott, 2006) to reflect reasonably the relative bioavailable and leachable P for most biosolids. The critical role of Al and Fe in determining P solubility and release from biosolids-amended soils has been documented by several researchers (Elliott et al., 2005; Maguire et al., 2001; Shober and Sims, 2003). The poultry manure had little total Al and Fe (2.4 g kg^{-1}) and was dominated by calcium (102 g kg^{-1}), a basic ingredient of poultry diet (Barnett, 1994).

We also calculated the PSI to estimate the degree to which biosolids P is potentially bound with Fe and Al. Elliott et al. (2002) suggested that PSI values $<$1 indicate excess Fe and Al for binding of P (little available P), whereas values $>$1 suggest available P beyond that associated with Fe and Al precipitates. Obviously, P-binding soil components will affect P availability once biosolids are land applied (Elliott et al., 2002), but the PSI value of biosolids can be useful as an index of biosolids-P lability in soils. In short- and long-term studies, Miller and O'Connor (2009) showed that biosolids

PSI values were strongly correlated (r^2 ~0.82) with biosolids relative P phytoavailability (RPP), which is an estimate the plant availability of biosolids-P compared to fertilizer P. The strong relationship between biosolids RPP estimates and biosolids PSI values suggest that biosolids PSI values could be used as an *a priori* estimate of biosolids-P phytoavailability. The PSI values observed in the present study (Table 2) suggest that P contained in the GRU biosolids was highly labile.

Sweet Sorghum Dry Matter Yield

The effects of N and GA application on DMY of the sweet sorghum are provided in Figure 1. Without N addition, with or without GA application, DMY for the crop was relatively low (≤ 1 g pot^{-1}), consistent with the low native fertility of the soil. Without GA application, DMY increased with increasing N application rate, with the greatest yield (~11 g pot^{-1}) occurring at N application rate of 150 kg ha^{-1}. The essentially linear increase in DMY with increasing rates N fertilization was consistent with observations of Gardner et al. (1994) and Addy et al. (2010) in a field study involving sweet sorghum, and confirms that the soil we used for our study was N deficient and required N additions for biomass production.

With the exception of the N application rate of 150 kg ha^{-1}, application of GA significantly affected DMY, when N was applied to the soil (Figure 1). At an N application rate of 37.5 kg ha^{-1}, DMY increased linearly with

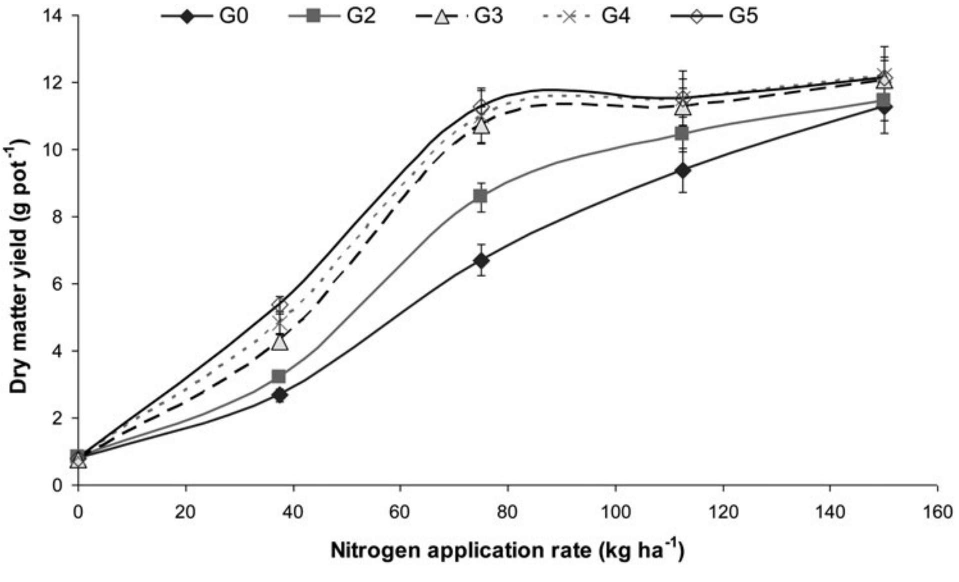

FIGURE 1 Sweet sorghum dry matter yield as a function of nitrogen and gibberellins (GA) application rates (G0 = no GA application, G2 = 2 g GA ha^{-1}, G3 = 3 g GA ha^{-1}, G4 = 4 g GA ha^{-1}, G5 = 5 g GA ha^{-1}).

increasing rates GA application. However at N application rates $\geq$75 kg ha^{-1}, no significant differences in DMY were observed when GA was applied at rates $\geq$3 g ha^{-1}. The optimum yield was obtained for the treatment with N and GA application rates of 75 kg N ha^{-1} and 3 g GA ha^{-1}. At these rates of application, DMY was similar to yields obtained at the maximum N application rate, with or without GA application.

