NUTRIENT MANAGEMENT OF ELEPHANTGRASS FOR BIOMASS PRODUCTION

By

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To my family

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High-yielding bioenergy crops remove relatively large amounts of nutrients at harvest. It is important that nutrients in soil be replenished in a manner that minimizes environmental impact and production costs. In this work, three studies were conducted in Florida to evaluate the use of class AA municipal biosolids (MBS) on plant and soil responses. In Experiment 1, two elephantgrass (*Pennisetum purpureum* Schum.) entries received four differing proportions (0, 33, 66, and 100%) of N from surface-applied MBS, with the remainder from ammonium nitrate, to supply a total annual rate of 350 kg N ha^{-1} yr^{-1}. Two additional treatments were 1) 100% MBS with soil incorporation and 2) twice the rate of the 100% MBS treatment (i.e., 700 kg N ha^{-1} yr^{-1}) surface-applied. Dry matter (DM) yield, tissue N and P concentrations and removal, and soil C and P concentrations were assessed. Experiment 2 investigated the effect of season of application (spring versus summer) of MBS on organic N mineralization and DM decomposition patterns when MBS were incubated under field conditions. Finally, in Experiment 3 the effect of method of MBS application (soil-incorporated versus surface-applied) on organic N mineralization and DM decomposition were investigated under field conditions. Elephantgrass DM yield decreased linearly as the percentage of total N supplied from MBS increased in Experiment 1. Yield reductions were 7.4 and 4.1 Mg ha^{-1} for Chinese Cross and Merkeron elephantgrass, respectively,

as percentage of total N from MBS increased from 0 to 100. Incorporation of MBS had a positive effect on DM yield and N and P removal. When MBS were incorporated, DM yield was not different than when N was supplied solely as ammonium nitrate fertilizer, and yield of those treatments was greater than when MBS were surfaced applied. Nitrogen and P removal increased approximately 39 and 15 kg ha^{-1} yr^{-1} respectively, when MBS were incorporated versus surface applied. There was no effect of addition of MBS or method of MBS application on water extractable soil P, Mehlich-1 soil P, total soil P, and total soil C concentration in the Ap horizon over a 2-yr period. Nevertheless, results suggest a gradual accumulation of total P in the Ap horizon when comparing Year 2 to Year 1. Field incubation studies of MBS showed lower temperatures and rainfall were associated with lower rates of organic N mineralization and DM decomposition for spring-applied MBS. Field incubation of MBS also demonstrated the positive effect of soil incorporation on organic N mineralization. Mineralization of N was 22% greater when MBS were incorporated. Results show that MBS can be used to reduce inorganic fertilizer inputs for bioenergy crops, but greatest yield and organic N mineralization occur when MBS are soil incorporated as opposed to surface applied.

CHAPTER 1
INTRODUCTION

The world energy demand is satisfied primarily by fossil fuels, whose known reserves are limited and located in geographically concentrated and often politically unstable areas. The large-scale dependence on fossil fuels has raised concerns because their use releases to the atmosphere C that was previously stored below ground. In the USA, 85% of the energy consumed is derived from fossil fuels (EIA, 2006); a fact that prompts research to identify alternative sources of energy, strategies to save energy, and opportunities to re-use waste products that otherwise would require energy for disposal as well as pose a threat to the environment.

The documented increasing concentration of atmospheric carbon dioxide (CO_2) is related to human activities and, particularly, to the burning of fossil fuels (Longinelli, 2005). Combustion of fossil fuels has been recognized as one of the main contributors to climate change and global warming. Cellulosic biomass, the fibrous and human-inedible portion of plants, is a potential alternative to conventional energy sources because unlike fossil fuels it is renewable and its use may not add previously sequestered CO_2 to the atmosphere.

High yielding perennial C_4 grasses represent a potential source of cellulosic biomass and can store significant amounts of C belowground (Fisher, 1994). Elephantgrass (*Pennisetum purpureum* Schum.) is one such grass. It is native to tropical Africa and was introduced to the USA in 1913 (Thompson, 1919). Early research focused on the potential as forage for cattle; however, the tall growth habit, low leaf/stem ratio, and high cost of establishment limited its use for grazed pastures in the USA (Sollenberger, 1987). Research initiatives to identify herbaceous plants with the highest biomass yields for renewable energy purposes have consistently reported elephantgrass to be the highest yielding or among the highest yielding C_4 grasses. Dry matter

(DM) yields in the range of 20 to 41 Mg ha^{-1} yr^{-1} (Woodard and Prine, 1993a; Bouton, 2002) are commonly obtained. The Gulf Coast region of the southeastern USA, bounded on the north where temperatures do not drop below -6 to -9°C in winter, has been identified as the optimum zone in the USA for sustained survival and high yield of elephantgrass (Woodard and Sollenberger, 2008).

Plant biomass production is affected by nutrient quantity and availability. In biomass production systems, the ability to supply plants with the required nutrients relies on natural soil fertility and on external inputs such as fertilizers. The high cost of fertilizers, a pattern of land use that allocates grasslands to soils of low fertility, and the increasing pressure on available land associated with crop production for both food and bioenergy, call for a re-evaluation of how nutrients will be supplied for efficient production of biomass.

Biosolids represent an alternative source of nutrients for biomass production, not only due to lower prices compared to commercial fertilizers, but also because the material already exists as a by-product of municipal sewage sludge treatment plants. Thus, biosolids can be beneficially recycled via land application (EPA, 1999). Nevertheless, concerns exist relative to long-term application of biosolids. Accumulation of soil P above concentrations needed for optimum crop yield and associated water quality impairment leading to eutrophication have caused some states to adopt policies and laws aimed at reduction of P losses to surface waters from agricultural land (Sharpley et al., 2003; Shober and Sims, 2003). At the national level, the Code 590 standards require that animal wastes be applied on a N basis if risk of P loss is low and on a P basis (P removal by the crop) if P loss risk is high (Pierzynski and Gehl, 2005). Thus, Elliot and O'Connor (2007) state that the future of land-based recycling of biosolids is threatened if policies fail to 1) consider critical issues including site indices that account for the sizeable

differences in P-loss potential among biosolids types compared to mineral fertilizers and livestock manures, 2) base soil-test P benchmarks upon environmental P loss risk rather than crop response, and 3) apply agronomic rate calculations that recognize different P fertilizer replacement values among biosolids products. Thus, despite their potential as a nutrient source for biomass production in renewable energy systems, the use of municipal biosolids requires site specific management strategies.

In summary, elephantgrass represents an important species for cellulosic biomass production in the southeastern USA. The possibility of reusing waste products as nutrient sources to produce biomass while maximizing environmental benefits and lowering production cost needs to be evaluated. Thus, the objectives of this study were: 1) to quantify DM production of elephantgrass using different ratios of total N provided by municipal biosolids and ammonium nitrate fertilizer (Chapter 3); 2) to quantify N and P concentration and removal by elephantgrass when different ratios of N from biosolids and ammonium nitrate are applied (Chapter 3); 3) to quantify changes in soil C and P concentrations when municipal biosolids are applied as a nutrient source (Chapter 3); 4) to quantify the DM decomposition and N mineralization rates of the Ocala municipal biosolids in litter bags placed on the soil surface (Chapter 4); and 5) to evaluate the effect of season of application and incorporation of the biosolids on patterns of decomposition and N mineralization (Chapter 4).

CHAPTER 2
LITERATURE REVIEW

Background

Use of Grasses as Bioenergy Crops

The conversion of forage plants into biofuels, industrial products, and human-use products, referred to as the *biorefinery concept*, has enhanced the value of perennial crops such as grasses beyond their traditional use for animal feed and conservation (Sanderson et al., 2007). The technology to produce fuel ethanol from renewable lignocellulosic biomass is available, although building a cost-effective integrated process remains a challenge (Ingram et al., 1998; Ingram et al., 1999). Significant progress has been made in the past several years in all aspects of lignocellulosic conversion to ethanol (Hamelink et al., 2005; Galbe and Zacchi, 2007). Agricultural residues, forest residues, and dedicated crops, among other sources, represent potential lignocellulosic feedstocks. The literature review that follows discusses the potential of grasses as lignocellulosic feedstocks, explores the potential of elephantgrass as a bioenergy crop, describes the effects of management on biomass production and composition, and evaluates the potential of organic amendments as sources of nutrients for biomass production.

Grasses as Lignocellulosic Feedstock

Lignocellulose represents a high percentage of the dry weight of most plant material (Ingram et al., 1999). It is a renewable resource in which solar energy is stored in the form of carbohydrate (cellulose, hemicellulose, pectin) and aromatic polymers (lignin) (Ingram et al., 1999). Thus, lignocellulose is a chemically complex polymer and consists of fibrous bundles of crystalline cellulose encased in a polymeric matrix of hemicellulose and lignin. Although composition varies, this material is generally composed of 20 to 50% cellulose, 20 to 40% hemicellulose, 2 to 20% pectin, and 10 to 20% lignin on a dry weight basis (Ingram et al., 1999).

For bioconversion, the carbohydrate portion must be solubilized while the lignin and insoluble residues can be used to provide energy by combustion (Ingram and Doran, 1995; Somerville et al., 2004).

Currently almost all the bioethanol produced is from sugar or starch-based crops. The ethanol production processes based on the use of these crops are well developed on a commercial scale. However, sugar and starch-based crops have a high value for food use, and their sugar yield per hectare is very low compared to cellulose and hemicellulose yield (Hamelinck et al., 2005). Thus, cellulose is an attractive energy feedstock because supplies are abundant, renewable, and its use does not result in release of previously stored C to the atmosphere.

According to the USA Department of Energy (USDOE, 2006) there are three distinct goals for a new generation of lignocellulosic energy crops: 1) maximizing the total amount of biomass produced per hectare per year, 2) maintaining sustainability while minimizing inputs, and 3) maximizing the amount of fuel that can be produced per unit of biomass. From the four major biomass feedstock categories: urban wood waste/residues, agricultural residues, forestry residues, and dedicated energy crops (USDOE-EIA, 2006), the latter category produced on managed plantations of herbaceous crops or short-rotation woody crops is not only considered a promising renewable energy source but also a viable C-sequestering option (Sartori et al., 2006).

Potential Genotypes for Use as Lignocellulosic Feedstock

Basic characteristics desired in plant species to be suitable for use as a biomass crop include a high yield of dry matter (DM), low moisture concentration, and low concentrations of minerals at harvest (Christian et al., 2002). The proportions of cellulose and lignin in biomass are important in biochemical conversion processes. The biodegradability of cellulose is greater than that of lignin; therefore, the overall conversion of plant material with a greater proportion of cellulose is greater than for plants with a higher proportion of lignin, a determining factor when

selecting biomass plant species for biochemical processing (McKendry, 2002). In general, grasses have lower concentrations of lignin than trees (Badger, 2002). Nevertheless, high concentration of lignin is also associated with beneficial characteristics in plants such as pathogen resistance, reduced lodging, and allowance of delayed harvest which leads to a reduction in water concentration and leaching of water-soluble components such as K and Cl (Lewandowsky et al., 2003). A reduction in lignin concentration, whether resulting from mutations in lignin biosynthesis genes, from selection of traits associated with reduced lignin, or from transformation, needs to be evaluated in diverse genetic backgrounds and gene combinations, and in diverse environments to discover optimal combinations and a true measure of value to, and fitness in agricultural systems (Pedersen et al., 2005).

A variety of species have been proposed as bioenergy crops. Unlike maize (*Zea mays* L.) and soybean (*Glycine max* L.), which are annuals, lignocellulosic bioenergy crops are typically perennials, including both woody species such as willows (*Salix* spp.) (Volk et al., 2006), poplars (*Populus* spp.) (Hussain, 1998), and herbaceous species such as switchgrass (*Panicum virgatum* L.) (Fike et al., 2006), miscanthus (*Miscanthus* spp.) (Heaton et al., 2004), reed canarygrass (*Phalaris arundinacea* L.), elephantgrass (*Pennisetum purpureum* Schum.), and tall fescue (*Lolium arundinaceum* Schreb.) (Lemus and Lal, 2005). Several characteristics have been suggested for evaluation of bioenergy crop potential. These include high biomass yield, wide geographic range, efficient nutrient utilization, low erosion potential, high C sequestration capability, and reduced fossil fuel input requirements relative to annual crops (Christian et al., 2002; Lewandowski et al., 2003; Heaton, 2004; Lemus and Lal, 2005). Perennial rhizomatous grasses have been found to have comparative advantages versus other species such as trees. Advantages of grasses include the rapid development of high yield potential, relatively low input

demand, and multiple ecological benefits (Lewandowsky et al., 2003). Nevertheless, given the fact that the process of conversion of biomass into biofuels on a commercial scale remains a challenge, also remains a challenge to appropriately recognize and value the potential services of specific bioenergy systems.

Carbon Sequestration

Although CO_2 has the lowest global warming potential per mole among the greenhouse gasses, it is responsible for about half of the observed greenhouse effect because of its high absolute increase in concentration (Rodhe, 1990). This has led to greater emphasis on reducing atmospheric CO_2 levels by minimizing anthropogenic emissions to the atmosphere, and removing C from the atmosphere by sequestration in the biosphere (Ingram and Fernandes, 2001).

In agricultural soils, reducing net CO_2 emissions is synonymous with increasing soil C storage, a process usually referred to as C sequestration (Paustian et al., 1999). Soil C sequestration is a process whereby plants remove CO_2 from the atmosphere and incorporate it into soil C pool along with other nutrients (N, P, and S) (Lemus and Lal, 2005). Because a large part of the biomass produced in agricultural systems cycles through the soil decomposer community, the magnitude of CO_2 fluxes between agricultural soils and the atmosphere is large – several Pg (10^{15}) per year. The net difference between the photosynthetically-fixed CO_2 that enters the soil as plant residues and the CO_2 emitted from residue decomposition determines the net C balance of the ecosystem, i.e., whether it is a source or a sink of CO_2. Thus, management to increase C sequestration can be directed toward increasing residue inputs and/or reducing decomposition rates (i.e., heterotrophic soil respiration) (Paustian et al., 1999).

According to Paustian et al. (1999), the relationship between C inputs and soil organic carbon (SOC) levels is relatively straightforward; steady-state C content for many agricultural

soils has been shown to be linearly related to C-input levels (Rasmussen and Collins, 1991; Paustian et al., 1995). Nevertheless, this may not hold for soils with very high C levels, which may exhibit a C 'saturation' behavior (Hassink, 1996).

The prolific root system of perennial crops strongly influences C sequestration by adding significant quantities of organic matter (SOM) into the soil. The beneficial effects of the organic material containing soil C has been widely recognized for plant growth and maintenance of pH status, soil structure (Chen and Aviad, 1990; Stevenson and He, 1990; Blanco-Canqui and Lal, 2004), and enhancing soil's capacity to retain and provide water and nutrients to plants (Lemus and Lal, 2005).Ingram and Fernandez (2001) provided a review of factor limiting soil C sequestration and pointed out the importance of soil mineralogy in reaching potential soil C sequestration levels.

Switchgrass soil C sequestration rates have been reported between 1.7 to 3.0 Mg C ha^{-1} yr^{-1} (McLaughling et al., 2002; Zan et al., 2001). Lee et al. (2007) calculated soil C sequestration for the 0- to 90-cm depth under switchgrass to be 2.4 and 4.0 Mg C ha^{-1} yr^{-1} for NH_4NO_3 fertilizer and cattle manure treatments, respectively. In their experiment, soil C sequestration was consistently greater in the manure treatment. This has also been supported by Smith et al. (2000) and Tian et al. (2009). Using labeled C in a 15-yr experiment with *Miscanthus*, researchers accounted for 8.9 Mg ha^{-1} of sequestered SOC (Clifton et al., 2007). Ma et al. (2000) evaluated the effect of cultural practices on SOC. Their results showed that row spacing, N rate, switchgrass cultivar, and harvest frequency had no effect in the short term (2–3 yr). After 10 yr, SOC was 45 and 28% greater at depths of 0 to 15 cm and 15 to 30 cm, respectively, than in an adjacent fallowed area.

Potential of Elephantgrass as a Bioenergy Crop in the USA

Origin

Elephantgrass (also known as napiergrass) is a C_4 photosynthetic pathway plant of tropical African origin that has been introduced to all tropical areas of the world. It is indigenous to areas of equatorial Africa where annual rainfall exceeds 1000 mm (Hanna et al., 2004). There is great variability within the species (Bogdan, 1977; Skerman and Riveros, 1990).

Distribution and Adaptation

Elephantgrass grows best in hot temperature regions with temperatures between 30 to 35˚C (Ferraris, 1978), and growth stops when temperatures are below 10˚C (Bogdan, 1977). Frost kills leaves and above-ground stems, but unless the soil freezes, the underground parts will resume growth at the beginning of the spring. Elephantgrass is adapted to various soil conditions, from low fertility and acid to slightly alkaline, but it is better adapted to deep, well drained, fertile soils. It usually does not grow well on heavy clay soils and cannot tolerate soils that remain waterlogged for long periods of time. The species also has substantial drought tolerance owing to a deep fibrous root system, but responds to irrigation (Hanna et al., 2004). In the southeastern USA, after 15 yr of field trials conducted in Florida, Alabama, and Georgia, the northern limit for sustained survival and adequate elephantgrass yield was determined to be where temperatures do not drop below the range of -6 to -9°C (Woodard and Sollenberger, 2008). Elephantgrass has been observed growing along roadsides and in some row-crop fields, especially sugarcane (*Saccharum officinarum* L.), in South Florida. Potential for invasiveness has resulted in elephantgrass being listed on the "do not plant list" for South Florida (FLEPPC, 2005; IFAS Assessment of Non-native Plants, 2008). Ongoing research is assessing the genetic, reproductive, and morphological similarities and differences of weed type elephantgrass and that selected for bioenergy applications.

