



Incorporation of Municipal Biosolids Affects Organic Nitrogen Mineralization and Elephantgrass Biomass Production

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ABSTRACT

Municipal biosolids (MBS) represents an alternative source of nutrients for the production of bioenergy crops like elephantgrass (*Pennisetum purpureum* Schum.). Two experiments were conducted during 2 yr in Florida to evaluate the effect of soil incorporation vs. surface application of MBS on: (i) elephantgrass dry matter (DM) yield, tissue N and P concentration and removal, and soil C and P (Exp. 1); and (ii) organic N mineralization and DM decomposition rates of MBS measured in the field using a litter bag incubation technique (Exp. 2). In Exp. 1, three treatments supplied 350 kg total N ha⁻¹ yr⁻¹ from surface-applied municipal biosolids (MBS-SA), soil-incorporated municipal biosolids (MBS-INC), and surface-applied ammonium nitrate (NH₄NO₃). A fourth treatment provided 700 kg total N ha⁻¹ yr⁻¹ from MBS-SA (double rate of municipal biosolids, 2x-MBS). In Exp. 2, MBS was field incubated in litter bags placed on the soil surface or at a 5-cm soil depth. Elephantgrass DM yield, and N and P removal were greater for MBS-INC than MBS-SA. Dry matter yield for MBS-INC was not different than for NH₄NO₃ fertilizer (22.5 vs. 24.3 Mg ha⁻¹). Removal of N and P increased 39 and 10 kg ha⁻¹ yr⁻¹, respectively, for MBS-INC and MBS-SA. Total organic N mineralized was greater for MBS-INC (386 g kg⁻¹) than MBS-SA (308 g kg⁻¹). Incorporation of MBS increases elephantgrass DM yield and nutrient removal compared to surface application and allows MBS to replace a greater proportion of inorganic N fertilizer.

ADEQUATE NUTRIENT SUPPLY is necessary to meet the requirements of high-yielding bioenergy crops. Land application of MBS has been shown to be an effective alternative for reducing the amount of inorganic fertilizer used in bioenergy crop production systems (Heller et al., 2003; Castillo et al., 2010b). Use of MBS also recycles nonrenewable resources and addresses the problem of finding alternative options for MBS disposal.

Like many other organic sources of nutrients, when MBS is applied on an N-rate basis there is potential for overapplication of P due to the narrower N:P ratio in MBS vs. plant requirements. Typical concentrations of total N and P in MBS range from 35 to 68 and 18 to 39 g kg⁻¹, respectively (USEPA, 1999). Thus, in some cases application of MBS is limited to P-based rates. Another issue of significance when using MBS is that ~800 g kg⁻¹ of the total N is in organic forms (Sommers, 1977; Binder et al., 2002), meaning that this N is not immediately available for plant uptake. Plant-available nitrogen (PAN) in MBS is a function of the initial inorganic N concentration (NH₄-N and NO₃-N) and the proportion of organic N that mineralizes over time (Gilmour and Skinner, 1999).

Organic N mineralization occurs through the activity of nonspecific heterotrophic soil microorganisms (Jarvis et al., 1996; Pierzynski et al., 2000; He et al., 2003). The rate and

quantity of mineralization largely depends on the quantity and composition of the organic matter (Sommers et al., 1981) and the influence of the environment on biological activity (Goncalves and Carlyle, 1994; Castillo et al., 2010a). Thus, placement of the organic substrate (soil-incorporated vs. surface-applied), results in differences in microenvironmental conditions (e.g., contact of MBS with moist soil particles and microbes) and may influence the dominant forms of N (NH₄-N vs. NO₃-N) and rates of organic-N mineralization (Terry et al., 1978; Clark and Gilmour, 1983; Gilmour and Clark, 1988; He et al., 2003).

Experiments with poultry litter and cattle manure have demonstrated benefits of soil incorporation. These include reduction in N volatilization losses, odors, and residue cover as well as an increase in organic N mineralization and crop yields (Hanna et al., 2000; Pote et al., 2003; Sharpe et al., 2004). Several laboratory incubation studies have demonstrated the positive effects of incorporation of organic materials produced in wastewater treatment plants (King, 1973; Terry et al., 1978; Sommers et al., 1981; Adamsen and Sabey, 1987; He et al., 2003). Fewer data have been reported from actual field-incubation trials (He et al., 2000; Hanselman et al., 2004) and from studies that have measured plant responses (e.g., DM yield) as an indicator of organic N mineralization.