Nitrogen use efficiency was calculated as DMY per plant as a function of N supplied in fertilizer per plant. Consistent with the DMY data, optimum N use efficiency of ~49 g g^{-1} was obtained with N application rate of 75 kg ha^{-1} with co-application of GA ($\geq$3 g ha^{-1}). At the recommended N application rate (150 kg N ha^{-1}), N use efficiency was ~30 g g^{-1} (with or without GA application), similar to N use efficiency values (26–33 g g^{-1}) observed by Gardner et al. (1994). The data suggest that if the plant could be enhanced to utilize N efficiently, N could be applied at rates smaller than the recommended rates, without a significant reduction in DMY. The yield data, thus, suggest that, with a modest GA application rate of 3 g ha^{-1}, the N application rate could be reduced by one half the recommended rate and still obtain biomass production similar to those observed at the recommended N rate of 150 kg ha^{-1}.

Gibberellins are reported to repress the expression of several genes whose products inhibits cell elongation (Croker et al., 1990; Richards et al., 2001; Schwechheimer and Willige, 2009). Recent reports indicate that DELLA proteins inhibit cell elongation by binding to the DNA binding domain of a transcription factor called phytochrome interacting factor (PIF). This binding prevents the PIF from interacting with promoter elements and stimulating transcription of growth-related genes. Interaction with GA ultimately leads to ubiquitination and degradation of the DELLA repressor, and promotion of cell division and cell elongation (Hedden and Phillips, 2000; Schwechheimer and Willige, 2009). Thus, adequate concentration of GA within the plant cells is likely to promote cell division, stem elongation and the overall production of the plant.

Nitrogen and Phosphorus Uptake

As expected, N uptake increased with N fertilization (Table 3) suggesting that with the increased N supply, the plant had access to larger pools of N in the soil solution. At N application rates of 112 and 150 kg N ha^{-1}, GA application significantly affected N uptake (Table 3). At such rates, N uptake efficiency increased linearly from ~0.28 g g^{-1} (no GA) to ~0.44 g g^{-1} (3 g GA ha^{-1}) and to ~0.48 g g^{-1} (5 g GA ha^{-1}). At N application rate of 75 kg ha^{-1}, N uptake efficiency increased from 0.30 g g^{-1} (no GA application) to 0.52 g g^{-1} ($\geq$ 3 g ha^{-1}), suggesting that essentially >50% of the N applied at this rate was utilized by the plant. Without GA application, a substantial

TABLE 3 Sweet sorghum nitrogen uptake, phosphorus uptake, and mass of nitrogen and phosphorus leached as a function of nitrogen and gibberellins application rates. Numbers are mean values of five replicates

	GA application rate (g ha^{-1})				
N rate (kg ha^{-1})	0	2	3	4	5
N uptake (mg pot^{-1})					
0	1.04	1.12	1.51	2.08	1.93
37.5	33.3	39.4	42.6	42.9	43.1
75	44.8	62.2	73.5	74.2	74.3
112.5	62.3	86.4	101	105	108
150	98.9	112	147	147	149
P uptake (mg pot^{-1})					
0	6.23	7.14	7.05	7.21	6.56
37.5	13.3	14.8	18.6	20.3	22.0
75	34.4	42.3	63.6	64.2	65.4
112.5	41.0	49.2	68.5	69.5	70.5
150	47.9	55.1	71.4	72.4	74.1
Mass of N leached (mg)					
0	bdl†	bdl	bdl	bdl	bdl
37.5	1.24	1.81	0.82	1.23	0.64
75	12.1	9.06	1.82	2.43	1.21
112.5	41.4	36.6	21.4	19.2	16.2
150	58.8	52.8	27.3	24.4	21.0
Mass of P leached (mg)					
0	105	105	105	104	104
37.5	88.0	83.0	76.4	72.6	72.2
75	54.5	47.1	29.3	26.2	23.2
112.5	48.2	40.6	22.4	21.2	20.1
150	41.7	35.4	19.2	18.4	16.6

†Gibberellic acid.

‡Below instrument detection limit (0. 02 mg L^{-1}).

portion (>70%) of the applied N at ≥0.75X recommended rate was not utilized by the plant, and is subject to offsite losses.

Similarly to N uptake, P uptake increased linearly with increased N application rates (Table 3). Without GA application, P uptake values ranged from 6.23 mg pot^{-1} (no N application) to 47.9 mg pot^{-1} (N rate of 150 kg ha^{-1}) (Table 3). Several studies have shown that N application enhances P uptake (Adjei et al., 1998; Belanger et al., 2002; Newman et al., 2009). Newman et al. (2009) demonstrated that N fertilization could enhance P phytoremediation potential of warm season forage grasses. Adjei et al. (1998) reported that application of N fertilizer at 60 kg ha^{-1} after each harvest of limpograss increased P uptake of the grass five-fold, relative to an untreated control plots.