Taxonomy and Morphology

Elephantgrass is a robust, creeping rhizomatous plant that perennates in the tropics and subtropics. Plants can form bamboo-like clumps that grow up to 7 m in height. Stems can have 20 or more internodes, ranging from 20 to 25 cm in length and up to 3 cm in diameter (Hanna et al., 2004). Dwarf ecotypes produce the same number of leaves, but because of their short internodes, uncut plants will attain a maximum height of 1.6 m (Hanna and Monson, 1988; Sollenberger et al., 1987). Elephantgrass is a cross-pollinated species that sets little seed, but low seed set is partially due to self-incompatibility and the fact that a single genotype or clone may occupy a large area. Therefore, cultivars of elephantgrass are all propagated vegetatively.

Pennisetum purpureum Research in the USA

Elephantgrass was introduced into the USA by the U.S. Department of Agriculture in 1913 and it was distributed to farmers in 1915 (Thompson, 1919). Early research focused on the potential of elephantgrass as forage for cattle. A combination of factors, reviewed in Sollenberger et al. (1987), limited the use of early tall elephantgrass (2 to 5 m height) in Florida. The silage showed lower quality than sorghum (*Sorghum bicolor* L.), corn, and sugarcane (Neal et al., 1935; Shealy et al., 1941); continuous close grazing resulted in yield reduction and ultimately loss of stand (Blaser et al., 1955); harvesting it for hay was considered impossible due to large stems that were difficult to dry; and difficult, costly, and labor-intensive establishment precluded its use as forage for cattle compared to other grasses existing in the area.

Renewed interest in this species occurred when dwarf types were selected such as Tift N75 (Hanna and Monson, 1988) which was tested and released in Florida as 'Mott' elephantgrass (Sollenberger et al., 1989). Animal average daily gains of 0.97 kg d^{-1} were observed compared with 0.38 kg d^{-1} for cattle grazing bahiagrass (*Paspalum notatum* Flugge) pastures (Mott and Ocumpaugh, 1984; Sollenberger et al., 1987; Sollenberger and Jones, 1989). Flores et al. (1993)

concluded that the greater forage quality of Mott elephantgrass than Pensacola bahiagrass resulted from greater intake of digestible organic matter. This was associated with less cell wall and greater digestibility of cell walls in Mott as a consequence of a smaller proportion of sclerenchyma fibers and less developed girder structure. Also, as reported by Mott (1984), there was no "summer slump" for animals grazing dwarf elephantgrass. Further, Ruiz et al. (1991) reported that dwarf elephantgrass silage was able to substitute for corn silage with only a small decrease in milk yield. Nevertheless, Woodard et al. (1985) indicated that vegetative establishment of this genotype remained more difficult than with tall types. They indicated that even under the best conditions, initial emergence of Mott may be less than 80%, suggesting that in order to obtain satisfactory establishment in future plantings it may be necessary to overplant as much as 40 to 50%. The difficulties concerning mechanization of establishment and appropriate management to ensure persistence of the species for animal production were not totally overcome. As a result, other grasses such as bermudagrass [*Cynodon dactylon* (L.) Pers.] and bahiagrass, with less intensive management requirements, continued to be preferred to tall and dwarf elephantgrass genotypes.

In 1981, an agreement between the University of Florida Institute of Food and Agricultural Sciences (IFAS) and the Gas Research Institute, Chicago, IL, stimulated a 10-yr research effort to use crops for the production of methane. Scientists identified tall-growing perennial bunchgrasses as having the greatest potential as biomass plants (Stricker et al., 1993), and elephantgrass has consistently shown the highest DM production(20 to 48 Mg ha^{-1} yr^{-1} (Prine et al., 1984; Woodard and Prine, 1993a; Bouton, 2002). Woodard and Prine (1993b) attributed the yield superiority of elephantgrass over other C_4 plants to the extended period of linear DM accumulation (140 to 196 d) rather than to greater maximum crop growth rates than other C_4

grasses. Under the renewed interest to produce ethanol from lignocellulosic feedstocks, it is not certain whether mechanization of establishment, nutrient management, or other factors will limit the use of elephantgrass.

Effects of Management on Biomass Production and Composition

Establishment

Elephantgrass can be propagated by seed, stems, and rhizomes; however, improved cultivars are multiplied and propagated by stems and/or rhizomes because genotypes do not breed true from seed. In addition, plants from seed grow slowly and are weak (Hanna et al., 2004). Research done by Woodard et al. (1985) evaluated the effect of planting date [10 planting dates starting 4 July and ending 8 January, planting orientation of stem cuttings (horizontal in furrow, vertical, and at a 45˚ angle), the number of nodes on the stem cutting, and position of nodes on the cutting (2, 3, 4, 6, or 12-node stem cuttings from either the base or the apex of individual stalks) and planting depth of two cultivars (PI 300086 tall: 5-, 10-, 15-, and 20-cm deep; Mott dwarf: 2.5-, 7.5-, and 12.5-cm deep) at three locations (up to a latitude of 29˚ North) upon subsequent establishment. They indicated that establishment of dwarf elephantgrass was more difficult than tall types (PI 300086). They concluded that PI 300086 stem cuttings should be planted in July and August or in the late fall, November and December, in North and Central Florida, avoiding intermediate planting dates from late September to early November that results in winter kill. Planting orientation did not affect initial emergence or winter survival. Shorter cuttings (2 and 3 nodes) resulted in the highest primary shoot counts compared to stalks with six and 12 nodes. With horizontal furrow planting, Mott elephantgrass was found to be particularly sensitive to planting depth, while this factor was less critical for tall PI 30086.

Further research done by Woodard and Prine (1990) evaluated the effect of N-P-K fertilization on propagation quality of cuttings made from Mott and ‘Merkeron’ (tall)

elephantgrass stems. They reported that for both cultivars vigor scores of primary tillers recorded at 5-wk postplant improved to the highest fertilizer rate, suggesting that quality of elephantgrass stems can be improved by applying high rates of fertilization to nursery plants.

Sollenberger et al. (1990) evaluated the effect of five defoliation treatments prior to planting stems of Mott elephantgrass. They suggested that successful establishment of Mott is most likely if undefoliated stems are planted. Also, general patterns of shoot and root growth were observed in their study which were consistent with apical dominance theory explained by Tamas (1987). The removal of the apical meristem resulted in early initiation of shoot growth, and shoot emergence was greater at 2-wk postplant, but stems with meristems removed did not have sufficient nutrient storage to support growth of these shoots to the point where they were independent of the original stem cutting.

Genotypes

Five of the best adapted elephantgrass selections for biomass energy were planted in a holding nursery in 2000 at the Plant Science Research and Education Unit near Citra, FL. These are: Merkeron, N-51, N-43, N-13, and PI 300086 elephantgrasses. Merkeron, N-51, N-43, and N-13 are the more cold tolerant accessions that yielded well throughout Florida and have the potential to grow as perennials in the lower southeastern USA (Woodard and Sollenberger, 2008). Merkeron is a released cultivar and is a tall F1 hybrid that resulted from a dwarf × tall elephantgrass cross made in 1941 (Burton, 1989). Genotype PI 300086 was introduced in to the USA from South Africa in 1964 (USDA, 1968). Genotypes N51 and N43 were collected on the Georgia Coastal Plain Station at Tifton but were not designated as cultivars.

Harvest Management

Woodard and Prine (1991) evaluated DM production, crude protein (CP), in vitro digestible organic matter (IVDOM) concentration, and neutral detergent fiber (NDF) of four tall

elephantgrasses (PI 300086, Merkeron, N-43, and N-51), Mott dwarf elephantgrass, and a semi-dwarf pearl millet [*Pennisetum glaucum* (L.) R. Br.] × elephantgrass hybrid (Selection 3) as a response to three harvest frequencies (one, two, and three harvests per year). They concluded that plant growth and survival was greatly affected by harvest frequency and genotype. In addition, CP and IVDOM concentrations increased with more frequent harvesting, whereas NDF levels gradually decreased. For tall genotypes, forage yields declined ~15 and 30% with two and three harvests per season, respectively, compared to full-season growth. Tall genotypes Merkeron, N-43, and N-51 did not differ in DM yield during the 3-yr study. Tall elephantgrass PI 300086 yields did not differ from Merkeron, N-43, and N-51 during the first two years of harvests, but during the third year PI 300086 yields were lower when harvested multiple times. This reduction in yield of PI 300086 was primarily attributed to loss of stand that occurred during winter. Calhoun and Prine (1985) evaluated the response of elephantgrass PI 300086 to harvest interval and method of fertilization. They reported that PI 300086 survival through the winter of 1983 and 1984 was greater (85% survival) when the plots were harvested every 24 wk vs. 6, 8, or 12 wk (≤ 65% survival). Three-year mean DM yields for Merkeron, N-43, and N-51 were 24.3, 21.1, and 17.0 Mg ha^{-1} yr^{-1} for one, two, and three harvests per year, respectively. Two-year mean CP concentrations for the three harvest intervals (across genotypes) were 40.3, 57.5, and 75.7 g kg^{-1} DM, whereas IVDOM concentrations were 399, 492, and 555 g kg^{-1} organic matter (OM). For tall and dwarf genotypes, forage CP and IVDOM concentrations increased with more frequent harvesting.

Reduction of elephantgrass DM yield with increased harvest frequency has been reported consistently in the literature (Watkins and Severen, 1951; Vicente-Chandler et al., 1959; Vélez-

Santiago and Arroyo-Aguilú, 1981; Calhoun and Prine, 1985). The reduction in yield is probably due to interrupted light harvest in the linear growth phase (Woodard and Prine, 1991).

Fertilization

Elephantgrass can produce more DM than most perennial tropical grasses. Nutrient removal of high-yielding stands can be exceptionally high. In the absence of fertilizer in East Africa, napiergrass yields dropped rapidly starting in the second year after applications ended (Boonman, 1993).

Increasing N fertilization has been documented to affect successful establishment, DM production, and forage nutritive value. Stricker et al. (1993) reported DM production of PI 300086 and N51 of 36.2 and 45.2 Mg ha^{-1} yr^{-1}, fertilized with 134 kg N ha^{-1} and 112 kg K ha^{-1} on phosphatic clay in Florida. Woodard and Prine (1993a) reported DM production of PI 300086 and N51 of 46 and 47 Mg ha^{-1} yr^{-1} with fertilization rates of 200, 22, and 83 kg ha^{-1} of N, P and K, respectively. In a 4-yr study, Prine and Woodard (1986) fertilized PI 300086 elephantgrass plots that were harvested one time per year with annual N, P, and K rates of 224, 24, and 93 and 448, 48, and 186 kg ha^{-1}, respectively. Split applications of these rates made in spring and midseason did not improve forage yield over a single full-rate application made in spring. Average 4-yr DM production for the single application rates were 48.6 and 46.1 Mg ha^{-1} for the 224 and 448 kg N ha^{-1} treatments, respectively. Mislevy et al. (1986) reported a 14 to 24% decline in DM yield when fertilizer was reduced from 168-25-93 to 84-12-46 kg ha^{-1} of N, P, and K, respectively).

At an elephantgrass DM yield of 12.5 Mg ha^{-1} yr^{-1}, approximately 150, 30, and 375 kg of N, P, and K were removed. Thus, significant fertilizer inputs are needed in cut-and-carry systems that remove large quantities of nutrients from the soil (Boonman, 1993). Geus (1973) and Sanchez (1976) indicated that approximately 52 kg ha^{-1} yr^{-1} of P may be needed to match the

removal of P in cut and carry systems of tropical grasses. It has been suggested that tropical grasses can achieve up to 30% of their annual P uptake from soil below a 30-cm depth, and as a result soil-test P interpretation based on the top 30 cm of soil can be misleading (Nye and Foster, 1961). Elephantgrass is a luxury consumer of K, and concentrations of tall elephantgrass types at Kawanda, Kenya ranged from 31 to 67 g kg^{-1} (Foster, 1969). Montalvo et al. (1987) reported K concentrations of the dwarf type in Florida up to 50 g kg^{-1}. The critical level for K concentration is considered to be between 10 and 15 g kg^{-1}.

Organic Amendments as a Source of Nutrients for Biomass Production

Florida's rapidly increasing population is generating large quantities of wastes that must be disposed of in a manner that will not adversely impact the environment. Thus, alternative means of handling wastes (municipal effluent, biosolids, animal wastes, food processing wastes, and municipal solid wastes) have focused on utilization rather than disposal (Muchovej and Obreza, 2004). Further, increasing prices of commercial fertilizers encourage the use of organic amendments as an alternative and relatively inexpensive source of nutrients for land application and crop production.

Adequate nutrient supply is necessary to meet the requirements of high-yielding bioenergy crops, while minimizing environmental impact, and crop production costs are critical. The two nutrients of greatest environmental concern are N, a major risk for human and animal health, and P, a major cause of eutrophication of surface waters (Carpenter et al., 1998; Nelson, 1999). Controlling eutrophication requires that both N and P enrichment of surface waters be minimized. Studies have shown that the ratio of N:P in the water body (commonly referred to as the "Redfield" ratio) is an important indicator of which nutrient is limiting eutrophication. If the Redfield ratio is >16:1, P is most likely to be the limiting factor for algal growth; lower ratios indicate that N is of greater importance. The eutrophication threshold for most P-limited aquatic

systems (~20 to 100 µg P L^{-1}) is also much lower than for N (500 to 1000 µg N L^{-1}) (Pierzynski et al., 2000).

Nutrient management regulations were initially based on concerns regarding nitrate leaching and required estimates of plant available N, including potentially mineralizable N, to determine appropriate application rates. The issue of N needed vs. N removal set the basis for determining application rates (Pierzynski and Gehl, 2005). However, N-based application rates of organic amendments led to accumulation of soil P above concentrations needed for optimum crop yield due to narrower N:P ratio in organic amendments vs. plant uptake. These practices were associated with water quality impairment and accelerated eutrophication, causing some states in the USA to adopt policies and laws to reduce P losses to surface waters from agricultural land (Sharpley et al., 2003; Shober and Sims, 2003). Federal legislation has addressed various aspects of nutrient management, including land application of by-products. These include: 40 CFR Part 503 regulations for land-applied biosolids (USEPA, 1993), the USDA-NRCS Conservation Practice Standards for Nutrient Management Code 590 (USDA, 1999), the USEPA concentrated animal feeding operation (CAFO) regulations (USEPA, 2003), and the USDA National Organic Standards (USDA, 2000). At a national level, the Code 590 standards provide guidelines for nutrient management to be developed further and followed by each state using three possible approaches: two threshold approaches, one based on environmental criteria and the other on agronomic criteria, and a P index rating. Combinations of these approaches are permitted.

Currently, soil test P (STP) levels are used as criteria to limit or prohibit P applications when the soil P threshold is reached (Pierzynski and Gehl, 2005). Thus, the STP threshold level is generally set based on readily available, agronomic soil P testing procedures. This means that

once crop yield no longer increases in response to additional P input, no more P could be applied. However, research studies suggest that agronomic (the soil P value where an increase in P will not increase yield) and environmental thresholds (the soil P value where significant amounts of additional P will be mobilized by rain water) are not equivalent (Higgs et al., 2000). This is due to controlling processes by which plants access soil P that are quite different from those that determine soil P availability for removal by runoff (Sharpley et al., 2003). Thus, when a wide range of STP concentrations are considered, the relationship between runoff dissolved P and STP is curvilinear due to saturation of P-fixing sites in the soil (McDowell and Sharpley, 2001). Elliott and O'Connor (2007) suggest that the "change point" (approximately three to four times the agronomic optimum STP) ostensibly identifies the STP at which the soil P-sorbing sites become sufficiently filled that environmentally significant amounts of additional P will be mobilized by rainwater. Thus, below the change point of the slope, much of the P added is retained in the plow layer; beyond this change point, P losses are substantially greater due to the reduced P-fixing ability of the soil (Elliott and O'Connor, 2007).

The P index rating is a "work in process tool" within each state's particular requirements. Since phytoavailable P varies among biosolids (O'Connor et al., 2004) and P loss potential is different for biosolids when compared to commercial fertilizers and animal manures (Kleiman et al., 2000; Brandt et al., 2004; Elliott and O'Connor, 2007), it has been suggested that these differences must be accounted for when developing P rating indices.

Biosolids

Biosolids are a "nutrient-rich organic by-product of municipal wastewater treatment that can be beneficially recycled via land application" (EPA, 1999). The forms (solubilities), nutrient concentrations, and potentials for loss of N and P in the systems can vary depending upon the wastewater treatment process (Penn and Sims, 2002).

Typical concentrations of total N and P in the biosolids can range from 35 to 68 g kg^{-1} and 18 to 39 g kg^{-1}, respectively (EPA, 1999). On average, 800 g kg^{-1} of the total N in the biosolids occurs in organic forms (Sommers, 1977; Binder et al., 2002), whereas most of the P, 700 to 850 g kg^{-1}, is found in inorganic forms (Chae and Tabatabai, 1981). Relative P bioavailability when compared to commercial fertilizers can vary from 10 to 100% (de Hann, 1981; O'Connor et al., 2004).

Regardless of the chemical forms of N and P initially in biosolids, plant uptake of N and P from the soil substrate occurs mainly in the forms of ammonium (NH_4^+) and nitrate (NO_3^-), and primary orthophosphate ($H_2PO_4^-$) and secondary orthophosphate (HPO_4^{-2}), respectively. These forms are known as inorganic or mineral forms. Therefore, due to the initial forms of N (mainly organic) in the biosolids, the main factor controlling availability of N is the mineralization/immobilization process. If most P is already in an inorganic form, the availability of P is mainly influenced by soil pH, soil mineralogy, and the reaction with Fe, Al, and Ca which can be found in the biosolids as a product of the waste water treatment plant process and in the soil where the material is applied.