Although soil incorporation as a nutrient management practice has been studied and recommended for a variety of organic and inorganic sources, little attention has been given to the effect of incorporation of MBS on plant DM yield. Thus, two experiments were conducted under field conditions to assess the effects of incorporation of Class A MBS on plant and soil responses and on decomposition and organic N mineralization rates of MBS. The objectives of Exp. 1 were to: (i) quantify

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Abbreviations: BDR, biosolids decomposition rate; DM, dry matter; MBS, municipal biosolids; MBS-INC, municipal biosolids, soil incorporated; MBS-SA, municipal biosolids, surface applied; 2x-MBS, double rate of municipal biosolids; NMR, nitrogen mineralization rate; WEP, water-extractable phosphorus.

DM production, tissue N and P concentration, and N and P removal by elephantgrass when MBS was incorporated and surface-applied; and (ii) measure changes in total soil C and P concentration when MBS was applied as a nutrient source. The objective of Exp. 2 was to determine the effect of MBS method of application (incorporated vs. surface-applied MBS) on organic N mineralization and DM decomposition rates measured in the field using a litter-bag technique.

MATERIALS AND METHODS

Experiment 1

Experimental Site

The experiment was conducted during 2007 and 2008 at the Range Cattle Research and Education Center (RCREC) at Ona, FL (27°26'N, 81°55' W). The soil at the experimental site was classified as Ona sand (sandy, siliceous, hyperthermic Typic Alaquods). Initial soil characterization of the Ap horizon (to a depth of 20 cm) indicated average soil pH of 6.0 and Mehlich-1 extractable P, K, Mg, and Ca of 10, 42, 230, and 1510 mg kg⁻¹, respectively. Annual rainfall was 1006 mm in 2007 and 1141 mm in 2008, compared to the 30-yr average of 1392 mm. Temperatures were similar during both years with average low and high temperatures of 16 and 29°C, and average daily temperature of 22°C. Weekly precipitation and temperature data during 2007 and 2008 were previously reported for this location (Castillo et al., 2010a).

Treatments and Design

An elephantgrass of unknown origin, referred to as Chinese Cross, was chosen for use in this study because it had the most complete and uniform stands of several elephantgrass types available at the research location. The growth habit and morphological characteristics of Chinese Cross are similar to other tall elephantgrasses used in Florida. The experimental area contained four replicate 9- by 16-m plots of Chinese Cross established during the early 1990s and were used for experimentation during that decade. Thereafter, accumulated biomass was harvested and removed from the plots once per year at the end of the growing season. Each of the 9- by 16-m areas served as a block in the current study, and four 4- by 4-m plots, which served as the experimental units, were placed in each block. Plots were separated by alleys on all sides that were at least 1-m wide.

There were four treatments, and three of them supplied 350 kg total N ha⁻¹ yr⁻¹ from: (i) surface-applied NH₄NO₃, (ii) MBS-SA, and (iii) MBS-INC. The fourth treatment supplied 700 kg total N ha⁻¹ yr⁻¹ from surface-applied MBS (2x-MBS). Total amounts of MBS and N fertilizer applied were chosen based on earlier work by Woodard and Prine (1991). For all treatments, one-half of N was applied at the beginning of the growing season and the other half immediately after the first of two biomass harvests each year. The MBS were applied on 20 May and 3 Aug. 2007 and 4 Apr. and 8 Aug. 2008. Incorporation of the MBS in the MBS-INC treatment was accomplished by disturbing the top 5 cm of soil using a garden rake immediately after broadcasting the MBS on the soil surface.

The quantity of MBS required to achieve the target N amount was calculated based on total N analysis of each batch of MBS. The amount of P supplied was a function of P concentration in the MBS. Total P applied was 118 kg ha⁻¹ in 2007 and 142 kg ha⁻¹ in 2008 for both the MBS-SA and

Table 1. Composition of the Class A municipal biosolids used as a nutrient source for elephantgrass plots and the incubation study.†

Item	Batch§			
	1	2	3	4
pH‡	6.3 ± 0.2	6.4 ± 0.1	6.5 ± 0.0	6.6 ± 0.1
	g kg ⁻¹			
Dry matter	950 ± 2	990 ± 1	980 ± 2	980 ± 1
Total C#	352 ± 0.1	373 ± 0.1	338 ± 0.1	340 ± 0.1
Total N¶	58 ± 1	65 ± 0	54 ± 0	54 ± 0
Organic N§	57	64	53	52.6
C/N ratio	6.1	5.8	6.2	6.3
Total P††	20 ± 2	21 ± 1	20 ± 2	23 ± 1
	mg kg ⁻¹			
NH ₄ -N‡‡	881 ± 20.0	618 ± 0.1	966 ± 17.9	1317 ± 1.6
NO ₃ -N	37 ± 0.6	9 ± 0.3	37 ± 0.6	28 ± 0.5
WEP§§	37 ± 2.6	186 ± 2.9	30 ± 0.5	64 ± 2.4

† Data are means and standard deviations of two samples per batch, on a 100% dry matter basis.