Addition of GA at ≥3 g GA ha^{-1} significantly enhanced P uptake, particularly when N was applied at ≥75 kg ha^{-1} (Table 3). Similarly to the DMY data trends, an optimum P uptake efficiency of 0.58 g g^{-1} was observed with GA application rate of 3 g ha^{-1} and N application rate of 75 kg ha^{-1}.

Enhancement of P uptake efficiency, through N and GA applications, could be useful to P phytoremediation of contaminated sites.

Leachate Nitrogen and Phosphorus Concentrations

Improved N and P uptake efficiency, through the use of GA, significantly reduced masses of P and N lost to leaching. Without GA application, ~ 60 mg of the N applied at the recommended rate leached (Table 3). However, application of GA at $\geq$3 g ha^{-1} reduced the mass of N leached from ~60 mg to ~27 mg. At N application rate of 75 kg ha^{-1}, GA application of $\geq$3 g ha^{-1} reduced N leaching losses by ~3 fold, and >10 fold compared to treatments that received N at the recommended rate of 150 kg ha^{-1} (without GA application; Table 3).

Consistent with P uptake data (Table 3), significant reduction in masses of P leached was observed with increasing N application. With N application rate of <37.5 kg ha^{-1}, P uptake was impacted, resulting in >80% of the applied P being lost in leaching. The leaching P losses decreased with increasing N application, and co-application with GA further decreased P losses in leachate (Table 3), as plant uptake was increased.

Substituting Manure and Biosolids for Inorganic Fertilizers

The ultimate goal of the study was to identify a cheaper means of producing biomass for biofuel production. Costs of commercial fertilizers (particularly nitrogen) account for a substantial portion of the total production cost of cellulosic biomass, and tend to be a major obstacles to widespread biofuel production (Bennett and Anex, 2008; Castillo et al., 2010). Organic nutrient sources such as manures and biosolids can be substituted for commercial N fertilizers (and incidentally supply P) to further reduce the cost of nutrient supply for biomass production (Castillo et al., 2010). However, several studies have shown that applying organic sources of nutrients based on crop N needs simultaneously supplies excess P to an ecosystem (Chinault and O'Connor, 2008; Oladeji et al., 2008; Agyin-Birikorang et al., 2009). Excess P can cause undesirable environmental effects, threatening surface or ground waters with eutrophication when the receiving waters are P-limited (Daniel et al., 1998). The preliminary study clearly shows that application of GA enhanced N and P uptake from nutrient (N and P) sources, which can lead to reduced application rates of N and P and reduced off-site N and P losses from applied sources.

Consistent with the preliminary study, without GA application, dry matter yields obtained from treatments receiving reduced N application rate (75 kg ha^{-1}; corresponding to 50% of recommended N application rate for sorghum) were generally low (5.1 ± 1.3 kg DMY pot^{-1}) (Figure 2). Within this rate of N application rate (and no GA application), yields obtained from

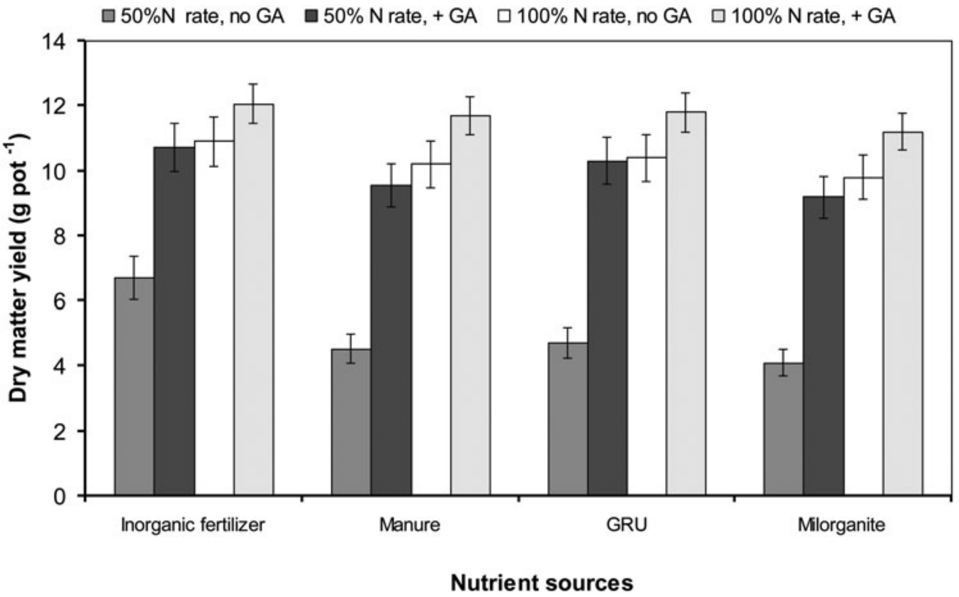

FIGURE 2 Sweet sorghum dry matter yield as a function of nitrogen sources co-applied with gibberellins (GA) at application rates of 3 g GA ha^{-1}. GRU = Gainesville Regional Utilities biosolids.