Mineralization/Immobilization Process

In the mineralization process, organic materials (e.g., proteins, chitins, amino acids, amino sugars, and nucleic acids) are initially degraded to NH_4^+, to produce the inorganic forms for subsequent N uptake by the plant. The N mineralization process, under both aerobic and anaerobic conditions, is mediated by the activities of nonspecific heterotrophic soil microorganisms (bacteria, fungi, actinomycetes), that use the N for their metabolism (He et al., 2003). The microorganisms produce extracellular enzymes that can degrade proteins and non proteins to NH_4^+ (Pierzynski et al., 2000).

The immobilization process is basically the reverse of mineralization. It is the conversion of inorganic N into organic N. Although both NO_3^- and NH_4^+ can be assimilated by microorganisms, immobilization occurs predominantly from the NH_4^+ pool when available (Jarvis et al., 1996).

Nitrogen mineralization and immobilization processes occur simultaneously in the soil, with the relative magnitudes determining whether the overall effect is net N mineralization or net N immobilization (Cabrera et al., 2005). Although biological transformations of N in soils are complex, mineralization largely depends on the quantity and quality (composition) of OM and reflects the influence of the environment, principally temperature and moisture, on biological activity (Goncalves and Carlyle, 1994).

A commonly used index of substrate quality and mineralization-immobilization potential is the C:N ratio. This is based on the premise that for the assimilation of C to occur, N also has to be assimilated in an amount determined by the C to N ratio of the microbial biomass. If the amount of N present is larger than that required by the microbial biomass, mineralization occurs with the release of inorganic N. If the amount of N is equal to that required by the microbial biomass, there will be no net N mineralization. On the other hand, if the amount of N in the material is lower than that required by the microbial biomass, there will be immobilization (Cabrera et al., 2005). Research suggests that the break-even point between net N mineralization and N immobilization can be found between C:N ratios of 20 to 40 (Whitmore, 1996). Nevertheless, in some cases the break-even point has been found to be near 15 (Gilmour, 1998). The existence of a range instead of a single value for the break-even point has been related to variation in the C:N ratio of the decomposing microbial biomass as well as the existence of organic components with different susceptibility to decomposition (Cabrera et al., 2005).

Further, organic residues with similar C:N ratios may be mineralized to different extents because of differences in composition that are not described effectively by the C:N ratio alone (Cabrera et al., 2005).

Because C:N alone cannot explain all differences in N mineralization, much effort has been spent on characterizing the different compounds or groups of compounds present in organic residues (Cabrera et al., 2005). Other properties such as pH, salinity, and heavy metal concentration of the material have been proposed as factors that affect microbial activity and N mineralization (Sikora and Szmidt, 2001). In addition to the inherent characteristics of the initial material, physical characteristics of the soil such as: moisture, temperature, porosity, texture, and chemical characteristics also influence the mineralization process (Parr and Papendick, 1978).

In general, as temperature and moisture increase microbial activity increases. Most soil microorganisms are mesophyllic and prefer moderate temperatures, with optimum activity between 25 and 37˚C (Jarvis et al., 1996). Optimum conditions of soil moisture for mineralization are in the range of 50 to 75% of soil water holding capacity, although the actual optimum moisture concentration varies with soil texture (Pierzynski et al., 2000). Standford and Epstein (1973) determined that highest mineralization occurs when matric suction is in the 0.03 to 0.01 MPa range, where 80 to 90% of the total pore space is filled with air.

Incorporation of organic amendments increases NH_4^+-N in the soil, due to reduced NH_3 volatilization and enhanced mineralization. Once organic N has been mineralized, NH_4^+ can be taken up by plants, nitrified, immobilized by soil microorganisms, lost as a gas by ammonia volatilization, held as an exchangeable ion by clays or other soils colloids, or fixed in the interlayers of certain clay minerals (Pierzynski et al., 2000). A 12-wk laboratory incubation study of surface-applied liquid sludge was conducted (Adamsem and Sabey, 1987) using glass tubes

and 1 M H_2SO_4 traps for NH_3 titrated with standardized acid after steam distillation into boric acid (Bremner, 1965b). Temperatures were set at 30°C for 14 h d^{-1} and 12°C for 10 h d^{-1}. The authors reported that surface-applied liquid sludge lost 403 g kg^{-1} of its NH_4^+-N as NH_3 in the first 2 wk (95% of the total loss), whereas subsurface applied sludge (25 mm below surface) lost 35 g kg^{-1} of added NH_4^+-N as NH_3 in the same period (Adamsem and Sabey, 1987). Similar patterns of loss were reported by Ryan and Keeney (1975) and Terry et al. (1978). Also, Adamsen and Sabey (1987) reported that apparent organic mineralization (change in organic-N in the treated sample minus the change in organic-N in the control divided by the amount of organic-N added in the sludge) was 298 and 325 g kg^{-1} for the surface and subsurface treatments, respectively. Those values are greater than the 200 g kg^{-1} reported for an entire growing season (Mosier et al., 1982; Keeney et al., 1975). Using a constant temperature of 30°C during a 180-d incubation period, He et al. (2003) reported that incorporation of biosolids, in a soil with pH of 7.9, increased N mineralization by 60% (221 and 362 g kg^{-1} mineralized N for surface-applied and incorporated biosolids, respectively). The researcher attributed the increased mineralization to reduced volatilization and enhanced microbial activity as evidenced by a greater presence of NO_3-N in the incorporated treatment versus surface applied.

Plant-Available Nitrogen from Biosolids

Plant-available N release from biosolids is a function of N mineralization (potentially mineralizable N) over time and the extent of losses of mineralized N. Several chemical indices and laboratory incubations have been used for estimating the amount of N that mineralizes. However, uncertainty exists regarding the extrapolation of laboratory N mineralization data to field conditions where dynamic and site-specific factors like drying and rewetting events and temperature changes are expected to alter mineralization rates. The uncertainty has lead to the

measure of N mineralization rates and plant-available N in actual production fields with in situ incubation devices (Hanselman et al., 2004).

Estimates of N mineralization are distributed over a wide range of values due to quality differences in the initial material as well as the technique used to measure mineralization. Therefore, predictions resulting from greenhouse and growth chambers must be validated with actual field data (Tester, 1989). Hanselman et al. (2004) reviewed the characteristics and limitations of current methods used to measure mineralization and proposed a "new" soil-resin trap method that tracked field soil water content better than all other in situ methods evaluated. Barbarick et al. (1996) reported that net N mineralization ranged from 250 to 570 g kg^{-1} for five to six applications of 6.7 Mg biosolids ha^{-1} yr^{-1}on dryland winter wheat (*Triticum aestivum* L., 'Vona' or 'TAM107'). Field studies by Cogger et al. (1999) found apparent biosolids N recovery of 280 to 400 g kg^{-1} for forage grasses and 110 to 440 g kg^{-1} for dryland winter wheat. Sullivan et al. (1997) reported a cumulative apparent N recovery (carryover mineralization) of 90 g kg^{-1} in the year following application of biosolids. Gilmour and Skinner (1999) estimated plant-available N from six biosolids using decay-series modeling. Slopes of the relationships of plant-available N to organic N and total N were 45 and 40%. The researchers suggested that the percentages represented the amounts of biosolids-N made plant available during the growing season. He et al. (2000) estimated N mineralization in the field using three methods: the cumulative amount of NH_4-N and NO_3-N in the sample, the cumulative amount of NH_4-N and NO_3-N in both the sample and the soil directly beneath the sample, and the difference in the organic N concentration of the sample before and after incubation. They reported N mineralization of 49, 135, and 484 g kg^{-1}, respectively. They concluded that the organic N decrease method estimates total N mineralization of a compost or biosolid, including mineralized

N lost through leaching, volatilization, or denitrification during incubation; however, this method may not indicate the amount of mineralized N actually available to plants, whereas the incubation-extraction measures the mineral N (NH_4-N + NO_3-N) that remains in soil.

Impact on Soils and the Environment

The potential benefits of applying organic amendments to land are well documented (DeLuca and DeLuca, 1997; Moss et al., 2002; Epstein, 2003) and include increases of crop nutrient content and growth. These benefits have been reported for biosolids (Smith, 1996; Butler and Muir, 2006). The addition of biosolids can improve soil physical properties (Metzger and Yaron, 1987) as well as alter soil chemical properties. In general, biosolid application increases water retention capacity (Hall and Coker, 1983), increases hydraulic conductivity (Epstein, 1975; Epstein et al., 1976), reduces soil bulk density, increases total porosity (Chang et al., 1983), and increases the number and size of water-stable aggregates through the increase of soil OM concentration (Tisdall and Oades, 1982).

After 3 yr of biosolid application, Tsadilas et al. (2005) reported that OM concentration, water retention capacity, available water, and infiltration rate significantly increased, whereas bulk density and aggregate instability index decreased. Further, soil physical properties were significantly correlated with OM concentration. Thus, OM concentration was positively correlated with yield and water retention capacity and negatively correlated with bulk density and aggregate index.

Concerns regarding the use of biosolids and its effect on the environment are mainly focused on its nutrient content. In a 2004 conference entitled “Sustainable Land Application” held in Orlando, FL, USA, papers addressed biosolid trace elements (Basta et al., 2005), organic chemicals (Overcash et al., 2005; Xia et al., 2005), pathogens (Gerba and Smith Jr., 2005; Godfree and Farrell, 2005), odors (Schiffman and Williams, 2005), and nutrients (Pierzynski and

Gehl, 2005). Nevertheless, the major challenge when biosolids are used either as an alternative nutrient source to commercial fertilizers or as part of a land application disposal program, is precisely the concentration and ratios of nutrients that are present in the material. When the material is applied on a N-based rate there is potential for over-application of P; on the other hand, application of biosolids on a P-based rate generally is an uneconomical option due to reduced plant response and cost of transporting the material.

Ratio of Organic and Inorganic Nutrient Sources

Combining organic nutrient sources with inorganic fertilizers, when appropriately managed to meet crop requirements, has potential benefits over the use of either nutrient source alone. Benefits include 1) reducing the use of expensive commercial fertilizers without compromising yield, 2) enhancing physical and chemical properties of the soil, 3) preventing over-application of nutrients.

While non-availability of P to plants is a serious problem in heavily leached tropical soils (Brady and Weil, 2002), P loss from soil can be a major problem in heavily fertilized agricultural enterprises located on coarse-textured, poorly drained soils with either surface or tile drainage through which soil drainage water ultimately enters surface waters (Nair and Graetz, 2004). Specifically, animal manures and fertilizers pose a serious threat to surface and groundwater quality in the Suwannee River and the Lake Okeechobee Basins of Florida. The Lower Suwannee River Basin (LSRB) was designated by the Florida Department of Environmental Protection as a 'Group 1' basin (Florida Department of Environmental Protection, 2001), primarily because of increased nitrate concentrations in surface and groundwater in the Upper Floridan Aquifer attributed to dairy wastes, poultry operations, and fertilizer applications (Andrews, 1994; Hatzell, 1995; Katz et al., 1999, Woodard et al., 2003). In the Lake Okeechobee Basin in South Florida, dairy farming and beef ranching are major contributors of P to the lake

(Fonyo and Flaig, 1995), where except for highly manure-impacted intensive area soils, the Mehlich 1-P concentrations are lower than the IFAS agronomic recommended high rate of 30 mg P kg^{-1} (Kidder et al., 2002). However, Nair and Graetz (2002) point out that low Mehlich 1-P concentrations in the surface horizon of these Spodosols is not an indication that more P can be added without detrimental effects on the quality of water leaving the farm. This is the case because P moves through the sandy soil profile of the Spodosols of this region and could eventually reappear in surface and groundwaters.

The USDA Natural Resources Conservation Service (NRCS) policy for nutrient management in Florida, uses the P index rating to determine whether manure and biosolids utilization may be nitrogen or phosphorus based. In areas where there is high risk of P loss the fertilization program has to be on a P-application basis instead of N. The amount of total P applied has to reflect the amount of P removed by the crop. In Florida, N and P application rates are expected to be less than or to match any recommended rates of application by the Institute of Food and Agricultural Sciences, University of Florida (UF-IFAS) (Kidder et al., 2002). Thus, areas such as the Everglades, Green Swamp, and the Okeechobee Basin, which are designated as P limited by legislation, are to have P-based nutrient budgets, irrespective of the nutrient source or the type of soil (Nair and Graetz, 2002).

Summary

Nutrient management to obtain high crop productivity and sustained economic returns represents one of the key management challenges in any agricultural system. The increased cost of fertilizers and environmental concerns associated with their use have focused attention on the use of alternative sources of nutrients. One such nutrient source is municipal biosolids. The re-use of waste products such as municipal biosolids as nutrient sources for crop production while maximizing environmental benefits and lowering production costs, represents a multidisciplinary

challenge. The following chapters describe experiments that evaluate the use of biosolids as a nutrient source for a bioenergy crop. These experiments measure DM yields, N and P removal rates, characterize N mineralization rate of the biosolids, and monitor increases of C and P in the soil profile due to the addition of biosolids at differing proportions with commercial fertilizers.

CHAPTER 3
MUNICIPAL BIOSOLIDS AS A NUTRIENT SOURCE FOR ELEPHANTGRASS BIOMASS PRODUCTION

Introduction

Eighty-five percent of the energy consumed in the USA is derived from fossil fuels, and 60% of that is imported (EIA, 2006). The large-scale dependence on imported fossil fuels has implications for economic stability and national security, and the associated conversion of C formerly stored below ground to atmospheric CO_2 impacts global climate.

These concerns have stimulated research on alternative sources of energy. Biomass from plants has long been recognized as a potential renewable energy source and a candidate to help reduce fossil fuel dependence. Research conducted in the 1980s focused on the identification of plants with high biomass yields for use in the production of methane (Prine et al., 1984; Prine and Woodard, 1986; Woodard and Prine, 1991; Woodard and Prine, 1993a). Currently, the focus has shifted to the production of fuel ethanol from lignocellulosic feedstocks (Ingram et al., 1998; Ingram et al., 1999; Hamelink et al., 2005; Galbe and Zacchi, 2007). Tropical grasses, because of their C_4 carbon fixation pathway, are widely recognized for their biomass production potential. Across the southeastern USA, elephantgrass (*Pennisetum purpureum* Schum.) has consistently shown the highest or near the highest dry matter (DM) production, with yields of 20 to 48 Mg ha^{-1} yr^{-1} (Prine et al., 1984; Woodard and Prine, 1993a; Bouton, 2002; Woodard and Sollenberger, 2008).

Energy crops have not only been considered a promising renewable energy source but also a viable C sequestering option (USDOE-EIA, 2006; Sartori et al., 2006). Soils of the southern regions of the USA have significant potential for soil organic carbon (SOC) sequestration due to current low SOC concentration caused by a combination of a long history of crop production and exposure to high temperatures and precipitation (Paustian et al., 1999; Tolbert et al., 2002).

Increases in SOC of greater than 1 Mg ha^{-1} yr^{-1} under grasslands have been reported (Trumbore et al., 1995; Franzluebbers, 2007).

High-yielding bioenergy crops remove relatively large amounts of nutrients at harvest. Nutrients replenished must occur in a manner that minimizes environmental impact and production costs. Biosolids are a "nutrient-rich organic by-product of municipal wastewater treatment that can be beneficially recycled via land application" (EPA, 1999). Typical concentrations of total N and P in biosolids can range from 35 to 68 g kg^{-1} and 18 to 39 g kg^{-1}, respectively. Thus, biosolids can supply significant quantities of nutrients for crop production, but like many other organic sources of nutrients, when the material is applied at an N-based rate there is potential for over-application of P, due to narrower N:P ratio in biosolids versus plant requirements.

Soil test P (STP) is used as a criterion to limit or prohibit P application when the soil P threshold is reached (Pierzynski and Gehl, 2005). However, research has shown that agronomic (the soil P level where an increase in P will not increase crop yield) and environmental thresholds (the soil P level where significant amounts of additional P will be mobilized by rain water) are not equivalent (Higgs et al., 2000). When a wide range of STP concentrations are considered, the relationship between runoff dissolved P and STP is curvilinear due to saturation of P-fixing sites in the soil (Kleinman et al., 2000; McDowell and Sharpley, 2001; Elliott and O'Connor, 2007).

Water extractable P (WEP) has been highly correlated with P concentrations in runoff and leaching from manure and manure-amended soils (Pote et al., 1996; Kleinman et al., 2002; Sharpley and Moyer, 2000). Brandt et al. (2004) concluded that a good measure of the environmentally relevant portion of total P in biosolids and manures is the percentage of total P

that is water extractable (PWEP). The PWEP in 41 biosolids, representing a variety of wastewater and solids treatment processes, averaged 24 g kg^{-1}, compared to inorganic fertilizer (850 g kg^{-1}), dairy manure (520 g kg^{-1}) and poultry manure (210 g kg^{-1}). Water extractable P is seen as a key means to develop a weighting factor that differentiates P sources based on their relative potential to release P into runoff. This has been termed the P-source coefficient, or PSC (Leytem et al., 2004).

If municipal biosolids are to play an important role in fertilization of energy crops, information is needed on their ability to support crop growth without soil P accumulation to levels that have negative environmental consequences. Thus, the objectives of this study were 1) to quantify DM production of elephantgrass when supplying differing proportions of N from municipal biosolids (MBS) and ammonium nitrate fertilizer; 2) to quantify tissue N and P concentration and removal by elephantgrass when N is applied in differing proportions from MBS and ammonium nitrate; and 3) to quantify changes in total soil C and P concentrations (total P, water extractable P and Mehlich-1 P) when MBS are applied as a nutrient source.

Materials and Methods

Experimental Site

The experiment was conducted at the Range Cattle Research and Education Center (RCREC) at Ona, FL (27° 26' N and 81° 55' W). Plots used were part of an experiment conducted during the early 1990s that evaluated the effect of defoliation treatments on six elephantgrass entries. Plot size in the earlier experiment was 9 x 16 m and there were four replicates of each entry arranged in a randomized block design. 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 15-20 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. The

current study was conducted during 2007 and 2008. Average weekly maximum and minimum temperatures, as well as precipitation during the 2 yr are shown in Figure 3-1.