‡ pH determined using 15 g wet sample + 200 mL deionized water. Sample was stirred and allowed to stabilize for 5 min.

§ Organic N calculated as: Total N - (NH₄-N + NO₃-N).

Total C determined by dry combustion (Nelson and Sommers, 1996) on a Thermo Flash EA1112-NC elemental analyzer.

¶ Total N determined using a micro-Kjeldahl method, a modification of the aluminum block digestion procedure (Gallaher et al., 1975) followed by semi-automated colorimetric determination (Hambleton, 1977).

†† Phosphorus determined by ashing samples and digesting using 6 M HCl. Analyzed using ICP spectrometer.

‡‡ NH₄-N and NO₃-N determined by shaking 2.5 g of biosolids with 25 mL of 1 M KCl for 1 h and analyzing concentrations with a Rapid Flow Analyzer (RFA, method A303-S020, Alpchem Corporation, 1989).

§§ Water-extractable phosphorus (WEP) determined using a 1:200 ratio with DDI water (Kuo, 1996).

MBS-INC treatments. These amounts were doubled for the 2x-MBS treatment. For the NH₄NO₃ treatment, P was supplied at a rate of 60 kg P ha⁻¹ yr⁻¹ from triple superphosphate (460 g P₂O₅ kg⁻¹). Expected P removal of elephantgrass herbage was 40 to 60 kg P ha⁻¹ yr⁻¹ based on previous research (Vicente-Chandler et al., 1959; Sanchez, 1976; Boonman, 1993). In addition, all treatments received 145 kg K ha⁻¹ yr⁻¹ from a potassium-magnesium sulfate source "Sul-Po-Mag" (220 g K₂O kg⁻¹, and 110 g Mg kg⁻¹); half was applied at each date when MBS and NH₄NO₃ fertilizer application occurred.

The MBS consisted of anaerobically digested sludge blended with undigested waste-activated sludge that was dried to an average 950 g DM kg⁻¹ product (Hicks et al., 2007). The heat drying (150–200°C) serves to further reduce pathogens and results in Class A biosolids (USEPA, 1995) that are marketed as a slow-release soil amendment. The MBS required for each treatment application date was collected separately from four different batches obtained from the municipality (two per year). Two composite subsamples from each batch were analyzed before application of treatments in the field (Table 1). Detailed nutrient and heavy metal concentration of the MBS can be found in Castillo et al. (2010b).

Response Variables

Dry matter yield was measured two times per year in each plot by harvesting all tillers within a 3- by 1.5-m quadrat to a 10-cm stubble height. Harvests occurred on 26 July and 27 Nov. 2007, and 24 July and 21 Nov. 2008. A bush cutter with a metal blade was used to cut elephantgrass tillers. The material was collected and weighed fresh in the field. A

subsample of the tillers was weighed fresh and dried at 60°C until constant weight to determine DM concentration and to calculate DM yield. Following removal of the yield sample, the remainder of the plot was clipped to 10-cm stubble and the harvested material was removed from the plots.

Dried subsamples were ground first in a hammer mill and then through a 1-mm screen in a Model 4 Wiley mill (Thomas Scientific, Swedesboro, NJ) before laboratory analyses. Total N and P concentrations in the tissue were determined using a micro-Kjeldahl method (Gallaher et al., 1975) and semi-automated colorimetry (Hambleton, 1977). Crop N and P removals were calculated for each harvest as the product of tissue N and P concentration and DM yield of that harvest, and summed across the two harvests per year to determine the annual total.