the inorganic fertilizer treatments were significantly greater than yields obtained from the treatments with the organic sources of N (Figure 2). Application of GA not only increased yields of sorghum, but masked the differences in yields between the treatments with organic and inorganic sources of N. With GA application, DMY from the treatment with inorganic sources of N increased by ∼40%, and by >2 fold when the crops received N from the organic sources (manure and biosolids). Across N sources, there were no significant differences between DMY of treatments with co-application of the 50% N rate plus GA, and the treatments with 100% N rate without GA application (Figure 2). Thus, the organic sources of N could be co-applied at one-half the N-based application rate, with a modest GA application of 3 g ha^{-1}, and obtain similar yields as those obtained from inorganic N sources applied at the recommended N rate. Reduced application of N sources, particularly the organic sources, will not only reduce bioenergy crop production costs, but reduce offsite losses of N and P.

With and without GA application, N uptake by the plants from the organic sources of N applied at 50% the recommended N rate (75 kg ha^{-1}) were similar. Across N sources applied at 75 kg ha^{-1}, N uptake efficiency increased by an average of 0.7 g g^{-1} when GA was applied (Figure 3). Although total N uptake from the plants receiving N applied at the recommended rate of 150 kg ha^{-1} was significantly greater than the N uptake of the treatments with 50% N rate, the increased N uptake did not significantly increase yield,

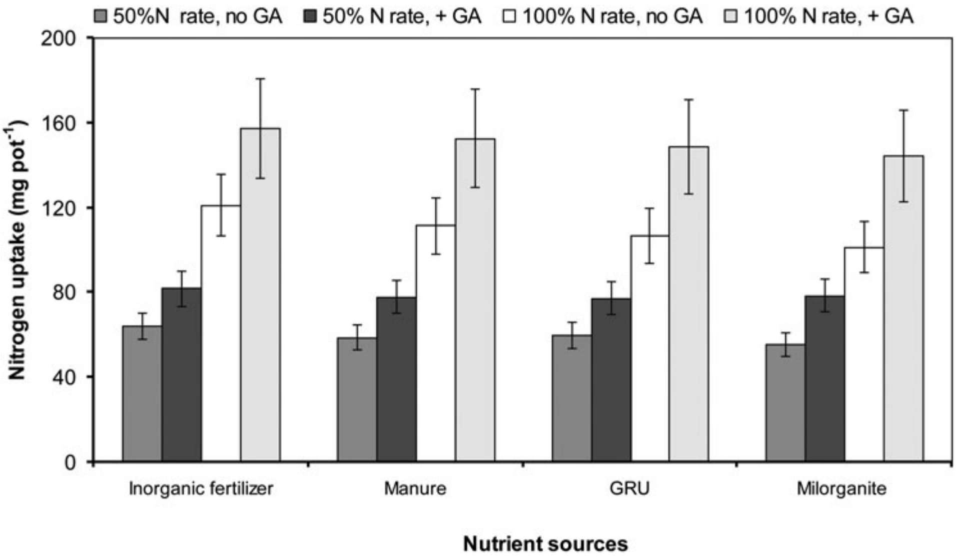

FIGURE 3 Sweet sorghum nitrogen uptake as a function of two application rates (75 and 150 kg PAN ha^{-1}) of different nitrogen sources co-applied with gibberellins at 3 g GA ha^{-1}. GRU = Gainesville Regional Utilities biosolids.

and the N uptake efficiencies were smaller (~ 0.32 g g^{-1} vs. 0.48 g g^{-1}) than the treatments receiving GA with the 50% N rate (Figure 3).

Similarly, GA application significantly increased P uptake from the manure and the biosolids treatments. Application of GA (3 g ha^{-1}) increased P uptake efficiency from 0.36 g g^{-1} to 0.55 g g^{-1} when N was applied at one-half the recommended N rate; and from 0.33 to 0.57 g g^{-1} when N was applied at the recommended rate of application. Without GA application, P uptake from the treatment receiving the inorganic fertilizer (ammonium nitrate) was significantly greater than from the manure and biosolids. However, with GA application, P uptake from both the organic and inorganic sources of nutrients was similar (Figure 4). Thus, with GA application, the concern that land application of organic sources of nutrients for production could negatively impact the environment can be addressed.