Treatments and Design

Two of the six elephantgrass entries in the original study were chosen for inclusion in the current study. These were elephantgrass cultivar 'Merkeron' and an elephantgrass of unknown origin named "Chinese Cross". These two entries were selected because their plots had the most complete and uniform stands. For the purposes of the current research, each elephantgrass entry was considered a separate experiment. This was done because Chinese Cross plots had more useful experimental area and could accommodate more treatments than Merkeron and because the Chinese Cross plots were added sometime after the five original entries had been planted. Thus they were not assigned randomly to experimental units, and all Chinese Cross plots were at the north end of a north-south row of plots in each block.

The same four treatments were imposed on both grasses, plus two additional treatments were imposed on Chinese Cross. Each 9- x 16-m plot from the original study served as a block in the current study and contained either four or six, 4 x 4 m plots with a 1-m alley between plots. The four treatments imposed on both grasses were proportions of total N applied that came from class AA municipal biosolids (MBS) vs. ammonium nitrate (NH_4NO_3; 340 g N kg^{-1}) fertilizer. Levels were 0, 33, 66, and 100% of total N from MBS. The MBS and fertilizer were surface-applied, to supply a total annual rate of 350 kg N ha^{-1} yr^{-1}. One half was applied at the beginning of the growing season and the other half after the first harvest of each year. The MBS were applied on 20 May and 3 Aug. 2007 and 4 Apr. and 8 Aug. 2008. The two additional treatments, applied to Chinese Cross only, included plots where all N was applied as MBS, but it was soil incorporated (100% MBS-INC) instead of surface applied, and plots where MBS were surface

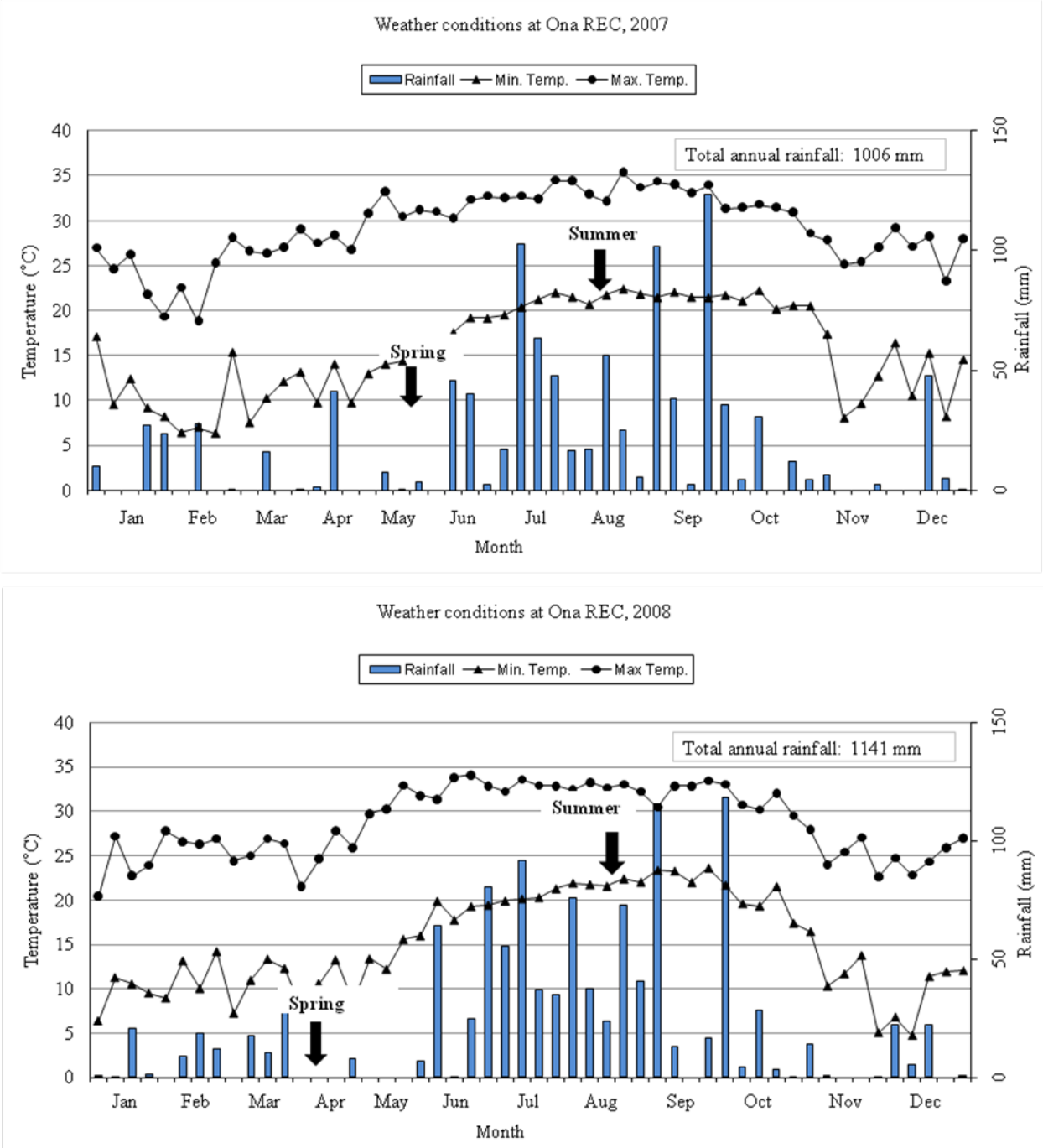

Figure 3-1. Weekly total precipitation and average weekly maximum and minimum air temperatures at the Range Cattle Research and Education Center at Ona, FL during 2007 and 2008. Arrows indicate when treatmens were applied. Spring and summer dates were 20 May and 3 Aug. 2007 and 4 Apr. and 8 Aug. 2008, respectively.

applied but at twice the rate of the 100% MBS treatment (2x-MBS surface). Incorporation of the MBS in the 100% MBS-INC treatment was accomplished by slightly disturbing the top 5 cm of soil using a home-garden rake. Thus, all six treatments (four differing proportions of N from MBS and NH_4NO_3, 100% MBS-INC, and 2x-MBS surface) were allocated to Chinese Cross plots and only four treatments (the four differing proportions of N from MBS and NH_4NO_3) were allocated to Merkeron plots.

Proportions and total amounts of MBS and N fertilizer applied were chosen based on earlier work conducted by Woodard and Prine (1991) and bracketed the range of expected P removal by the crop. Expected P removal of elephantgrass herbage was 40 to 60 kg ha^{-1} yr^{-1} (Vicente-Chandler et al., 1974; Sanchez, 1976; Boonman, 1993). The 33, 66, and 100% MBS treatments supplied 39, 79, and 118 kg P ha^{-1} yr^{-1} in 2007, and 47, 95, and 142 kg P ha^{-1} yr^{-1} in 2008, respectively. The treatment where N was supplied using 100% NH_4NO_3 fertilizer received 60 kg P ha^{-1} yr^{-1} from triple superphosphate (TSP; 460 g P_2O_5 kg^{-1}) each year. This amount was chosen to be intermediate between the total amounts of P supplied by the 33 and 66% MBS treatments. In addition, all treatments received 145 kg K ha^{-1} yr^{-1} from a potassium-magnesium sulfate source "Sul-Po-Mag" (220 g K_2O kg^{-1}); half was applied at each date when MBS application occurred.

The MBS were obtained from Water Reclamation Facility #3 of the City of Ocala, FL. The material was the result of blending anaerobically digested sludge and undigested waste-activated sludge. The mixture was fed to a belt press and ultimately sent to an indirect drier system and dried to an average 950 g DM kg^{-1} product (Hicks et al., 2007). The end product is classified as AA biosolids and is marketed as a slow-release soil amendment. The quantity of material required at each treatment application date was collected separately from four different batches.

Two sub-samples from each batch were analyzed following the procedures used by a commercial laboratory (Dairy One Lab., Ithaca, NY), before application of treatments in the field (Table 3-1).

Table 3-1.Composition of the class AA municipal biosolids used as a nutrient source for elephantgrass plots.[†]

Date of application	20 May 2007	3 Aug. 2007	4 Apr. 2008	8 Aug. 2008
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 N[§]	58 ± 1	65 ± 0	54 ± 0	54 ± 0
Total P[¶]	20 ± 2	21 ± 1	20 ± 2	23 ± 1
K	1.6 ± 0	1.6 ± 0	1.5 ± 0	1.7 ± 0
Ca	1.9 ± 0	3.1 ± 0	1.6 ± 0	2.2 ± 0
Mg	1.8 ± 0	3.5 ± 0	2.4 ± 0	2.6 ± 0
Na	0.6 ± 0	0.7 ± 0	0.5 ± 0	0.7 ± 0
	---------- mg kg^{-1} ----------			
Fe	17600 ± 707	21450 ± 919	18950 ± 2757	37800 ± 1131
Zn	709 ± 65.0	871 ± 17.7	525 ± 60.8	873 ± 0.7
Cu	197 ± 6.4	184 ± 3.5	127 ± 14.1	193 ± 3.5
Mn	127 ± 5.7	117 ± 2.8	62 ± 6.4	100 ± 0.7
Mo	14 ± 0.1	16 ± 0.6	11 ± 1.2	17 ± 1.0
NH_4-N[#]	1435 ± 0.0	975 ± 0.0	1315 ± 0.0	1710 ± 0.0
NO_3-N[††]	<1	<1	65 ± 2.1	<1
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. Mehlich-1 P values are on an air dry basis.
[‡]pH determined using 15 g wet sample + 200 ml de-ionized water. Sample was stirred and allowed to stabilize 5 min.
[§]Total N determined using a Combustion Analyzer - AOAC 990.03.
[¶]Minerals were determined by ashing samples and digesting using 6 M HCl. Analyzed using ICP spectrometer.
[#]NH_4-N determined by titration and distillation - AOAC 941.04.
[††]NO_3-N: RQflex® Reflectometer using Relectoquant® Nitrate test strips.
[‡‡]WEP (Water-extractable P) determined using a 1:200 ratio with DDI water (Kuo, 1996).

Response Variables

Dry matter yields were measured two times per year in each plot by harvesting all tillers within a PVC quadrat of 3 × 1.5 m to a 10-cm stubble height. Harvests occurred on 26 July and 27 Nov. 2007, and 24 July and 21 Nov. 2008. A bushwacker was used to cut elephantgrass tillers inside the quadrat to the target 10-cm stubble height. The material was collected and weighed fresh in the field. A subsample of representative tillers was weighed fresh and dried at 60°C until constant weight to determine DM concentration and calculate DM yield. After each sample collection was completed, all plots were cleared by cutting the remaining standing material using a crop chopper and a silage wagon. The material was cut to a 15-cm height on a first pass and to 10 cm when running in the opposite direction on the second pass.

The dried subsample was ground in a hammer mill and then through a Wiley mill (1-mm screen) prior to laboratory analyses. Total N and P in the tissue were 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). Crop N and P removal were calculated for each harvest as the product of N and P concentration in the tissue 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 monitoring were taken before treatments were applied in 2007, at the end of the 2007 growing season, and after the last harvest in 2008. Samples were collected from the Ap horizon which was consistently between 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 corresponding two samples per plot on an equal volume basis. The soil samples were analyzed for 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, following the procedure described by Kuo (1996). For total P determination, 0.3 g of air-dried soil was ashed and digested according to Anderson (1976). Water extractable P and total P concentrations were determined using the ascorbic acid method by Murphy and Riley (1962). Mehlich-1 P determinations were done at the Analytical Research Laboratory of the University of Florida. Total soil C was determined by dry combustion (Nelson and Sommers, 1996) on a Thermo Flash EA1112-NC elemental analyzer on soil samples that were previously homogenized to a powder using a mortar and pestle.

Statistical Analysis

Data were analyzed using mixed-model methods (SAS Institute, 1996). In all models percent of N from MBS and year were considered fixed effects. Blocks were considered random effects. When there was percent MBS × year interaction, data were analyzed by year. Polynomial contrasts (linear, quadratic, and cubic) were used to determine the nature of responses to percent of N from MBS. The 100% MBS-INC and 2x-MBS surface treatments were compared to others using the pdiff test of LSMEANS. For analysis of soil data, initial values (pre-treatment application) were used as covariates. All means reported in the text are least squares means. Treatments were considered different if $P \leq 0.05$.

Results and Discussion

Dry Matter Yield

There was no percentage MBS × year interaction for DM yield of either entry ($P > 0.05$). Dry matter yield of elephantgrass decreased linearly as percentage of N from MBS increased ($P \leq 0.05$) for both entries (Table 3-2). The decrease in yield from 0 to 100% MBS was 7.4 Mg ha^{-1} for Chinese Cross but only 4.1 Mg ha^{-1} for Merkeron. In addition, the 33% MBS treatment of Merkeron performed quite well relative to 0% MBS, but this was not the case for Chinese Cross.

Elephantgrass DM yields obtained in this study were comparable to the two harvests per year treatment yields reported by Woodard and Prine (1991) and less than those of a single harvest per year reported by the same authors.

Table 3-2. Dry matter (DM) yield of elephantgrass cultivars Chinese Cross and Merkeron fertilized with municipal biosolids (MBS) and ammonium nitrate (NH_4NO_3) at differing proportions to supply a total of 350 kg N ha^{-1} yr^{-1} but differing amounts of plant-available N. Data are means across 2 yr with plots harvested two times per year, and DM yield summed within year.

Percent of N from MBS	Plant-available N[†]	Cultivar	
		Chinese Cross	Merkeron
	---- kg N ha^{-1} ----	----------Mg ha^{-1} yr^{-1} ----------	
0	350 (100)	24.3 (100)[‡]	24.2 (100)
33	277 (79)	19.2 (79)	24.2 (100)
67	203 (58)	18.5 (76)	21.3 (88)
100	130 (37)	16.9 (70)	20.1 (83)
SE		1.5	1.3
Polynomial effects[§]		L**	L*

[†]Plant-available N considers all N from NH_4NO_3 to be plant available while MBS have 370 g plant-available N kg^{-1} (based on incubation studies reported in Chapter 4). Numbers in parentheses are the percentage of plant-available N provided compared to the 0% MBS treatment.

[‡]Numbers in parentheses are the yield of that treatment as a percentage of the yield of the 0% MBS treatment.

[§]Linear (L) effect of percent MBS; *, $P \leq 0.05$; **, $P < 0.01$.

Initial chemical characterization of the MBS used in the current study indicated that inorganic N forms (NH_4-N and NO_3-N) were ~ 20 g kg^{-1} of total N in the material; therefore, mineralization of N was expected to have a major impact on crop yield. The organic N mineralization process can be affected by a number of dynamic and site-specific factors including temperature, water, and aeration (Hanselman et al., 2004), and few studies have validated laboratory incubation data with field experiments. This is unfortunate because in order for measures of plant-available nutrients (e.g., organic N mineralized) to relate to crop response they must occur in comparable environments. Sikora and Enkiri (2000) indicated that field

mineralization of organic N in manures or composts can be different than that found in laboratory studies, and strongly recommended field evaluations. Tester (1989) determined that in the greenhouse, 135 Mg ha^{-1} of sewage sludge compost application was equivalent in yield to N and P fertilizer rates of 200 kg ha^{-1}. Using the same compost and crop (tall fescue, *Lolium arundinaceum* Schreb.) in the field, a 60 Mg ha^{-1} application rate equaled a 200 kg ha^{-1} N and P fertilizer rate. Sikora and Enriki (2000) conducted an experiment to evaluate the effect of biosolids compost-fertilizer blends on tall fescue. Plant-available N blends of 50% NH_4NO_3 and 50% biosolids compost or 67% NH_4NO_3 and 33% biosolids compost produced greater yields and N uptake by tall fescue than predicted from incubation studies of compost alone. The authors used ^{15}N and showed that NH_4NO_3 fertilizer stimulated compost soil and/or compost N mineralization. They concluded that compost N can replace one-third of the fertilizer N required by fescue without decreasing yield (Sikora and Enkiri, 1999).

In the current study, MBS in the fertilizer-MBS blend was applied based on total N, not on the organic N expected to mineralize in a growing season. Mineralization of organic N during the 2 yr was investigated under field conditions using polyester bags (Chapter 4). Total organic N mineralization averaged 369 and 366 g kg^{-1} across years for spring and summer MBS applications, respectively. Using 370 g kg^{-1} as an estimate of total N mineralization from MBS during a growing season and considering N from NH_4NO_3 to be 100% plant available, the plant-available N applied was 350, 277, 203, and 130 kg ha^{-1} for the 0, 33, 67, and 100% MBS treatments, respectively (Table 3-2). Yields of MBS treatments were proportionally greater than these numbers might suggest, especially for higher % MBS treatments of Chinese Cross and across all treatments for Merkeron. It is not clear if the combination of NH_4NO_3 and MBS resulted in greater N mineralization from MBS than for MBS alone, although this has been

reported previously for NH_4NO_3 and biosolids compost by Sikora and Enriki (2000). Thus, a remaining challenge is to accurately establish correlations between organic N mineralization rates determined in field incubation studies and DM yields obtained when MBS are applied on a total N basis, or even when MBS are applied taking into account mineralization values from field incubation studies.

Nitrogen and Phosphorus Concentration in the Tissue

There was percentage MBS × year interaction for N concentration for Merkeron ($P \leq 0.05$). There was no effect of percent MBS on N concentration in Chinese Cross ($P > 0.05$). Year effect was significant for Chinese Cross ($P \leq 0.05$). Interaction occurred for Merkeron because tissue N concentration decreased linearly as percent MBS increased in 2007 ($P \leq 0.05$) but not in 2008 (Table 3). Tissue N concentration was greater in 2007 than 2008 for Chinese Cross (8.6 vs. 7.0 g kg^{-1}, respectively) and for three of four levels of percent MBS for Merkeron (Table 3-3).