Soil samples for C and P analysis were taken before treatments were applied in 2007 and at the end of the 2007 and 2008 growing seasons. Samples were collected from the Ap horizon that ranged from 15- to 20-cm deep. Two samples per plot were collected, air dried, crushed, and passed separately through a 2-mm sieve. Plant roots and solid particles that did not pass the sieve after crushing were discarded. For laboratory analysis, one sample per plot was obtained by compositing the two samples from that plot on an equal volume basis. The soil samples were analyzed for water-extractable phosphorus (WEP), total P, Mehlich-1 P, and total soil C. Water-extractable P was determined by adding 20 mL of distilled/deionized water to 2 g of air-dried soil (Kuo, 1996). For total P determination, 0.3 g of air-dried soil was ashed and digested according to Andersen (1976). Water-extractable P and total-P concentrations were determined using the ascorbic acid method of Murphy and Riley (1962). Mehlich-1 P concentrations were determined according to Mylavarapu (2009). For total soil C analysis the samples were further ground using a mortar and pestle. Total soil C was determined by dry combustion (Nelson and Sommers, 1996) on a Thermo Flash EA1112-NC elemental analyzer (CE Elantech, Lakewood, NJ).

Statistical Analysis

Data were analyzed using mixed-model methods (SAS Institute, 1996). Year and treatment were considered fixed effects. Year was considered fixed due to potential carry-over effects in Year 2 from application of MBS in Year 1. Blocks were considered random effects. When there was treatment \times year interaction, data were analyzed by year. Treatments were compared using the pdiff test of LSMEANS. For analysis of soil data, initial P and C concentrations (before treatment application) were tested as covariates, but the covariates were not significant and were removed from the model. All means reported in the text are least squares means. Treatments were considered different if $P \leq 0.05$.

Experiment 2

Because of the positive elephantgrass yield response to soil incorporation of MBS in 2007, a litter bag experiment was implemented in 2008 to determine if this response could be explained by organic N mineralization from soil-incorporated MBS. For logistical reasons (i.e., regular bag collection and hand weeding of experimental site), the litter bag study was conducted at the University of Florida Beef Research Unit (29°43' N; 82°21' W) near

Gainesville, FL. Timing of the trial and location were selected so that environmental conditions would be similar there and at the site where the elephantgrass yield study was ongoing.

Experimental Site

Experiment 2 was conducted during 2008 and 2009 on Chipley sand (thermic, coated Aquic Quartzipsamments). Initial characterization of the topsoil (to a depth of 20 cm) indicated soil pH of 6.1 and Mehlich-1 extractable P, K, Mg, and Ca of 82, 49, 72, and 473 mg kg⁻¹, respectively. Although the soils at the two locations (Exp. 1 vs. Exp. 2) are from different series, they both have an Ochric epipedon to a depth of ~20 cm (Ap horizon) and share similar physical and chemical characteristics. Temperature and rainfall conditions at this site during the 144-d period that the experiment was conducted were similar during both years (2008 and 2009), and on average, were similar to the conditions at Ona in Exp. 1 (Years 2007 and 2008). Average low and high temperatures were 14 and 27°C, respectively (compared to 16 and 29°C at Ona), and average daily temperature was 20°C (compared to 22°C at Ona). Total rainfall during the 144-d experimental period was 469 mm in 2008 and 423 mm in 2009, compared to the 30-yr average during that period of 424 mm, and was similar to the rainfall conditions during the same period in the summer season of 2007 (441 mm) and 2008 (432 mm) at Ona. Therefore, no major differences were expected in organic N mineralization due to different locations for Exp. 1 and 2.

Treatments and Design

Polyester bags containing MBS were used for the field incubation study. Similar bags were used in previous studies of grass and legume litter and MBS decomposition (Dubeux et al., 2006; Castillo et al., 2010a; Mulvaney et al., 2010). Enough MBS from Batch 4 (Table 1) was acquired in 2008 and stored in closed plastic containers at room temperature to be used for the 2 yr of this experiment. Laboratory analysis of MBS composition each year before treatment application showed that storage conditions successfully protected the MBS against changes in chemical composition.

Based on an application amount of 350 kg total N ha⁻¹ yr⁻¹ from MBS in Exp. 1, 25 g of MBS were placed in 15- by 20-cm polyester bags of 75- μ m mesh. Bags were placed in the field on 16 August each year and collected at seven incubation times following placement. Incubation times were 3, 6, 12, 24, 48, 96, and 144 d. Initiation of the study was timed to correspond with the application of MBS that followed the first harvest of Exp. 1. Selection of incubation times and duration of the experiment was based on a previous study conducted at the same location as Exp. 1 that showed that organic N mineralization from MBS leveled off ~50 d after application during high temperature, high rainfall conditions (Castillo et al., 2010a).