As a consequence of the improvement in N and P uptake efficiency through co-application of the nutrient sources with GA, a significant reduction in the quantity of N and P lost was observed, particularly when the nutrient sources were applied at N-based rates. With GA application, the mass of N lost from the treatment with the organic sources of N applied at N-based rates were similar to the losses that occurred in the inorganic fertilizer treatments (Figure 5). Application of the organic sources (biosolids and manure) at N-based rates resulted in differential total P application rates (154 kg P ha^{-1} for GRU biosolids; 133 kg P ha^{-1} for Milorganite biosolids; and 113 kg P ha^{-1} for manure). Therefore, P measured in leachate was

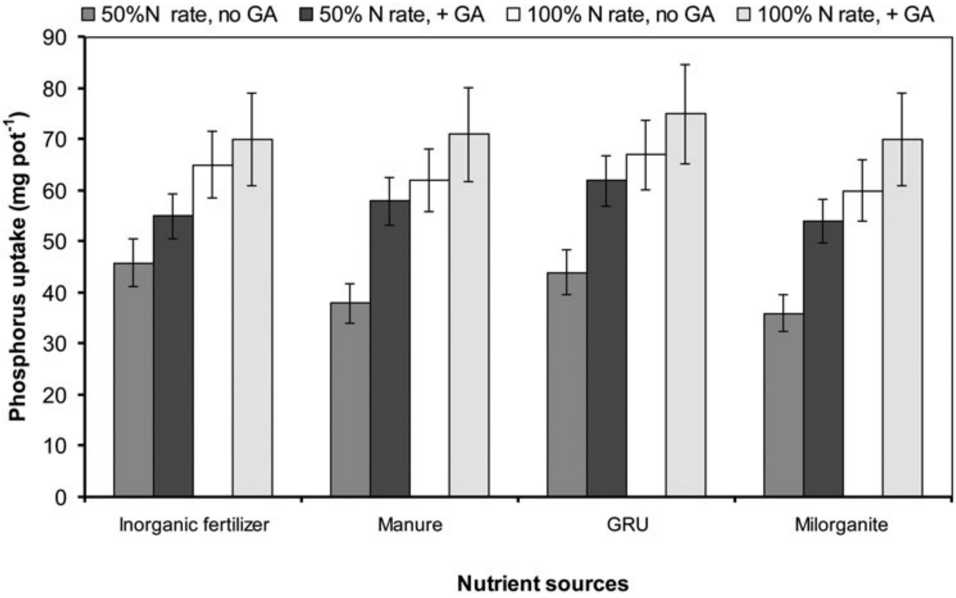

FIGURE 4 Sweet sorghum phosphorus uptake as a function of two application rates (75 and 150 kg PAN ha^{-1}) of different nitrogen sources co-applied with gibberellins at 3 g GA ha^{-1}. GRU = Gainesville Regional Utilities biosolids.

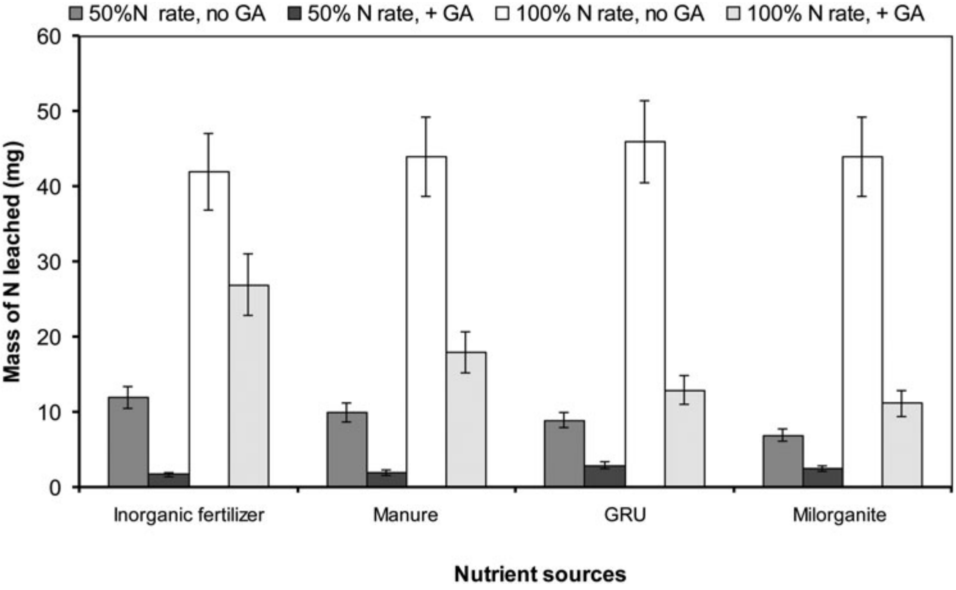

FIGURE 5 Total nitrogen measured in leachate collected as a function of two application rates (75 and 150 kg PAN ha^{-1}) of different nitrogen sources co-applied with gibberellins at 3 g GA ha^{-1}. GRU = Gainesville Regional Utilities biosolids.