Greater N concentration in 2007 compared to 2008 may be attributed to the shorter growing period before the first harvest in 2007 (67 d) than in 2008 (111 d). The shorter growing period was due to a severe spring drought, which delayed onset of spring growth and application of MBS and fertilizer. This likely translated into less mature herbage harvested, and the literature includes numerous examples of less mature elephantgrass herbage having greater N concentration (Gomide et al., 1969; Woodard and Prine, 1991; Schank and Chynoweth, 1992). As plants grow they contain increasing proportions of structural and storage materials that are low in N, so the concentration of N in the plant declines with increasing maturity (Greenwood et al., 1991).

Table 3-3. Nitrogen concentration of Chinese Cross and Merkeron elephantgrass fertilized with municipal biosolids (MBS) and ammonium nitrate (NH_4NO_3) at differing proportions, to supply a total of 350 kg N ha^{-1} yr^{-1}. Data are weighted means within year for plots harvested two times per year.

Percent of N from MBS	Cultivar			
	Chinese Cross	Merkeron		
	2007 & 2008	2007	2008	2007 vs. 2008
	---------- g kg^{-1} DM ----------			*P* – value
0	8.1	8.9	7.7	< 0.01
33	8.2	9.3	7.7	< 0.01
67	7.5	8.1	7.3	< 0.01
100	7.4	8.1	8.0	0.49
SE	0.3	0.3	0.2	
Polynomial effects[†]	NS	L*	NS	

[†]Linear (L) effect of percent MBS; *, $P \leq 0.05$; NS = not significant, $P > 0.05$.

There was percentage MBS × year interaction for P concentration for both entries ($P \leq 0.05$). There were linear, quadratic, and cubic effects of percent MBS on tissue P concentration for Chinese Cross and linear and quadratic effects for Merkeron (Table 3-4). Quadratic and cubic effects were significant due to greater, or trends toward greater, herbage P concentration of the 0% MBS treatment relative to the 33% treatment. For the 0% MBS treatment, P was added as triple superphosphate at a rate of 60 kg P ha^{-1} yr^{-1}, which is greater than the amount of P supplied by 33% MBS (average of 43 kg P ha^{-1} yr^{-1}). The data were analyzed subsequently with the 0% MBS treatment removed from the dataset, and the response with three levels of the treatment was linear. Thus, quadratic and cubic effects were likely due to the different phytoavailability of P in the biosolids compared to triple superphosphate (O'Connor et al., 2004) and the fact that the rate of P in the 0% MBS treatment was higher compared to the following level of the treatment factor (33% MBS).

Phosphorus concentration in elephantgrass has been reported to range from 1 to 5 g kg^{-1} (Vicente-Chandler et al., 1959; Gomide et al., 1969). In the current study, the range was from 1.4

to 2.4 g kg^{-1}, with greatest concentrations occurring for the highest proportions of MBS (in which P applications were greater).

Table 3-4. Phosphorus concentration of Chinese Cross and Merkeron elephantgrass fertilized with municipal biosolids (MBS) and ammonium nitrate (NH_4NO_3) at differing proportions, to supply a total N rate of 350 kg ha^{-1} yr^{-1}. Data are weighted means within year of plots harvested two times per year.

Percent of N from MBS	Cultivar			
	Chinese Cross		Merkeron	
	2007	2008	2007	2008
	g kg^{-1} DM			
0	1.7	1.9	1.7	1.7
33	1.4	1.4	1.6	1.6
67	1.7	2.0	1.7	1.7
100	1.8	2.4	1.9	2.4
SE	0.07	0.09	0.05	0.11
Polynomial effects[†]	L*Q**C*	L**Q**C*	L*Q*	L**Q**

[†]Linear (L), quadratic (Q), and cubic (C) effects of percent MBS; *, $P \leq 0.05$; **, $P < 0.01$

Nitrogen and Phosphorus Removal

There was no percentage MBS × year interaction for N or P removal of either entry ($P > 0.05$). Nitrogen removal decreased linearly ($P \leq 0.05$) with increasing percentage MBS for Chinese Cross, and for Merkeron there were linear ($P < 0.01$) and cubic effects ($P \leq 0.05$), the latter occurring because N removal increased slightly for the 33% MBS treatment compared to 0% (Table 3-5). Higher N removal in 2007 than 2008 ($P \leq 0.05$; data not shown) was due to higher tissue N concentration in 2007, as discussed previously.

Nutrient removal is a function of DM yield and nutrient concentration. The DM yield of elephantgrass was lower when harvested more frequently, but because the grass was less mature, it contained a higher concentration of minerals and nutrient removal was the same or larger than with less frequent harvest (Boonman, 1993).

Table 3-5. Nitrogen and phosphorus applied and removal by Chinese Cross and Merkeron elephantgrass fertilized with municipal biosolids (MBS) and ammonium nitrate (NH_4NO_3) at differing proportions, to supply a total of 350 kg N ha^{-1} yr^{-1}. Data are means across 2 yr.

Percent of N from MBS	Total N applied	N removal		Total P applied	P removal	
		Cultivar			Cultivar	
		Chinese Cross	Merkeron		Chinese Cross	Merkeron
	kg ha^{-1} yr^{-1}	------- g kg^{-1} DM -------		kg ha^{-1} yr^{-1}	------- g kg^{-1} DM -------	
0	350	195	199	60	43	42
33	350	156	208	43	28	39
67	350	139	165	87	34	36
100	350	127	162	130	35	43
SE		12	7.6		2.1	2.8
Polynomial effects		L**	L**C*		Q**C*	NS

†Linear (L), quadratic (Q), and cubic (C) effects of percent MBS; *, $P \leq 0.05$; **, $P < 0.01$; NS = not significant, $P > 0.05$.

Phosphorus removal showed quadratic and cubic effects ($P \leq 0.05$) for Chinese Cross but not for Merkeron (Table 3-5). When data were analyzed without the 0% MBS treatment (due to a different P source in this treatment), the percentage MBS effect on P removal of Chinese Cross was linear but there was no effect for Merkeron.

At a DM yield of 12.5 Mg ha^{-1} yr^{-1} Boonman (1993) reported that 30 kg P ha^{-1} yr^{-1} were removed. Sanchez (1976) and de Geus (1973) indicated that 40 to 64 kg P ha^{-1} yr^{-1} may be needed to match the removal of P in intensively zero-grazed grasses. Elephantgrass P removal as high as 136 kg ha^{-1} yr^{-1} was reported by Gomide et al. (1969), but in that experiment fertilization was 900 and 340 kg ha^{-1} yr^{-1} of N and P, respectively, well beyond what can be considered practical from economic and environmental perspectives. It has been suggested that elephantgrass has little tendency for luxury consumption of P (Pant et al., 2004).

In our experiment there was no zero P or N addition treatment to account for N or P uptake from an untreated soil and allow calculation of a true percent recovery. If percent N removal is calculated based on N removal divided by N applied, then the 0% MBS treatment, where all N was applied as NH_4NO_3, showed the highest removal percentage (560 and 570 g N removed kg^{-1} applied for Chinese Cross and Merkeron, respectively). In the 100% MBS treatments the apparent removal was 360 and 460 g N kg^{-1} for Chinese Cross and Merkeron, respectively. As noted earlier, the plant-available N concentration in MBS was approximately 370 g kg^{-1}, thus a relatively large amount of N in MBS was likely not mineralized and made available for plant uptake. These data and those for DM yield suggest, however, that the N mineralization measured in Chapter 4 may have underestimated the actual amount.

In the case of P, the plots were located in a soil considered deficient based on an initial Mehlich-1 extractable P concentration in topsoil (0 to 15 cm) of 10 mg P kg^{-1}. Considering only

the total amounts of P applied and removed by the treatments across the 2-yr period, apparent P removal decreased from 638 to 268 g P removed kg^{-1} P applied for the 33 to 100% MBS treatments in Chinese Cross, and from 890 to 330 g P removed kg^{-1} P applied, respectively, for Merkeron. Apparent P removal in the 0% MBS treatment, where all P was applied using triple superphosphate at rate of 60 kg P ha^{-1} yr^{-1}, were 717 and 690 g P removed kg^{-1} P applied in Chinese Cross and Merkeron, respectively. Again, caution is required when interpreting these calculations because this study was not specifically designed to evaluate percent recovery. Also, as reported by Ibricki et al. (1999), the source of plant-available P and active uptake for tropical grasses growing in a Spodosol may be deeper in the profile and not necessarily in the horizon sampled for soil tests.

Effect of MBS Incorporation

There was no treatment × year interaction for DM yield ($P > 0.05$). When the MBS were incorporated in the top 5 cm of soil, DM production was ~33% greater than when surface applied (Table 3-6). Further, DM production from the 100% MBS-INC treatment was not different from the 0% MBS treatment in which all N was supplied by NH_4NO_3 fertilizer. Dry matter yields from the 2x-MBS surface treatment were not greater than the 100% MBS-INC and the 0% MBS treatments.

Several laboratory incubation studies have demonstrated the positive effect of incorporation on organic amendments (King, 1973; Terry et al., 1978; Adamsem and Sabey, 1987; Sommers et al., 1981; He et al., 2003). This positive effect has been attributed to an increase in organic N mineralization and nitrification processes due to enhanced contact of MBS with moist soil particles and microbes and reduced volatilization losses. Few data have been reported on actual field trials (He et al., 2000; Hanselman et al., 2004). In an experiment designed to evaluate the effect of incorporation of poultry litter on quantity and quality of runoff

Table 3-6. Effect of incorporation and double rate of surface-applied municipal biosolids (MBS) on dry matter (DM) yield of Chinese Cross elephantgrass. Data are means across 2 yr, with plots harvested two times per year and DM yield summed across harvests within years.

Treatment[†]	DM yield
	Mg ha^{-1} yr^{-1}
0% MBS	24.3 a[‡]
100% MBS	16.9 b
100% MBS-INC	22.5 a
2x-MBS surface	21.6 a
SE	1.2

[†]All treatments supplied a total of 350 kg N ha^{-1} yr^{-1}, except treatment 2x-MBS surface where total N rate was doubled. Also, MBS in all treatments were surfaced applied except for 100% MBS-INC where MBS were incorporated. Nitrogen in 0% MBS treatment was supplied using NH_4NO_3 fertilizer.

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

water, Pote et al. (2003) reported that yields were approximately 25% higher on plots where litter was incorporated versus plots where it was surface-applied. In a companion study to the current research, a field incubation of MBS showed that incorporated material had greater organic N mineralization (by ~ 70 g kg^{-1}) than MBS that were surface applied (Chapter 4). Data from the current experiment and the associated conditions suggest that incorporated MBS can replace inorganic sources of N without significant reduction in DM yield of elephantgrass.

There was no treatment × year interaction for N concentration and N removal ($P > 0.05$). Nevertheless, year effect was significant ($P \leq 0.05$) for both response variables. There was no effect of incorporation and doubling the rate of MBS on tissue N concentration (Table 3-7). The 100% MBS treatment showed the lowest N removal compared to the other treatments ($P \leq 0.05$). When MBS were incorporated (100% MBS-INC), N removal was not different from 0% MBS and 2x-MBS surface treatments ($P > 0.05$). The effect of incorporation on N removal followed the same trend as DM yields. These results can be explained by the fact that N removal is a function of N concentration and DM yield.

Table 3-7. Effect of incorporation and doubling the rate of municipal biosolids (MBS) on tissue N concentration and N removal in Chinese Cross elephantgrass. Data are weighted means across 2 yr and two harvests per year.

Treatment[†]	N concentration	N removal
	---- g kg^{-1} DM ----	--- kg ha^{-1} yr^{-1} ---
0% MBS	8.1[‡] a	195 a
100% MBS	7.4 a	127 b
100% MBS-INC	7.6 a	166 a
2x-MBS surface	7.4 a	170 a
SE	0.3	14.8

[†]All treatments supplied a total of 350 kg N ha^{-1} yr^{-1}, except treatment 2x-MBS surface where total N rate was doubled. Also, MBS in all treatments were surface applied except for 100% MBS-INC where MBS were incorporated. In the 0% MBS treatment, N was supplied using NH_4NO_3 fertilizer and P was supplied using TSP at a rate of 60 kg P ha^{-1} yr^{-1}.
[‡]Means within a column not followed by the same letter are different ($P \leq 0.05$).

There was a treatment × year interaction for tissue P concentration and P removal ($P \leq 0.05$). In 2007, there was no effect of treatments on P concentration and the average across treatments was 1.8 g P kg^{-1} DM (Table 3-8). In 2008, the 100% MBS treatment was not different from the 2x-MBS surface and 100% MBS-INC treatments. The 0% MBS showed the lowest P concentration ($P \leq 0.05$) and it was consistent in 2007 and 2008 with a value of 1.7 g P kg^{-1} DM. In contrast, P concentration in the other treatments was consistently greater in 2008 compared to 2007. Thus, the increased P concentration in 2008 is attributed to carry over effects of MBS application in 2007.

Incorporation of MBS increased P removal in 2007 and 2008 compared to MBS surface applied ($P > 0.05$). This effect is due to greater DM yields in the incorporated treatment and similar P concentration in the tissue compared to the 100% MBS surface applied treatment. Calculating the P removal divided by P applied each year, the values were 240 and 300 g P removed kg^{-1} P applied when MBS were surface applied, and 320 and 380 g P removed kg^{-1} P applied when MBS were incorporated in 2007 and 2008, respectively.

Table 3-8. Effect of incorporation and doubling the rate of municipal biosolids (MBS) on tissue P concentration and P removal in Chinese Cross elephantgrass. Data are weighted means per year of harvest, with plots harvested 2 times per year.

Treatment[†]	P concentration		P removal	
	2007	2008	2007	2008
	----- g kg^{-1} DM -----		------ kg ha^{-1} yr^{-1} ------	
0% MBS	1.7[‡] a	1.7 c	39 ab	47 b
100% MBS	1.8 a	2.4 ab	31 b	39 c
100% MBS-INC	1.8 a	2.3 b	41a	50 b
2x-MBS surface	1.7 a	2.6 a	37 ab	58 a
SE	0.1	0.1	2.4	3.0

[†]All treatments supplied a total of 350 kg N ha^{-1} yr^{-1}, except treatment 2x-MBS surface where total N rate was doubled. Also, MBS in all treatments were surface applied except for 100% MBS-INC where MBS were incorporated. In 0% MBS treatment N was supplied using NH_4NO_3 fertilizer and P was supplied using TSP at a rate of 60 kg P ha^{-1} yr^{-1}.
[‡]Means within a column not followed by the same letter are different ($P \leq 0.05$).

Soil P and C Concentrations

Soil P and C Concentrations

There was no percent MBS × year interaction for any of the soil variables measured ($P >$ 0.05) for either entry. Soil variables followed the same response in Chinese Cross and Merkeron. There was no effect of percentage of total N supplied by MBS on WEP, Mehlich-1 P, total soil P, and total soil C concentration, except for total soil C in Merkeron which showed a quadratic effect (Table 3-9). The quadratic effect was significant due to greater, or trends toward greater, total soil C concentration of the 0% MBS treatment relative to the 33% treatment. There was a year effect for total soil P ($P \leq 0.05$) for both cultivars. Total soil P was 114 and 135 mg kg^{-1} in 2007 and 2008 ($P = 0.01$, SE = 7.3), respectively, for Chinese Cross, and it was 93 and 132 mg kg^{-1} in 2007 and 2008 ($P < 0.01$, SE = 8.7), respectively, for Merkeron. This increase reflects the additional P supplied by a second year of MBS application. Year effect for WEP was significant ($P \leq 0.05$) for Chinese Cross and there was a strong tendency in Merkeron ($P = 0.06$). Water extractable P averaged 2.6 and 1.6 mg kg^{-1} in 2007 and 2008 ($P = 0.04$, SE = 0.4), respectively,

Table 3-9. Soil P and C concentrations in the Ap horizon (0 to 15 cm) after addition of municipal biosolids (MBS) and ammonium nitrate (NH_4NO_3) at differing proportions, to supply a total of 350 kg N ha^{-1} yr^{-1} for 1 yr (2007) and 2 yr (2008).

Percent of N from MBS	Chinese Cross				Merkeron			
	WEP	Mehlich-1 P	Total P	Total soil C	WEP	Mehlich-1 P	Total P	Total soil C
	---------- mg kg^{-1} ----------			--- g kg^{-1} ---	---------- mg kg^{-1} ----------			--- g kg^{-1} ---
0	3.2	19.0	118	19	1.2	11.0	113	17
33	1.5	11.0	128	18	2.2	7.0	114	15
67	1.9	14.0	121	20	0.9	9.0	97	14
100	1.9	13.0	132	18	1.7	15.0	129	16
SE	0.6	3.1	20	1.5	0.7	3.5	14	1.0
Contrast	NS	NS	NS	NS	NS	NS	NS	Q*

†WEP = water extractable P

Quadratic (Q) effect of percent MBS; *, $P \leq 0.05$; NS = not significant, $P > 0.05$.

for Chinese Cross, and 2.0 and 0.94 mg kg^{-1} in 2007 and 2008 ($P = 0.06$, SE = 0.5), respectively, for Merkeron. When the data set was analyzed without the 0% MBS treatment (because P was supplied from a different P source, triple superphosphate), there was a trend toward increased WEP as percent MBS increased ($P = 0.132$) in Chinese Cross. Also, total soil C in Merkeron showed a trend to increase as percent MBS increased ($P = 0.158$). Total soil P, Mehlich-1 P, and total soil C did not show any trends.

Results suggest a gradual accumulation of total P in the Ap soil horizon when comparing 2007 versus 2008; nevertheless no trend was evident among treatments. Given that the 100% MBS treatment supplied approximately three times more P than the 33% MBS treatment, lack of response is probably due to natural soil variability and the size of the total P pools. There was no trend in soil C between the first and second year after application of MBS for either cultivar. This is not surprising considering that these areas had been planted to elephantgrass for more than 15 yr and for many of those years the plots were not harvested and the residue was chopped and spread in situ.