The treatments were MBS-INC and MBS-SA. Bags for MBS-SA were placed on the soil surface, while for MBS-INC, the bags were buried to a depth of 5 cm below the soil surface. To prevent contamination by roots penetrating the bags, the entire area was tilled before bags were placed in the field and all plant seedlings were removed by hand weeding every other week. Additionally, the areas where bags were placed were caged with kennel wire to prevent disturbance by wildlife. Treatments were arranged in a randomized complete block design with

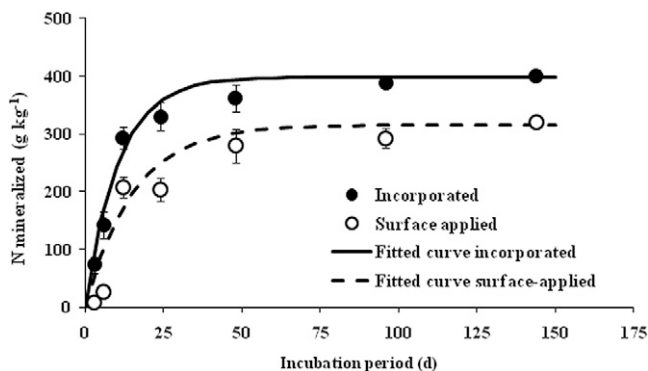


Fig. 1. Organic N mineralization from surface-applied and soil-incorporated municipal biosolids (MBS). Data are averages from 2 yr (2008 and 2009) when field incubation started on 16 August. Error bars represent treatment means ($n = 8$) \pm 95% confidence interval of the mean.

four replicates. Two complete sets of bags (sampling units) were included in each experimental unit, and each experimental unit was separately caged. Thus, for each incubation time there were a total of eight observations per treatment (four replicates \times two bags per replicate). Also, at each incubation time, two control (empty) bags per treatment were collected.

Following collection, the bags were immediately dried in a forced-air oven at 60°C for 72 h. Before weighing the dried bags, the outside was lightly brushed to remove extraneous materials. Each bag was weighed and weights were recorded individually. Final weights were corrected by the weight of the control bags. The contents of all bags from a single incubation time in a given experimental unit were composited and stored in Ziploc bags at room temperature awaiting subsequent analysis.

Before laboratory analysis, particle size homogenization of the collected MBS samples was achieved by grinding the samples using a mortar and pestle. Total N was determined as described in Exp. 1. Extractable $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ were determined by shaking 2.5 g of MBS with 25 mL of 1 M KCl for 1 h and analyzing concentrations of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ with a Rapid Flow Analyzer (RFA, method A303-S020, Alpkem Corporation, 1989).

Response Variables

Response variables calculated were biosolids decomposition rate (BDR) and organic nitrogen mineralization rate (NMR). The BDR was calculated as:

$$\text{BDR} = [(W_0 - W_x)/(W_0)] \times 100$$

where W_0 equals the initial weight of MBS and W_x equals the weight of MBS at a specific incubation time x . The final weights were corrected by the weights of the control bags. The NMR was calculated as:

$$\text{Organic NMR} = [(\text{Organic } N_0 - \text{Organic } N_x) / (\text{Organic } N_0)] \times 100$$

where N_0 equals the initial weight of organic N and N_x equals the weight of organic N at a specific incubation time x and where $\text{Organic N} = \text{Total N} - (\text{NH}_4\text{-N} + \text{NO}_3\text{-N})$. The approach was based on the work of He et al. (2000) and Castillo et al. (2010a).

Table 2. Regression coefficients for organic N mineralization estimated using NL MIXED procedures (see fitted models in Fig. 1).†

Treatment‡	N_0 g kg ⁻¹	k d ⁻¹
MBS-INC	386	0.08933
MBS-SA	308	0.05141
SE	12.4	0.00871
P value	<0.0001	<0.0001

† $N_{\min} = N_0 (1 - e^{-kt})$ where N_{\min} = amount of N mineralized at a specific time (t) and N_0 and k are regression coefficients.

‡ MBS-INC = soil-incorporated municipal biosolids; MBS-SA = surface-applied municipal biosolids.

Statistical Analysis

Incubation time was considered a repeated measure. The data were subjected to repeated measures analysis using mixed model procedures (SAS Institute, 1996; Littell et al., 1998), with correlation modeled via replicates by treatment within year. All means reported are least squares means. Treatment differences were obtained by using the ESTIMATE statement of SAS (Littell et al., 1991, p. 31–63). Differences were considered significant if $P \leq 0.05$.