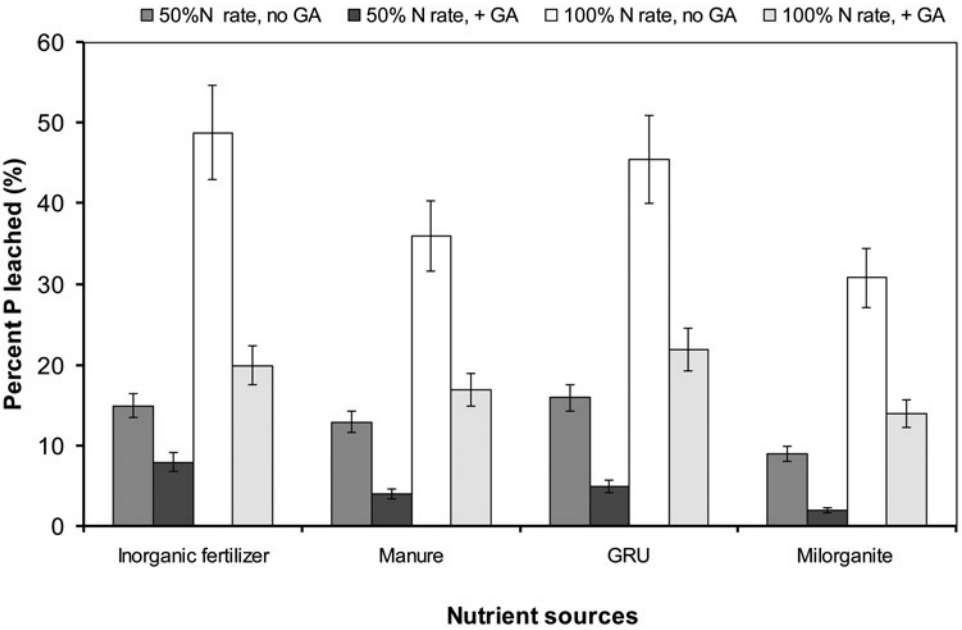

FIGURE 6 Percent of applied phosphorus in the organic and inorganic sources measured in leachate collected as a function of two application rates (75 and 150 kg PAN ha^{-1}), and co-application with gibberellins at 3 g GA ha$^{-1\cdot}$ PAN = Plant available nitrogen.

expressed as a percentage of P supplied in the nutrient sources. Without GA application, there were significant differences in percent P leached among the nutrient sources. The greatest percent P leached (~49%), occurred within the treatment having the inorganic nutrient source followed by GRU biosolids (~45%), manure (~36%), and Milorganite biosolids (~31%) in that order (Figure 6). The P leaching data were consistent with the PWEP values (Table 2), and also consistent with the observations of Brandt et al. (2004) and Agyin-Birikorang et al. (2008) that PWEP values of P sources were strongly related to off-site P losses emanating from the P sources. Application of GA significantly reduced P lost from all treatments, irrespective of the N source and the application rate of the N source (Figure 6).

Nitrogen fertilizer cost accounts for ~80% of the total nutrient cost for biomass production. The N requirements for most bioenergy crops range between 150 (sorghums) and 350 (elephantgrass) kg ha^{-1} (Mylavarapu et al., 2007). With the increases in fertilizer costs in recent times, particularly N fertilizers (from \$1.52 to \$1.94 kg^{-1} N in ammonium nitrate fertilizer over the last five years; United States Department of Agriculture (USDA, 2010), the cost of producing biomass for bioenergy production continues to increase. At N rate of 150 kg ha^{-1}, N cost alone could be as high as ~\$330 ha^{-1}. The current cost of GA is \$30 g^{-1}. Therefore, if N can be applied at one-half recommended rate, together with 3 g GA ha^{-1} applied to the plant, the combined cost will be ~\$255 ha^{-1}, a saving of ≥\$75 ha^{-1}. Most importantly, if the organic sources of nutrients are substituted for inorganic fertilizers, and applied at N-based rates, all the plant N and P requirements will be met at a cheaper cost and a saving of >90% of the total nutrient cost for biomass production.

SUMMARY AND CONCLUSIONS

Application of GA at a minimum rate of 3 g ha^{-1} significantly increased DMY and N and P uptake efficiencies of sweet sorghum, and reduced leaching losses of N and P. Co-application of N at a rate of 75 kg ha^{-1} (one-half of the recommended rate for sweet sorghum production), and GA rate at 3 g ha^{-1} optimized DMY, N and P uptake efficiencies, and minimized N and P leaching losses. Substituting organic nutrient sources such as manures and biosolids for commercial N fertilizers, which incidentally supplied all the required P, further reduced the cost of nutrient supply for biomass production. Co-application of the organic sources of N with GA enhanced N and P uptake and consequently reduced leaching losses. Thus, a low input technology, such as co-applying a modest rate of GA with one-half N-based application rate of manures and biosolids, could optimize biomass production without negatively impacting the environment. Field trials under a variety of soils, climatic conditions, and agronomic management practices are needed to validate the findings of the greenhouse studies.