There was no treatment × year interaction effect on WEP, Mehlich-1P, total soil P, and total soil C when MBS were incorporated and surfaced applied at the doubled rate ($P > 0.05$). Incorporation of MBS had no effect on WEP, Mehlich-1 P, total soil P, and total soil C values compared to surface application (Table 3-10). Total soil P concentration in the 2x MBS surface treatment was the highest ($P \leq 0.05$) compared to the other treatments reflecting the much greater amounts of P applied, but total soil P was not different in the 0% MBS compared to the 100%MBS and 100% MBS-INC treatments.

Table 3-10. Effect of incorporation and doubling the rate of municipal biosolids (MBS) on water extractable P (WEP), Mehlich-1 P, total P, and total soil C concentration.

Treatment[†]	WEP	Mehlich-1 P	Total P	Total soil C
	---------- mg kg^{-1} ----------			---- g kg^{-1} ----
0% MBS	3.2 a[‡]	19.0 a	118 b	19 a
100% MBS	1.9 ab	13.0 a	132 b	18 a
100% MBS-INC	1.7 b	14.0 a	116 b	18 a
2x-MBS surface	2.1 ab	15.0 a	232 a	22 a
SE	0.6	3.5	22	1.2

[†]All treatments supplied a total of 350 kg N ha^{-1} yr^{-1}, except treatment 2x MBS-INC where total N rate was doubled. Also, all treatments were surface applied except for 100% MBS-INC where MBS were incorporated. Phosphorus in the 0% MBS treatment was supplied using triple superphosphate at a rate of 60 kg P ha^{-1} yr^{-1}.

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

Summary and Conclusions

For both entries tested, elephantgrass yield decreased linearly as the percentage of N supplied by MBS increased, although the extent of the decline was greater for Chinese Cross than for Merkeron. Initial chemical characterization of the MBS used in the current study indicated that plant-available N forms (NH_4-N and NO_3-N) were ~ 20 g kg^{-1} of total N in the material; therefore, mineralization of N was expected to have a major impact on crop yield. Accounting for expected N mineralization based on a litter bag study (Chapter 4), the amounts of plant-available N applied were 350, 276, 203, and 130 kg ha^{-1} for the 0, 33, 67, and 100% of N applied as MBS treatments. Differences in plant-available N accounted for some of the differences in yields, but yields actually decreased proportionally less than did estimated plant-available N. This could occur because the incubation study underestimated N mineralized or because mixing of NH_4NO_3 with MBS increased N mineralization; the latter response has been reported previously (Sikora and Enriki, 2000). Thus, a challenge remains to appropriately correlating organic N mineralization values obtained from incubation studies with actual plant responses in the field.

Nitrogen removal in harvested biomass decreased linearly as the percentage of N supplied by MBS increased. This was primarily a function of yield, which followed the same pattern. Phosphorus removal response was less consistent. It generally decreased going from the zero to 33% MBS treatment, probably because of a decrease in total P applied (60 to 43 kg P ha^{-1}, respectively) and also a decrease in availability of P to the plant. Thereafter, the general pattern of response was increasing P removal with increasing percentage of N supplied by MBS and was associated with greater tissue P concentration and greater amounts of P applied. The MBS treatment for which removal most closely approximated application was the 33% level applied to Merkeron. In that treatment application averaged 43 kg ha^{-1} over the 2 yr and removal averaged 39 kg ha^{-1}. The gap between application and removal increased rapidly as percentage N applied in MBS increased above 33%.

Soil total P concentration, WEP, Mehlich-1 P, and total soil C were not different among treatments when MBS increased from 0 to 100. Incorporation of MBS did not have an effect on these responses either. The double rate treatment (2x-MBS surface) had no effect on WEP, Mehlich-1 extractable P, and total soil C; but it did increase total soil P ($P \leq 0.05$). Further, application of MBS caused an increase in total soil P in the Ap horizon by the end of Year 2 compared to Year 1.

Incorporation of MBS had a positive effect on DM yield and N and P removal. When MBS were incorporated, DM yield was not different than the treatment where N was supplied solely as ammonium nitrate fertilizer, and yields of both treatments were greater than when MBS were surfaced applied. The DM yield data suggest that mineralization of MBS is enhanced when MBS are incorporated. This conclusion is supported by litter bag studies described in Chapter 4. Phosphorus removal was increased approximately 15 kg ha^{-1} by incorporation, so there are

potential environmental benefits to MBS incorporation in addition to the agronomic benefits of yield increases. Results of this study showed that incorporation of MBS should be strongly recommended as a management tool when using MBS.

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CHAPTER 4
SEASON AND INCORPORATION EFFECTS ON NITROGEN MINERALIZATION AND DECOMPOSITION OF MUNICIPAL BIOSOLIDS IN FIELD STUDIES IN FLORIDA

Introduction

The focus of handling agricultural and municipal wastes has shifted from disposal to utilization (Muchovej and Obreza, 2004). According to EPA (1999), municipal biosolids (MBS) are a "nutrient-rich organic by-product of municipal wastewater treatment that can be beneficially recycled via land application". Thus, in recent years research has focused on the use of biosolids as an alternative to expensive commercial fertilizers for crop production.

Although various factors must be considered when using MBS (nutrient concentration and availability, waste water treatment process, environmental risk for nutrient loss), for agronomic purposes, the nutrient of greatest interest is N. Plant-available N (PAN) in MBS is a function of the initial inorganic N concentration (NH_4-N and NO_3-N) and the proportion of organic N that will mineralize over a specific period of time (Gilmour and Skinner, 1999). Significant research effort has been devoted to accurately estimating the organic N mineralization rates of organic amendments (Sommers et al., 1981; Hanselman et al., 2004).

Several laboratory techniques for determining N mineralization rates have been reported in the literature (Hanselman et al., 2004). However, there is uncertainty regarding the extrapolation of laboratory N mineralization data to field conditions due to dynamic and site-specific factors like drying and rewetting events and temperature changes (Gilmour and Skinner, 1999; Hanselman et al., 2004). The same biosolids incubated in different soils can decompose and mineralize N at different rates (Terry et

al., 1978; Ajwa and Tabatabai, 1994), and incubation conditions such as soil temperature and moisture status can markedly change the rate of decomposition and net N mineralization (Terry et al., 1978; Clark and Gilmour, 1983; Gilmour and Clark, 1988). Also, application method of MBS (incorporated versus surface applied) can influence the dominant forms of N during an incubation period (NH_4-N vs. NO_3-N), and rates of mineralization of organic N (He et al., 2003). This has lead to interest in measuring N mineralization rates and PAN in production fields with in situ incubation devices (Tester, 1989; Hanselman et al., 2004).

Estimating organic N mineralization rates is an important step in guiding application of MBS for crop production purposes. As shown in the experiment conducted at the Range Cattle Research and Education Center (RCREC), Ona (Chapter 3), when MBS were applied on a total-N basis without consideration of the mineralization of organic N, dry matter (DM) yields decreased linearly for two elephantgrass (*Pennisetum purpureum* Schum.) entries. Also, results from the same experiments suggested than when MBS were incorporated on a total-N basis, incorporation of MBS resulted in greater DM yields than surface application and similar yields to when ammonium nitrate fertilizer was used.

Because of the importance of MBS organic N mineralization to crop response and the specific responses to use of MBS observed in Chapter 3, field mineralization studies are needed. Thus, the objectives of this research were to 1) quantify the DM decomposition and organic N mineralization rates of the Ocala MBS in polyester litter bags incubated in the field, and 2) evaluate the effect of season and application method on MBS patterns of decomposition and organic N mineralization. Two experiments were

conducted to address the objectives. Experiment 1 evaluated the effect of season of application, and Experiment 2 evaluated the effect of method of application (incorporated vs. surfaced applied) on decomposition and organic N mineralization patterns of the Ocala MBS.

Materials and Methods

Experiment 1

Experimental Site

Experiment 1 was conducted at the RCREC at Ona, FL (27° 26' N and 81° 55' W) during 2007 and 2008. The soil at the experimental site is classified as a Spodosol (sandy, siliceous, hyperthermic Typic Alaquod from the Ona series). Native topsoil (0 to 15 cm) has an 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.

Treatments and Design

Polyester bags containing MBS were field incubated on the soil surface in experimental plots that were established within existing plots of elephantgrass PI 300086. Treatments were the season of the year when bags were placed in the field (spring vs. summer). Dates of treatment application were 20 May and 3 Aug. 2007 and 4 Apr. and 8 Aug. 2008. Spring dates were selected to represent the periods of typical drought conditions experienced in that season, and summer dates to represent high rainfall periods characteristic of that season (Figure 4-1). In addition, the dates corresponded to the initial and second application of the MBS to the plots in the study reported in Chapter 3. There were four replicates of each treatment, arranged in a completely randomized design.

The MBS were obtained from Water Reclamation Facility #3 of the City of Ocala, FL. The material is the result of blending anaerobically digested sludge and undigested

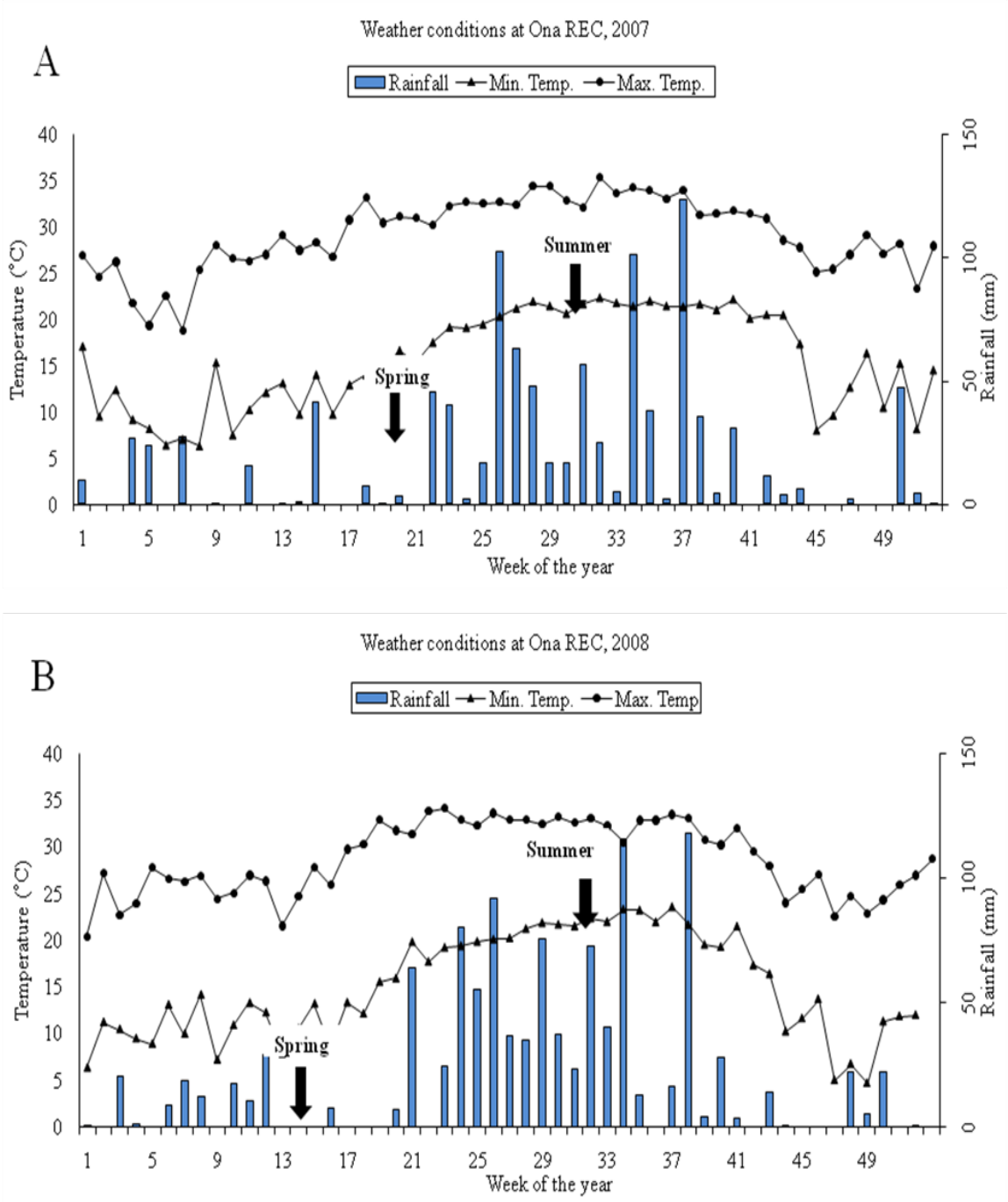

Figure 4-1. Weekly total precipitation and average weekly maximum and minimum air temperatures at the Range Cattle Research and Education Center at Ona, FL during 2007 (A) and 2008 (B). Arrows indicate when field incubation started. Spring and summer dates were 20 May and 3 Aug. 2007 and 4 Apr. and 8 Aug. 2008, respectively.

waste-activated sludge. After mixing, it is fed to a belt press and ultimately sent to an indirect drier system where it achieves an average 950 g DM kg^{-1} product (Hicks et al., 2007). The end product is classified as AA biosolids and is marketed as a slow-release soil amendment. The material for each application date represented a different batch from the reclamation facility (Table 4-1).

Table 4-1. Composition of municipal biosolids used in Experiment 1.

	Date of application			
	20 May 2007	3 Aug. 2007	4 Apr 2008	8 Aug 2008
pH	6.3	6.4	6.5	6.6
	---------- g kg^{-1} ----------			
Dry matter	950	990	980	980
Total C	352	373	338	340
Total N	58.3	65.0	54.0	54.0
Organic N	57.3	64.3	53.0	52.6
C:N ratio	6.1	5.8	6.2	6.3
P	20	21	20	23
K	1.6	1.6	1.5	1.7
Ca	1.9	3.1	1.5	2.2
Mg	1.8	3.5	2.4	2.6
Na	0.6	0.7	0.5	0.7
	---------- mg kg^{-1} ----------			
NH_4-N	881	618	966	1317
NO_3-N	37	9	37	28
Fe	17600	21450	18950	37800
Zn	709	871	525	873
Cu	197	184	127	193
Mn	127	117	62	100
Mo	14	16	11	17

Polyester bags of 75-µm mesh measuring 15 × 20 cm were used for the incubation study. These bags have been used in previous studies of grass litter decomposition (Dubeux et al., 2006). Based on an application rate of 350 kg N ha^{-1} yr^{-1} from MBS, 25 g of MBS were placed in each bag. The bags were collected at 10 and 9

incubation times during spring and summer, respectively. Collection dates were 3, 6, 12, 24, 48, 96, 144, 192, 240, and 336 d (336-d collection was last one for spring, 240-d collection was the last for summer) after placement in the field. These incubations times were chosen to provide information on the responses measured over the course of nearly 1 yr after placement in the field. During the incubation period, the bags were turned weekly to prevent roots from penetrating them.

Three complete sets of bags (sampling units) were included in each experimental unit. Thus, at each collection date there were a total of 12 observations (four replicates times three bags per replicate) per treatment. Also, at each collection date, two control (empty) bags per treatment were collected across the four replicates. The areas where bags were placed were caged with kennel wire to prevent disturbance from wildlife living in the surrounding area.

Following collection, the bags were immediately dried in a forced-air oven at 60°C for 72 h. Prior to 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 collection date in a given experimental unit were combined in a Whirl-pak bag and stored at room temperature awaiting subsequent analysis.

Chemical Analysis

Before laboratory analysis, particle size homogenization of the collected MBS samples was achieved by grinding the samples using a mortar and pestle. Total C was determined by dry combustion (Nelson and Sommers, 1996) on a Thermo Flash EA1112-NC elemental analyzer. Total N was determined by digesting the samples 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). Extractable NH_4-N and NO_3-N were determined by shaking 2.5 g of biosolids with 25 ml of 1 *M* KCl for 1 h and analyzing concentrations of NH_4-N and NO_3-N with a Rapid Flow Analyzer (RFA, method A303-S020, Alpkem Inc., 1989). Total P was determined by ashing and digesting 0.3 g of air-dried biosolids according to Anderson (1976). Analysis of total P was done using the ascorbic acid method by Murphy and Riley (1962).

Response variables calculated were biosolids decomposition rate (BDR, Equation 1) and organic N mineralization (NMR, Equation 2) rate. They were calculated using the following formulas:

(Equation 1)

$$BDR = (Initial\ weight_{time\ 0.} - Final\ weight_{time\ x}) / (Initial\ weight_{time\ 0}) \times 100$$

with the final weights corrected by the weights of the control bags.

(Equation 2)

$$Organic\ NMR = (Organic\ N_{time\ 0} - Organic\ N_{time\ x}) / (Organic\ N_{time\ 0)} \times 100$$

where *Organic N* = Total N - (NH_4-N + NO_3-N).

The approach was based on the work of He et al. (2000), who evaluated several techniques to measure organic N mineralization and transformations from MBS in a field incubation study. The authors concluded that the organic N decrease method was a useful method for estimating N mineralization potential of compost and biosolids under field conditions because it estimated total N mineralization including mineralized N lost through leaching, volatilization, and denitrification. They did point out that one limitation of this method is that it may not indicate the amount of mineralized N actually available to plants, whereas the incubation-extraction method measures the mineral N (NH_4-N +

NO_3-N) that remains in the soil. Nevertheless, they pointed out that the incubation-extraction method accounted only for 270 g kg^{-1} of the total N mineralized estimated by the organic N decrease method (He et al., 2000)

The data were subjected to repeated measures analysis using mixed model procedures (Littell et al., 1998). Collection date was used in the repeated statement with correlation modeled via replicates within season. All means reported are least squares means. Differences were considered significant if $P \leq 0.05$.