The nonlinear mixed models procedure (PROC NL MIXED) (Kratzer and Littell, 2006) was used to fit equations to the data describing organic N mineralization and MBS mass remaining. Organic N mineralization was described using the following equation (Stanford and Smith, 1972; Epstein et al., 1978; Wieder and Lang, 1982):

$$N_{\min} = N_0 (1 - e^{-kt})$$

where N_{\min} = percentage of organic N mineralized at a specific time t ; N_0 = constant that represents mineralizable organic N (asymptote); and k = rate constant. Municipal biosolids DM remaining was described as follows (Wieder and Lang, 1982):

$$X = a + (1000 - a) e^{-kt}$$

where X = percentage of MBS mass remaining at a specific time t , and a and k are constants.

RESULTS AND DISCUSSION

Organic Nitrogen Mineralization

Data from the litter bag study (Exp. 2) are presented first because of their relevance to subsequent discussion of the elephantgrass yield and composition data (Exp. 1). There were no year or incubation time interactions with treatment for organic N mineralization. Organic N mineralization was greater for MBS-INC than MBS-SA throughout the entire incubation period (Fig. 1). First-order kinetics equations were fitted to the data following the work by Sommers et al. (1981) who showed that this model described C and N mineralization. The maximum organic N mineralized, as estimated by the models, was 386 and 308 g kg⁻¹ for MBS-INC and MBS-SA, respectively (Table 2). Organic N mineralization leveled off ~50 d after initiation of field incubation for both treatments (Fig. 1), similar to the response reported for MBS placed in litter bags on the soil surface during summer (Castillo et al., 2010a).

Data from the current experiment indicated that incorporation of MBS increased organic N mineralization by 25% (308 and 386 g mineralized N kg⁻¹ organic N for surface-applied and

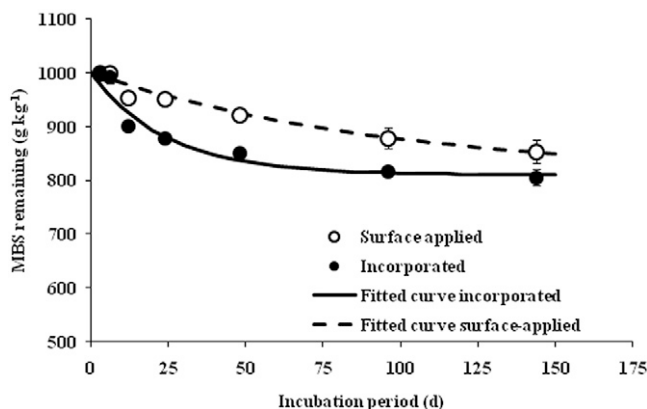


Fig. 2. Municipal biosolids (MBS) dry matter mass remaining following initiation of field incubation on 16 Aug. 2008 and 2009. Data are averages from the 2 yr. Error bars represent treatment means ($n = 8$) \pm 95% confidence interval of the mean.

incorporated MBS, respectively). Other studies have shown positive effects of soil incorporation, although the magnitude of the response has varied. He et al. (2003) reported that incorporation of biosolids increased N mineralization by 60% (221 and 362 g mineralized N kg⁻¹ for surface-applied and incorporated biosolids, respectively). Using a similar approach to that of the current experiment, Adamsen and Sabey (1987) reported that apparent organic mineralization was 298 and 325 g kg⁻¹ (9% increase) for the surface-applied and soil-incorporated treatments, respectively.

Biosolids Dry Matter Decomposition

Dry matter decomposition of MBS, expressed as the percentage of MBS mass remaining (Fig. 2), was faster initially for MBS-INC, but at the end of the 144 d of field incubation the mass of MBS remaining was similar for both treatments (Fig. 2). Approximately 100 and 150 g kg⁻¹ MBS mass disappeared when MBS was surface-applied and incorporated, respectively. Faster BDR in the MBS-INC treatment can be attributed to less variation in environmental conditions such as soil moisture and temperatures, compared to the MBS-SA treatment, and this more stable environment likely encouraged microbial population growth.

Table 4. Tissue N and P concentration of Chinese Cross elephantgrass fertilized with Class A municipal biosolids (MBS) or ammonium nitrate fertilizer (NH₄NO₃). Nitrogen concentrations are means across 2 yr and four replicates ($n = 8$) and P concentrations are means across four replicates ($n = 4$).

Treatment†	N concentration	P concentration	
		2007	2008
g kg ⁻¹ DM			
NH ₄ NO ₃	8.1a‡	1.7a‡	1.9c
MBS-SA	7.4a	1.8a	2.4ab
MBS-INC	7.4a	1.8a	2.3b
2x-MBS	7.9a	1.7a	2.6a
SE	0.4	0.1	0.1

† NH₄NO₃ = ammonium nitrate fertilizer as N source; MBS-SA = surface-applied municipal biosolids; MBS-INC = soil-incorporated municipal biosolids; 2x-MBS = twice the amount of surface-applied municipal biosolids as MBS-SA. All treatments supplied a total of 350 kg N ha⁻¹ yr⁻¹ except 2x-MBS where total N supplied was 700 kg N ha⁻¹ yr⁻¹.