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REFERENCES

Addy, S., C. E. Niedziela, and M. R. Reddy. 2010. Effect of nitrogen fertilization on stay-green and senescent sorghum hybrids in sand culture. *Journal of Plant Nutrition* 33: 185–199.

Adjei, M. B., J. E. Rechcigl, and R. S. Kalmbacher. 1998. Fertilization effects on the yield and chemical composition of limpograss pasture. *Procedure of Soil and Crop Science Society of Florida* 57: 66–73.

Agyin-Birikorang, S., G. A. O'Connor, and S. R. Brinton. 2008. Evaluating phosphorus loss from a Florida spodosol as affected by phosphorus-source application methods. *Journal of Environmental Quality* 37: 1180–1189.

Agyin-Birikorang, S., G. A. O'Connor, O. Oladeji, T. Obreza, and J. Capece. 2009. Drinking water treatment residual effects on the phosphorus status of field soils amended with biosolids, manure, and fertilizer. *Communications in Soil Science and Plant Analysis* 39: 1700–1719.

Andersen, J. M. 1976. An ignition method for determination of total phosphorus in lake sediments. *Water Research* 10: 329–331.

Barnett, G. M. 1994. Phosphorus forms in animal manure. *Bioresource Technology* 49: 139–147.

Belanger, G., A. Bregard, and R. Michaud. 2002. Phosphorus uptake and concentration of timothy genotypes under varying N applications. *Crop Science* 42: 2044–2048.

Bennett, A. S., and R. P. Anex. 2008. Farm-gate production costs of sweet sorghum as a bioethanol feedstock. *Transactions ASABE* 51: 603–613.

Box, G. E. P., and D. R. Cox. 1964. An analysis of transformations. *Journal of the Royal Statistical Society* 26: 211–243.

Brandt, R. C., H. A. Elliott, and G. A. O'Connor. 2004. Water extractable phosphorus in biosolids: Implications for land-based recycling. *Water Environment Research* 76: 121–129.

Castillo, M. S., L. E. Sollenberger, J. M. B. Vendramini, K. R. Woodard, G. A. O'Connor, Y. C. Newman, M. L. Silveira, and J. B. Sartain. 2010. Municipal biosolids as an alternative nutrient source for bioenergy crops: I. elephantgrass biomass production and soil responses. *Agronomy Journal* 102: 1308–1313.

Chinault, S. L., and G. A. O'Connor. 2008. Phosphorus release from a biosolids-amended sandy spodosol. *Journal of Environmental Quality* 37: 937–943.

Croker, S. J., P. Hedden, J. R. Lenton, and J. L. Stoddart. 1990. Comparison of gibberellins in normal and slender barley seedlings. *Plant Physiology* 94: 194–200.

Daniel, T. C., A. N. Sharpley, and J. L. Lemunyon. 1998. Agricultural phosphorus and eutrophication: A symposium overview Agricultural phosphorus and eutrophication: A symposium overview. *Journal of Environmental Quality* 27: 251–257.

Day, P. R. 1965. Particle fractionation and particle-size analysis. In: *Methods of Soil Analysis, Part 1,* eds. C. A. Black, D. D. Evans, and R. C. Dinauer, pp 545–567. Madison, WI: ASA.

Eghball, B., and J. W. Maranville. 1993. Root development and nitrogen influx of corn genotypes grown under combined drought and nitrogen stresses. *Agronomy Journal* 85: 147–152.

Elliott, H. A., R. C. Brandt, and G. A. O'Connor. 2005. Runoff phosphorus losses from surface applied biosolids. *Journal of Environmental Quality* 34: 1632–1639.

Elliott, H. A., G. A. O'Connor, and S. Brinton. 2002. Phosphorus leaching from biosolids amended sandy soils. *Journal of Environmental Quality* 31: 681–689.

Gallaher, R. N., C. O. Weldon, and J. G. Futral. 1975. An aluminum block digester for plant and soil analysis. *Soil Science Society of America Proceedings* 39: 803–806.

Gardner, W. H. 1986. Water content. In: *Methods of soil analysis, Part 1,* ed. A. Klute, pp. 493–545. Madison, WI: ASA.

Gardner, J. C., J. W. Maranville, and E. T. Paparozzi. 1994. Nitrogen use efficiency among diverse sorghum cultivars. *Crop Science* 34: 728–733.

Hedden, P., and A. L. Phillips. 2000. Gibberellin metabolism: New insights revealed by the genes. *Trends in Plant Science* 5: 523–530.

Kuo, S. 1996. Phosphorus. In: *Methods of Soil Analysis, Part 3,* ed. D. L. Sparks, Pp. 869–919. Madison, WI: SSSA.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. *SAS System for Mixed Models.* Cary, NC: SAS Institute.

Maguire, R. O., J. T. Sims, S. K. Dentel, F. J. Coale, and J. T. Mah. 2001. Relationships between biosolids treatment processes and soil phosphorus availability. *Journal of Environmental Quality* 37: 979–984.