The non-linear mixed models procedure (PROC NLMIXED) (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 (Epstein et al., 1978; Weider and Lang, 1982; Parker and Sommers, 1983; Chae and Tabatai 1986):

$$N_{min} = N_o\ [1\text{-}exp\ (\text{-}kt)] \qquad \text{(Equation 3)}$$

where N_{min} = amount of N mineralized at a specific time (t); N_o = constant that represents mineralizable organic N (asymptote); and k = rate constant. Municipal biosolids DM remaining was described as follows (Gilmour and Gilmour, 1980; Weider and Lang, 1982):

$$X = a + (1000 - a)\ exp\ (\text{-}kt) \qquad \text{(Equation 4)}$$

Where X = amount of MBS mass remaining at a specific time (t). a = constant.

Experiment 2

Experiment 2 was conducted at the Beef Research Unit (BRU) near Gainesville, FL (29° 43’ N and 82° 16’ W). The soil at this location is classified as an Entisol (thermic, coated Aquic Quartzipsamments from the Chipley series). Initial

characterization of the topsoil (0 to 15 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.

The treatments were MBS incorporated to a depth of 5 cm below the soil surface and surface-applied. The same polyester bag technique described in Experiment 1 was used in the same experimental design was the same as that described in Experiment 1. To prevent contamination of roots inside the bags, the entire area used for the study was completely tilled before bags placement. Thus, there was no active crop growing in the area during the time of the experiment, and all plant seedlings were removed by hand weeding every other week. This approach was chosen because an incorporation treatment attempted at the RCREC failed due to root penetration and contamination of the bags by plant roots.

The polyester bags were placed in the field on 15 Aug. 2008. There were 7 collection dates: 3, 6, 12, 24, 48, 96, and 144 d after placement in the field. The Ocala MBS corresponding to the last application date from Experiment 1 (8 Aug. 2008; Table 4-1) was used for this experiment. Weather conditions during the time the experiment was conducted are shown in Figure 4-2. Chemical analysis and data analysis were conducted following the same procedures described in Experiment 1.

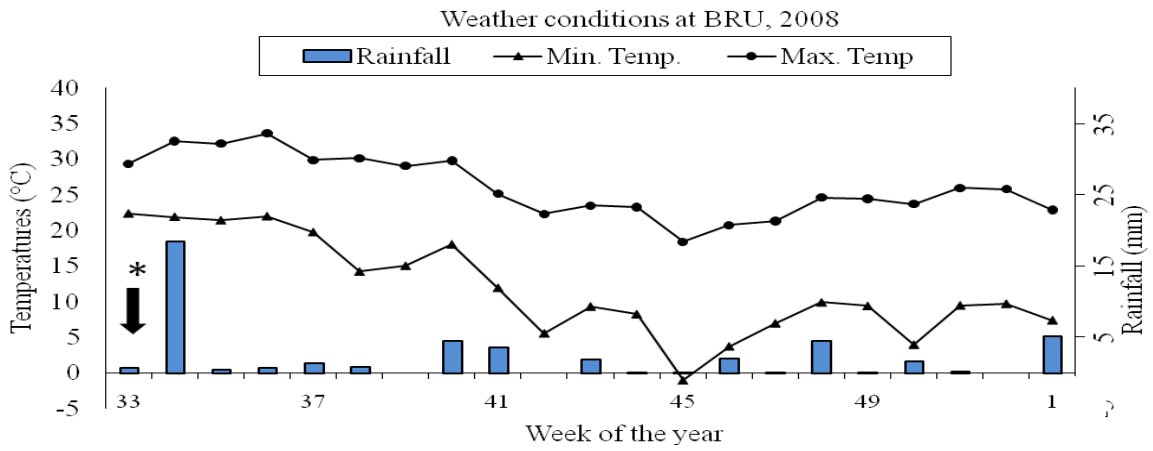

Figure 4-2. Weekly total precipitation and average weekly maximum and minimum air temperatures at the Beef Research Unit near Gainesville, FL. Arrow indicates the week of the year when field incubation started. Starting date was 15 Aug. 2008 (*).

Results and Discussion

Experiment 1

Organic N Mineralization

Analysis of season effect on N mineralization indicated year × season interaction ($P < 0.001$); therefore, data were analyzed by year. Within year, season × collection date interaction was significant ($P < 0.001$); thus, data were analyzed by collection date.

The amount of organic N mineralized followed a generally similar pattern of response during 2007 and 2008, with means greater for summer versus spring application except at the beginning and end of the 2008 incubation period (Table 4-2). When data were analyzed by collection date, mineralization of N was greater for all dates in summer versus spring 2007, and in 2008 there were differences favoring summer from Day 12 through 96. In 2007, the amount of organic N mineralized after 250 d in the field was greater ($P \leq 0.05$) for summer versus spring, 374 and 289 g kg^{-1}, respectively. In contrast,

after 196 d in 2008 the total amount of organic N mineralized was not different for the two seasons of initiation (average of 424 g kg^{-1}).

Although statistical differences were found when data were analyzed by collection date within year, generalization of the overall mineralization process based on point in time estimates of the differences between treatments may not be representative, and thus subject to bias. Sommers et al. (1981) indicated that the first order kinetics model described appropriately C and N mineralization. Therefore, generalizations about treatment responses were made based on the first order kinetics equations fitted to treatments (Figure 4-3).

Table 4-2. Organic N mineralization from municipal biosolids incubated in the field following application at the Range Cattle Research and Education Center in spring and summer 2007 and 2008.

Days in the field	2007			2008		
	Spring	Summer	SE	Spring	Summer	SE
d	---------- g kg^{-1} ----------			---------- g kg^{-1} ----------		
3	5.0 a[†]	102 b	11.4	104 a	97 a	14.6
6	2.3 a	202 b	11.4	116 a	115 a	14.6
12	63 a	218 b	11.4	90 a	228 b	14.6
24	168 a	281 b	11.4	125 a	261 b	14.6
48	221 a	349 b	11.4	194 a	301 b	15.7
96	268 a	347 b	11.4	332 a	379 b	14.6
144	289 a	345 b	11.4	400 a	366 b	14.6
196	313 a	377 b	11.4	420 a	427 a	14.6
250	289 a	374 b	11.4	428	ND[‡]	
336	320	ND		ND	ND	
Mean	180	288		223	272	

[†]Season means within a row and year followed by different letters are different ($P \leq 0.05$).

[‡]ND = not determined. Bags not placed in the field (Summer 2007 and 2008) or not collected yet (Spring 2008).

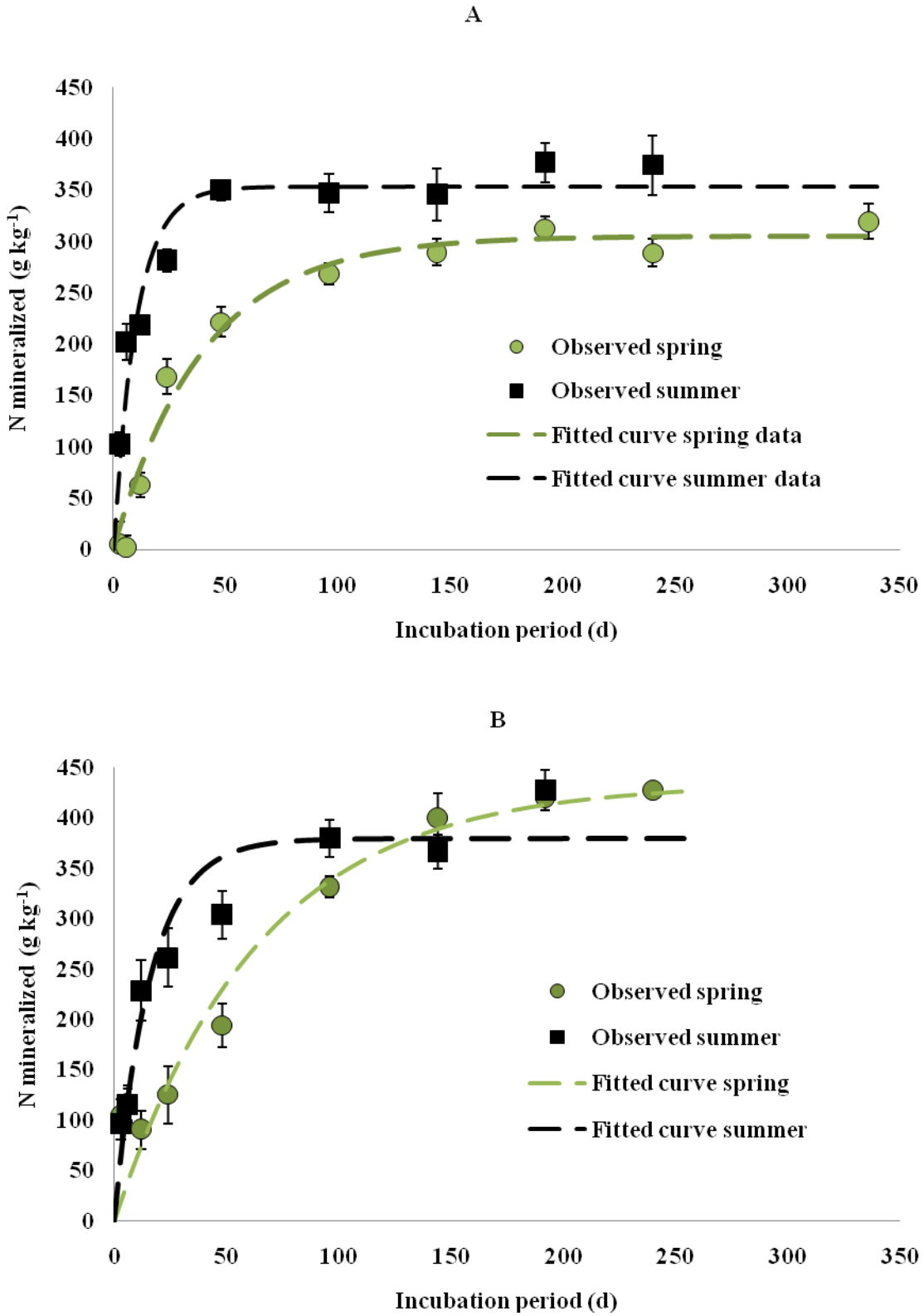

Figure 4-3. Effect of season of application of municipal biosolids on organic N mineralization patterns in 2007 (A) and 2008 (B).

The maximum amounts of organic N mineralized estimated by the first order kinetics models were 305 and 434 for spring 2007 and 2008, and 353 and 380 g kg^{-1} for summer 2007 and 2008 (Table 4-3).In 2007 the total organic N mineralized in spring did not reached the total amount mineralized when MBS were applied in summer, contrary to what occurred in 2008. Since MBS incubated at each season corresponded to different collection batches from the wastewater treatment plant, initial differences in chemical composition may explain this response.

Table 4-3. Regression coefficients for organic N mineralization estimated using NLMIXED procedures.

Season	Year			
	2007		2008	
	N_0	k	N_0	k
	-- g kg^{-1} --	-- d^{-1} --	-- g kg^{-1} --	-- d^{-1} --
Spring†	305	0.025	434	0.0156
Summer	353	0.0973	380	0.0628
P - value	0.0003	<0.0001	0.0711	0.0002

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

Estimates of N mineralization from organic sources vary widely due to factors such as initial chemical and physical characteristics of material, environmental conditions, and the technique used for measurements (Tester et al., 1977, Sommers et al., 1981, Hanselman et al., 2004). Sabey et al. (1975) reported mineralization of an anaerobically digested sludge of 360 to 410 g kg^{-1}, with no difference in mineralization due to different application rates. However, significantly lower mineralization rates (23 to 42 g kg^{-1} of initial organic N) have been found in some studies using both anaerobically digested and activated sludges (Premi and Cornfield, 1971). Sommers et al. (1981) indicated that in general the amount of mineralizable N in sewage sludges is proportional

to the total organic N. Further, they suggested that sewage sludge treatments (e.g., anaerobic digestion, composting) reduce organic N levels and thus, amounts of potentially mineralizable N. Expected organic N mineralization for sludge application rates for agronomic crops are: 250 g kg^{-1} for raw and primary sludges, 400 g kg^{-1} for waste-activated sludges, 150 g kg^{-1} for anaerobically digested sludges, and 80 g kg^{-1} for composted sludges (Sommers et al., 1981; USEPA, 1993). The Ocala MBS used in this experiment are the result of blending anaerobically digested sludge and undigested waste-activated sludge.

When MBS were applied in summer, organic N mineralization leveled off approximately 50 d after placement in the field in both years (Figure 4-3). When MBS were applied in spring, N mineralization reached its maximum at approximately 150 d in 2007 and 250 d in 2008. In general, as temperature and moisture increase, microbial activity increases. Soil microorganisms are primarily mesophyllic and prefer moderate temperatures, with optimum activity between 25 and 37˚C (Jarvis et al., 1996). During the first 24 d of incubation, total rainfall and average temperatures (Table 4-4) were similar when MBS were applied in summer of 2007 and 2008 (average daily temperature was 27°C for both years and total rainfall of 140 and 195 mm for 2007 and 2008, respectively). Thus, organic N mineralization followed similar patterns during this 24-d period in both years (Figure 4-3) with total amounts of N mineralized of 281 and 261 g kg^{-1} for 2007 and 2008, respectively. In contrast, when MBS were applied in spring, average daily temperatures during the first 24 d were 24°C in 2007 and 20°C in 2008, and total rainfall was 88 and 8 mm for 2007 and 2008, respectively (Table 4-4). Further the lowest temperature recorded during the first 24 d was 4°C in spring 2008 versus a low of

Table 4-4. Weather conditions during 24 and 48 d following application of municipal biosolids at the Range Cattle Research and Education Center, Ona, FL in 2007 and 2008.

Weather item	0 to 24 d				25 to 48 d			
	2007		2008		2007		2008	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
Rainfall (mm)	88	140	8	195	182	173	71	136
Temperature (°C)								
Avg. low	18	22	12	23	20	22	16	22
Avg. high	31	34	28	32	33	33	32	33
Avg. daily	24	27	20	27	26	26	24	27
Period lowest	12	20	4	21	17	20	8	16
Period highest	35	37	32	35	35	35	34	36

12°C in 2007 compared to period lows of 20 and 21°C during summer of the 2 yr. These weather differences likely explain the differences between seasons early in the incubation periods, and may explain the differences in mineralization during the first 24 d between spring 2007 (168 g kg^{-1}) and 2008 (125 g kg^{-1}).

From Day 25 to 48 of incubation for spring, rainfall was more than twice as great in 2007 (182 mm) than 2008 (71 mm). Although average daily temperatures during this period were comparable (26°C in 2007 and 24°C in 2008), the lowest temperature for this period was recorded in 2008 (8°C) compared to 2007 (17°C). These factors may explain in part why mineralization during Days 25 to 48 of 2008 (221 versus 194 g kg^{-1} for 2007 and 2008, respectively) did not compensate for the slower mineralization during Days 0 through 24 of 2008. From Day 25 to 48 of incubation time for summer, average temperatures were nearly the same in 2007 (26°C) and 2008 (27°C), but rainfall was 37 mm greater in 2007 than in 2008, possibly explaining greater organic N mineralization from Day 25 to 48 in 2007 versus 2008 (68.2 versus 43.2 g kg^{-1}, respectively). The total amounts of organic N mineralized after 48 d of summer incubation in the field were 349 in 2007 and 301 in 2008.

Seasonal effects on organic N mineralized (N_{min}) were also evaluated based on estimates of the difference of predicted values from the models for the two seasons within a year (Figure 4-4). The greatest difference in N mineralized between spring and summer occurred after the first 24 d of incubation in both years (approximately 170 g kg^{-1}) ($P \leq 0.05$). After 96 d of incubation, the difference between seasons in organic N mineralized leveled off in 2007 at 50 g kg^{-1}, whereas after 96 d in 2008 N mineralization was not different ($P > 0.05$) for summer versus spring.