‡ Means within a column not followed by the same letter are different by pdiff in LSMEANS ($P \leq 0.05$).

Table 3. Dry matter (DM) yield and N and P removal of Chinese Cross elephantgrass fertilized with Class A municipal biosolids (MBS) or ammonium nitrate (NH₄NO₃) fertilizer. Data are means across four replicates and 2 yr ($n = 8$) for DM yield and N removal and across four replicates for P removal ($n = 4$).

Treatment†	DM yield	N removal	P removal	
			2007	2008
kg ha ⁻¹ yr ⁻¹				
NH ₄ NO ₃	24.3a‡ (100)§	195a	39ab	47b
MBS-SA	16.9b (70)	127b	31b	39c
MBS-INC	22.5a (93)	166a	41a	50b
2x-MBS	21.6a (89)	170a	37ab	58a
SE	1.2	16	2.4	3

† NH₄NO₃ = ammonium nitrate fertilizer as N source; MBS-SA = surface-applied municipal biosolids; MBS-INC = soil-incorporated municipal biosolids; 2x-MBS = twice the amount of surface-applied municipal biosolids as MBS-SA. All treatments supplied a total of 350 kg N ha⁻¹ yr⁻¹ except 2x-MBS where total N supplied was 700 kg N ha⁻¹ yr⁻¹.

‡ Means within a column not followed by the same letter are different by pdiff in LSMEANS ($P \leq 0.05$).

§ Numbers in parentheses are percentages expressing the yield of that treatment as a percentage of the yield of the NH₄NO₃ treatment.

Elephantgrass Dry Matter Yield

There was no treatment \times year interaction or year effect for DM yield in Exp. 1. Elephantgrass DM yield increased from 16.9 for MBS-SA to 22.5 Mg ha⁻¹ for MBS-INC (Table 3). The DM yield of the MBS-INC treatment was not different from that of the NH₄NO₃ treatment or the 2x-MBS treatment. Elephantgrass DM yield was similar to that reported previously for two harvests per year and less than that of a single harvest per year (Woodard and Prine, 1991).

Initial chemical characterization of the MBS showed that ~980 g kg⁻¹ of total N was in organic forms (Table 1); therefore, mineralization of organic N was expected to have the greatest impact on crop yield for treatments where MBS was applied. Soil incorporation of MBS increased mineralization of organic N by 25% (386 vs. 308 g kg⁻¹ of organic N) over surface application (Table 2). The elephantgrass yield increase due to incorporation (MBS-INC vs. MBS-SA) was 33%, indicating that greater organic N mineralization following incorporation likely accounted for most of this increase. The magnitude of differences between treatments did not change with year, indicating that release of N in the second year was minimal from MBS applied in the first year. This conclusion is supported by the data showing that organic N release leveled off 50 d after application (Fig. 1).

Tissue Nitrogen Concentration and Removal

There was no treatment \times year interaction for N concentration or N removal, but both responses were greater in 2007 than 2008. There was no treatment effect on tissue N concentration (Table 4), and it averaged 8.1 and 7.2 g N kg⁻¹ DM in 2007 and 2008, respectively. Greater N concentration in 2007 compared to 2008 may be attributed to the shorter period from fertilization to first harvest in 2007 (67 d) than in 2008 (111 d). The shorter period was due to severe spring drought in 2007, which delayed onset of spring growth and application of MBS and fertilizer. The literature includes several examples of less mature elephantgrass herbage having greater N concentration (Gomide et al., 1969; Woodard and Prine, 1991; Schank and Chynoweth, 1993).

Elephantgrass N removal was 176 and 153 kg N ha⁻¹ yr⁻¹ in 2007 and 2008, respectively. Greater N removal in 2007 than in 2008 reflected the higher tissue N concentration in

Table 5. Soil P and C concentrations in the Ap horizon (0–20 cm) after addition of municipal biosolids (MBS) and ammonium nitrate (NH₄NO₃) fertilizer. Data are means across 2 yr, and samples were taken at the end of the 2007 and 2008 growing seasons.