McLean, E. O. 1982. Soil pH and Lime Requirement. In: *Methods of Soil Analysis, Part 2,* ed. A. L. Page, pp 199–224. Madison, WI: ASA.

Mehlich, A. 1984. Mehlich-3 soil test extractant: A modification of Mehlich-2 extractant. *Communications in Soil Science and Plant Analysis* 15: 1409–1416.

Miller, M., and G. A. O'Connor. 2009. The longer-term phytoavailability of biosolids phosphorus. *Agronomy Journal* 101: 889–896.

Moll, R. H., E. J. Kamprath, and W. A. Jackson. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen-utilization. *Agronomy Journal* 74: 562–564.

Monti, A., and G. Venturi. 2003. Comparison of the energy performance of fibre sorghum, sweet sorghum and wheat monocultures in northern Italy. *European Journal of Agronomy* 19: 35–43.

Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27: 31–36.

Mylavarapu, R., D. Wright, G. Kidder, and C. G. Chambliss. 2007. UF/IFAS standardized fertilization recommendations for agronomic crops. Cooperative Extension Service IFAS, University of Florida. SL129. Available at http://edis.ifas.ufl.edu/SS163 (accessed 16 July 2013).

Nelson, D. W., and L. E. Sommers. 1996. Total carbon, organic carbon, and organic matter. In: *Methods of Soil Analysis, Part 3,* ed. D. L. Sparks, pp. 961–1010. Madison, WI: SSSA.

Newman, Y. C., S. Agyin-Birikorang, M. B. Adjei, J. M Scholberg, M. L. Silveira, J. M. B. Vendramini, J. E. Rechcigl, and J. E. Sollenberger. 2009. Nitrogen fertilization effect on phosphorus remediation potential of three perennial warm-season forages. *Agronomy Journal* 101: 1243–1248.

O'Connor, G. A., and H. A. Elliott. 2006. The agronomic and environmental availability of biosolids-P (Phase II). Rep. 99-PUM-2T. Alexandria, VA: Water Environment Research Foundation.

Oladeji, O. O., G. A. O'Connor, and J. B. Sartain. 2008. Relative phosphorus phytoavailability of different phosphorus sources. *Communications in Soil Science and Plant Analysis* 39: 2398–2410.

Richards, D. E., K. E. King, T. Ait-Ali, and N. P Harberd. 2001. How gibberellin regulates plant growth and development: A molecular genetic analysis of gibberellin signaling. *Annual Review of Plant Physiology and Plant Molecular Biology* 52: 67–88.

Santner, A., L. I. A. Calderon-Villalobos, and M. Estelle. 2009. Plant hormones are versatile chemical regulators of plant growth. *Nature Chemical Biology* 5: 301–307.

Schoumans, O. F. 2009. Determining the degree of phosphate saturation in non-calcareous soils. In: *Methods of Phosphorus Analysis for Soils, Sediments Residuals and Waters,* eds. J. L. Kovar, and G. M. Pierzynski, pp. 29–32. Southern Cooperative Series Bull. 408. Raleigh, NC: Cooperative Extension Service, North Carolina State University.

Schwechheimer, C., and B. C. Willige. 2009. Shedding light on gibberellic acid signaling. *Plant Biology* 12: 57–62.

Sharpley, A. N., and B. Moyer. 2000. Phosphorus forms in manure and compost and their release during simulated rainfall. *Journal of Environmental Quality* 29: 1462–1469.

Shober, A. L., and J. T. Sims. 2003. Phosphorus restrictions for land application of biosolids: Current status and future trends. *Journal of Environmental Quality* 32: 1955–1964.

Thomas, G. W. 1996. Soil pH and soil acidity. In: *Methods of Soil Analysis, Part 3,* ed. D. L. Sparks, pp. 475–490. Madison, WI: SSSA.

USDA (United States Department of Agriculture). 2010. The economics of food, farming, natural resources and rural America. Economic Research Service. Available at: http://www.ers.usda.gov/Data/FertilizerUse/ (accessed 16 July 2013).

USEPA (United States Environmental Protection Agency). 1983. Methods for chemical analysis of water and wastes. EPA-600/4-79-020. Washington, DC: USEPA.

USEPA (United States Environmental Protection Agency). 1986. Acid digestion of sediments, sludges, and soils. Section A, Part I, Chapter Three – Metallic analytes. Method 3050. SW-846, test methods for evaluating solid waste. Washington, DC: USEPA.

USEPA (United States Environmental Protection Agency). 1995. Process design manual: Land application of sewage sludge and domestic septage. EPA/625/R-95/001. Cincinnati, OH: Office of Research and Development, USEPA.

Yabuta, T., and T. Hayasi. 1940. Biochemical studies of 'bakanae' fungus of rice. *Journal of the Imperial Agricultural Experimental Station, Nisigahara, Tokyo* 3: 365–400.