Treatment†	WEP‡	Mehlich-1 P	Total P	Total C
		mg kg ⁻¹		g kg ⁻¹
NH ₄ NO ₃	3.2a§	19.0a	118b	19a
MBS-SA	1.9ab	13.0a	132b	18a
MBS-INC	1.7b	14.0a	116b	18a
2x-MBS	2.1ab	15.0a	232a	22a
SE	0.6	3.5	22	1.2

† NH₄NO₃ = ammonium nitrate fertilizer as N source; MBS-SA = surface-applied municipal biosolids; MBS-INC = soil-incorporated municipal biosolids; 2x-MBS = twice the amount of surface-applied municipal biosolids as MBS-SA. All treatments supplied a total of 350 kg N ha⁻¹ yr⁻¹ except 2x-MBS where total N supplied was 700 kg N ha⁻¹ yr⁻¹.

‡ WEP = water extractable phosphorus.

§ Means within a column not followed by the same letter are different by pdiff in LSMEANS ($P \leq 0.05$).

2007, as discussed previously. Nitrogen removal was 127 and 166 kg ha⁻¹ yr⁻¹ for MBS-SA and MBS-INC, respectively (Table 3). Nitrogen removal from the MBS-INC treatment was not different from NH₄NO₃ or 2x-MBS treatments. These results followed the same trend as DM yield.

Tissue Phosphorus Concentration and Removal

There was a treatment × year interaction for tissue P concentration and P removal. In 2007, there was no effect of treatments on P concentration, but in 2008 all treatments receiving MBS had greater tissue P concentration than the NH₄NO₃ treatment (Table 4). In 2008, tissue P concentration in the 2x-MBS treatment was greater than MBS-INC and NH₄NO₃ treatments but not different from MBS-SA. Tissue P concentration was greater in 2008 than 2007 for all but the NH₄NO₃ treatment, indicating cumulative effects of the greater amounts of P applied for MBS treatments.

Incorporation of MBS increased P removal by 10 to 11 kg ha⁻¹ in both years compared to MBS-SA (Table 3). This effect is attributable to greater DM yields in the MBS-INC treatment because tissue P concentration was not different between MBS-SA and MBS-INC. Thus, incorporation of MBS results in greater P uptake. This may reduce soil P accumulation over time where MBS is applied, and it could also result in greater allowable rates of MBS application where these rates are P based.

Soil Phosphorus and Carbon Concentrations

There was no treatment × year interaction effect for any of the soil variables. Incorporation of MBS had no effect on WEP, Mehlich-1 P, total soil P, and total soil C compared to MBS-SA (Table 5). There was a year main effect for WEP and total soil P. Water-extractable P averaged 2.4 and 1.6 mg P kg⁻¹ soil in 2007 and 2008 ($P = 0.03$, SE = 0.34), respectively. Total soil P concentration for 2x-MBS was greater than the other treatments reflecting the much greater amounts of P applied. Total soil P was not different among NH₄NO₃, MBS-SA, and MBS-INC treatments. Given that the amount of total P applied in the MBS-SA and MBS-INC treatments was at least two times greater than the NH₄NO₃ treatment, the lack of response may be due to soil variability and size of the total P pool. Averaged across treatments, soil total P concentration was 127 and 156 mg P kg⁻¹ soil in 2007 and 2008 ($P = 0.04$, SE = 13.4), respectively. The greatest year-to-year increase in soil

total P concentration was 80 mg P kg⁻¹ soil for the 2x-MBS treatment ($P = 0.02$, SE = 32.1). There was no year or treatment effect on soil C. This result was not surprising given that the plots had been planted to a perennial grass for more than 15 yr before initiation of the study and soil C had likely reached equilibrium.

SUMMARY AND CONCLUSIONS

Incorporation of MBS increased elephantgrass DM yield and N and P removal compared to surface application. When MBS was incorporated, elephantgrass DM yield was not different than for plots receiving the same amount of total N from NH₄NO₃. Removal of N and P increased an average of 39 and 10 kg ha⁻¹ yr⁻¹, respectively, when MBS were soil incorporated vs. surface applied.

Total organic N mineralized was 386 and 308 g kg⁻¹ for MBS-INC and MBS-SA, respectively. The 25% increase in N mineralization was associated with a 33% increase in elephantgrass DM yield for MBS-INC compared to MBS-SA, indicating that most of the yield increase following soil incorporation was associated with greater N mineralization. The results of this study indicate that incorporation of MBS has positive effects on elephantgrass DM yield and nutrient removal compared to surface application. Increasing the proportion of organic N released for plant uptake through soil incorporation reduces the amount of MBS that need to be applied and the extent of soil P accumulation.

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