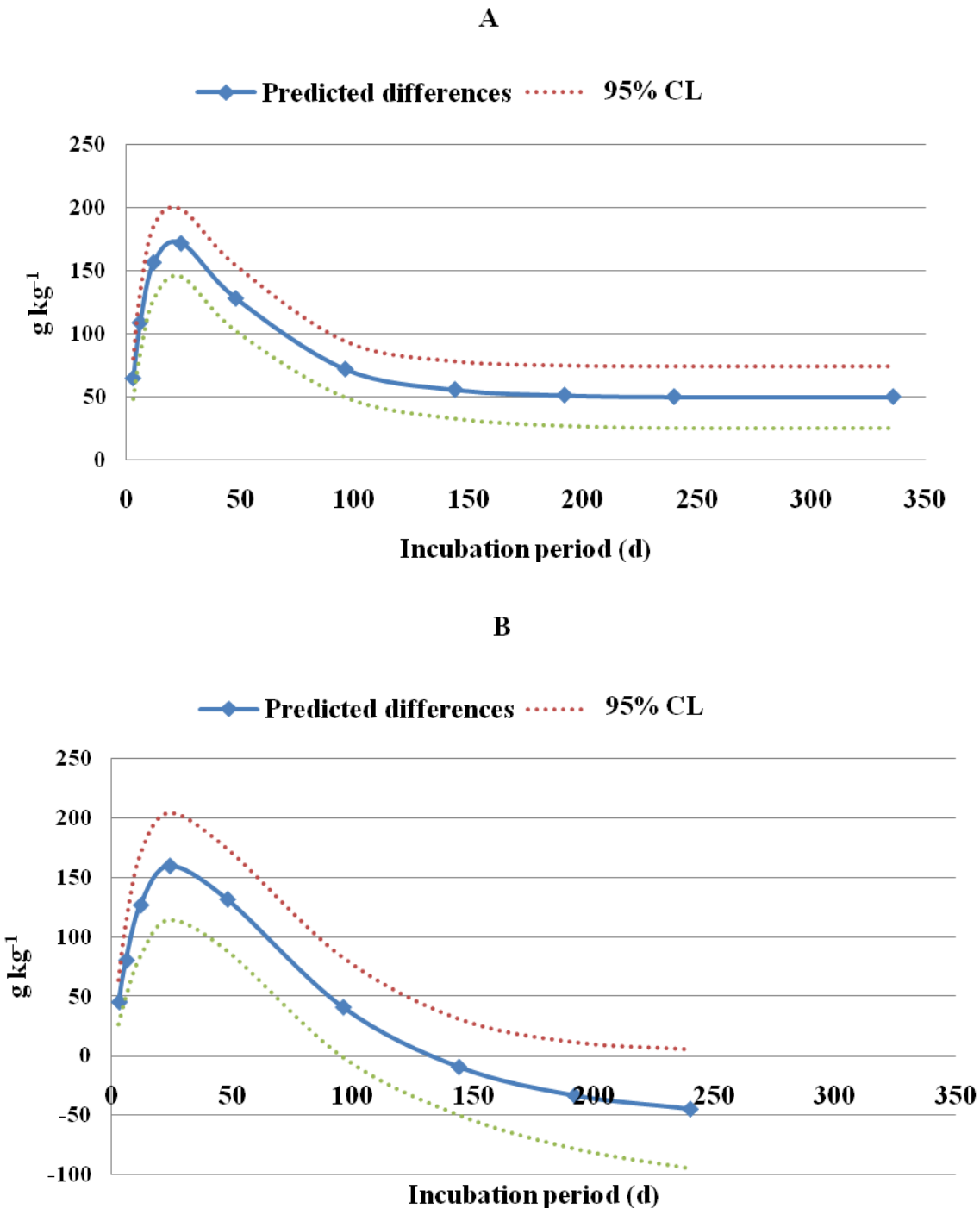

Figure 4-4. Predicted difference between summer and spring (summer minus spring) N mineralization using the fitted curves in 2007 (A) and 2008 (B).

Biosolids Dry Matter Decomposition

Decomposition of organic matter, expressed as percentage MBS mass remaining (Figure 4-5), followed the same patterns as organic N mineralization when MBS were applied in spring and summer in 2007. In 2008, there was a lag period during the first 48 d when MBS was applied in spring, which also occurred when measuring organic N mineralization. The response can be attributed to lower rainfall (86 mm lower) and lower air temperatures during the first 48 d of incubation when MBS were applied in spring versus summer (period lowest temperature was 4°C for spring versus 16°C for summer in 2008). Thus, decomposition of MBS in spring 2008 approached linearity, and the rate constant (*k* value) in Equation 4 approached zero.

In a laboratory study conducted for 32 wk with soils from the northern hardwood forest in the Great Lakes region, MacDonald et al. (1995) reported that microbial respiration (CO_2 release) and net N mineralization nearly doubled when temperatures increased from 15 to 25°C. Sommers et al. (1981) measured biosolids decomposition as the amount of CO_2 evolved. They reported a range of 0.33 to 3.42 for the C:N ratio of the materials tested in their study. Further, they indicated that from 150 to 610 g kg^{-1} of the sludge organic C was decomposed to CO_2 during a 16-wk incubation period. Decomposition of organic matter (microbial respiration, CO_2 evolution) is expected to respond to the same conditions that enhance organic N mineralization, and the mass loss will approximate the pattern of organic N mineralization. Others have recommended the use of the first order kinetic model to describe both responses (Sommers et al., 1981; MacDonald et al., 1995). At the end of 1 yr of MBS incubation under field conditions at the Indian River Research and Education Center, Fort Pierce, FL, He et al. (2000) reported total mass loss of 250 g kg^{-1} of dry weight from biosolids with an initial C:N ratio similar to the Ocala MBS (~ 6).

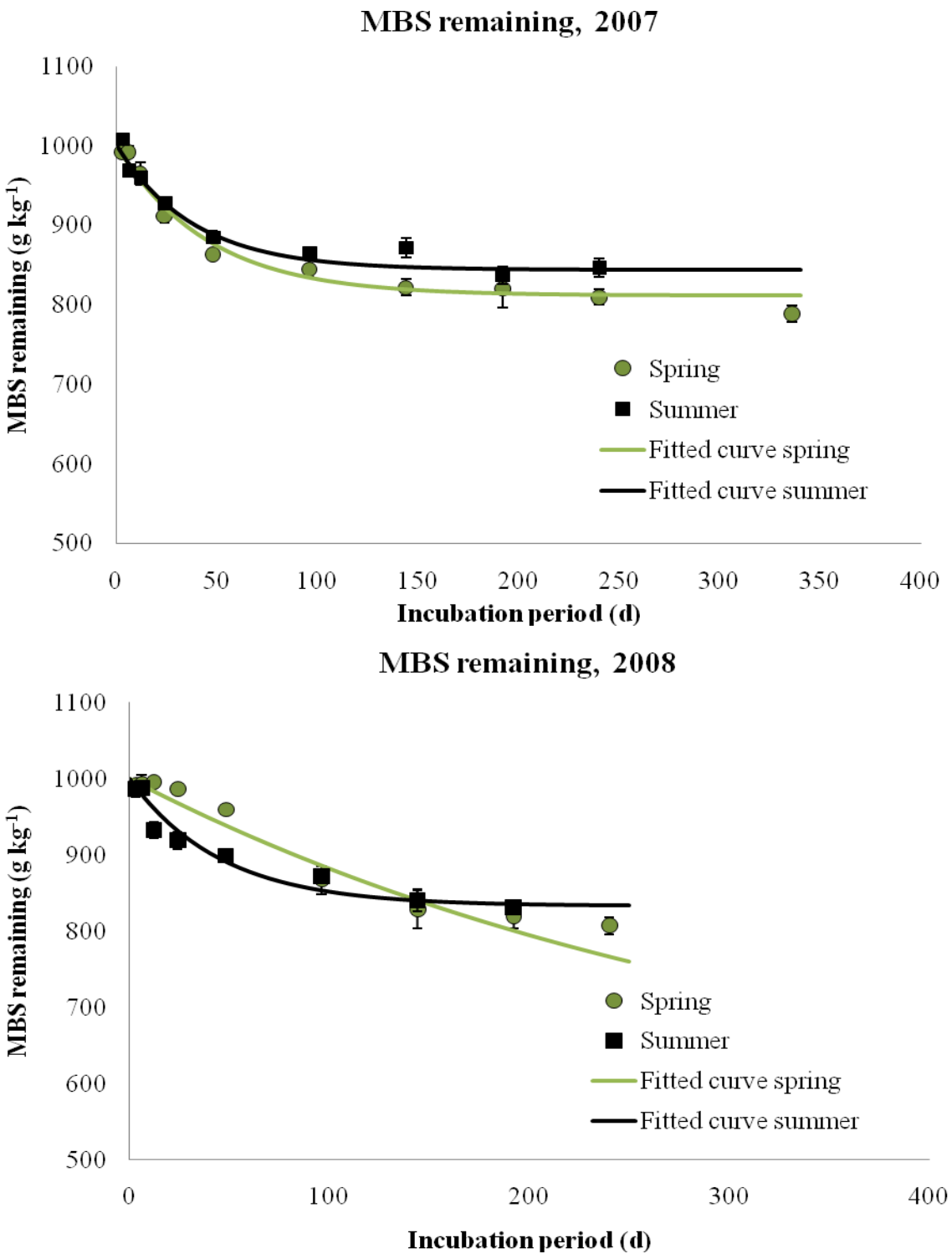

Figure 4-5. Municipal biosolids (MBS) mass remaining during incubation periods that started in spring and summer 2007 and 2008 at the Range Cattle Research and Education Center, Ona, FL.

Experiment 2

Effect of incorporation on organic N mineralization patterns: There was no treatment (incorporation vs. surface-applied) × collection date interaction ($P > 0.05$) for N mineralization, and organic N mineralization was greater when MBS were incorporated versus surface-applied. Mean organic N mineralization across sampling dates when MBS were incorporated was 273 versus 174 g kg^{-1} for surface application ($P \leq 0.05$) (Table 4-5).

Table 4-5. Organic N mineralization from surface-applied or soil-incorporated municipal biosolids incubated in the field at the Beef Research Unit in 2008.

Days in the field	Incorporated	Surface-applied	SE
--d--	---------- g kg^{-1} ----------		
3	70 a[†]	0 b	11.4
6	149 a	19 b	11.4
12	274 a	190 b	12.3
24	302 a	192 b	11.4
48	333 a	240 b	11.4
96	380 a	282 b	12.3
144	404 a	312 b	11.4
Mean	273 a	173 b	4.2

[†]Means within a row followed by different letters are different ($P \leq 0.05$).

The first order kinetics equation ($N_{min} = N_o\ [1\text{-}exp(\text{-}kt)]$) predicted greater maximum organic N mineralization when MBS were incorporated (374 g kg^{-1}) versus surface-applied (304 g kg^{-1}) ($P \leq 0.05$). Organic N mineralization followed the same pattern for both treatments (Figure 4-6). Organic N mineralized leveled off at approximately 50 d after placement in the field, similar to the response during summer in Experiment 1.

Because mineralization of organic N results in transformation of organic N to plant-available forms (NH_4-N and NO_3-N), most of the methods to calculate N mineralization have focused on monitoring the increase of recoverable quantities of NH_4-N and NO_3-N. He et al. (2000) described organic mineralization of N, measured as recovered NH_4-N and NO_3-N, from a

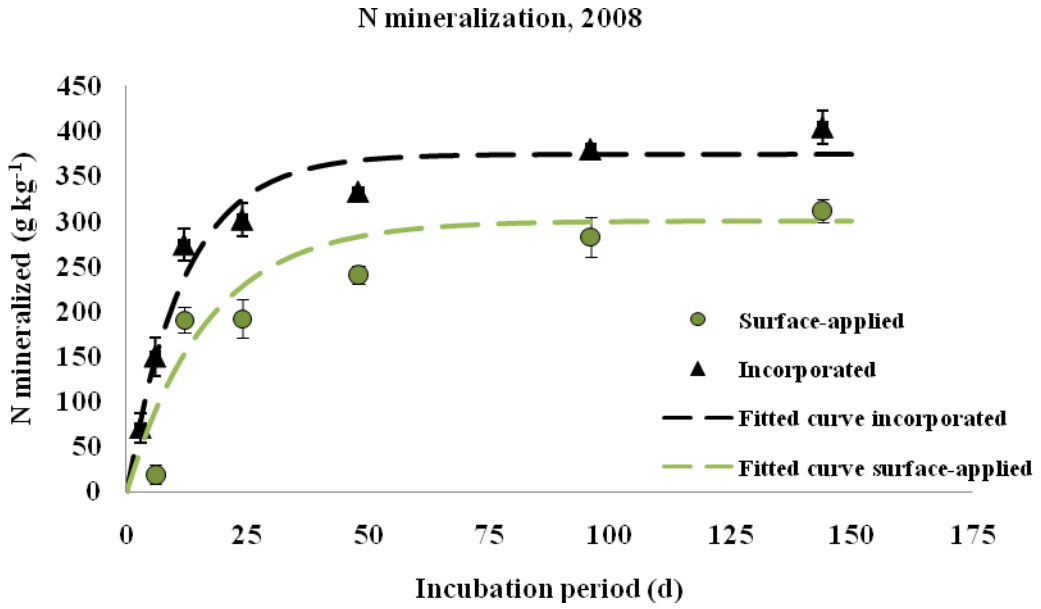

Figure 4-6. Effect of placement of municipal biosolids on N mineralization during 2008 at the Beef Research Unit, Gainesville, FL.

pelletized commercial biosolids with similar chemical and physical characteristics to the Ocala MBS. The biosolids were incubated in the field within PVC columns in Florida. Nitrogen mineralization leveled off after 90 d of field incubation, but the authors pointed out that measuring organic N mineralization using recovery of NH_4-N and NO_3-N accounted for only 280 g kg^{-1} compared to when using the organic decrease method. In another study, He et al. (2003) evaluated the effect of incorporation on mineralization. The NH_4-N concentration in the soil was greater when MBS were incorporated than when surface-applied, probably due to reduced NH_3 volatilization and enhanced N mineralization associated with soil incorporation.

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). Data collected in the current experiment suggested that incorporation of MBS increased organic N mineralization by 22% (304 and 374 g mineralized N kg^{-1} for surface-

applied and incorporated biosolids, respectively). Using a similar approach to the one used in our experiment, Adamsen and Sabey (1987) reported that apparent organic mineralization (change in organic N in the treated sample minus the change in organic N in the control divided by the amount of organic N added in the sludge) was 298 and 325 g kg^{-1} for the surface-applied and incorporated treatments, respectively.

When DM yield was measured as an indicator of the effect of incorporation of MBS, elephantgrass DM yield (Chapter 3) in the MBS incorporated treatment was greater than when MBS were surface-applied (22.5 vs. 16.9 Mg ha^{-1} yr^{-1}, respectively). Sommers et al. (1981) reported that incorporation of sewage sludge reduced volatilization losses of NH_4-N from 70 to 90 g kg^{-1} to less than 10 g kg^{-1} when compared to surface-applied. Others have noted similar decreases of NH_3 loss when liquid sewage sludges are incorporated into the soil (King, 1973; Terry et al., 1978). In a field study, Beauchamp et al. (1978) reported losses of 600 g kg^{-1} from surface-applied sludge in 5 d. Houck and Smith (1977) indicated that subsurface injection or incorporation immediately after sludge application reduced volatilization losses of NH_3 in the same manner as from fertilizers. Mechanisms proposed to explain this process are: interaction of NH_4-N with the cation exchange complex of the soil and thereby a reduction in the quantity of NH_4-N in the soil solution subject to volatilization (Sommers et al., 1981; Pierzynski et al., 2000); and favorable conditions for nitrification due to less NH_4-N availability and the acidifying effect from the nitrification process (He et al., 1999).

Decomposition (MBS mass remaining) of incorporated MBS followed the same pattern of response described in the Experiment 1 for summer applied material. In contrast, when MBS were surfaced-applied, decomposition approached linearity (Figure 4-7). Thus, the rate constant

(*k* value) in Equation 4 for spring 2008 at RCEC and surface-applied MBS treatment at BRU approached zero.

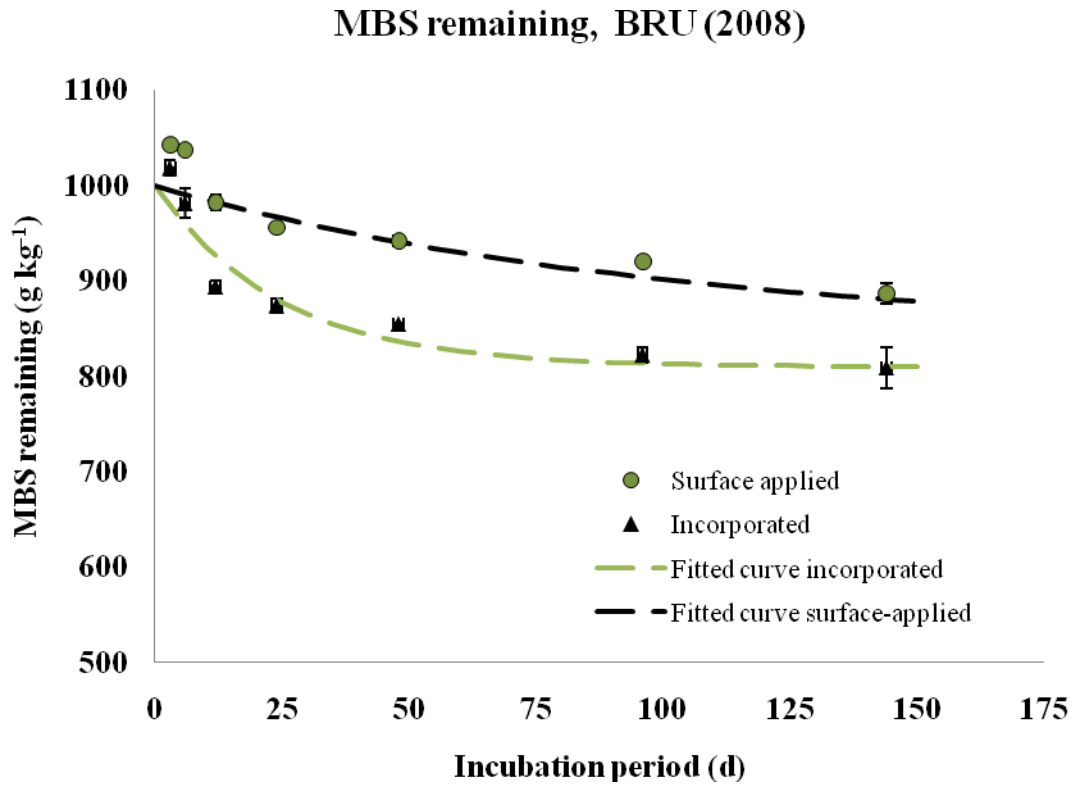

Figure 4-7. Municipal biosolids (MBS) mass remaining during the incubation period that started on 15 Aug. 2008 at the Beef Research Unit (BRU), Gainesville, FL.

Summary and Conclusions

Data suggest that there was a seasonal effect on organic N mineralization from MBS. Lower temperatures and rainfall were associated with lower rates of N mineralization for spring-applied MBS. When MBS were applied in summer, organic N mineralization leveled off approximately 48 d after application in the field during 2007 and 2008 with total amounts of 353 and 380 g kg^{-1}, respectively, as estimated by the models fitted to the data. In contrast, when MBS was applied in spring mineralization leveled off approximately 150 d after incubation in the field

in 2007 and after 200 d in 2008. The greatest differences in organic N mineralization between seasons occurred mainly in the first 50 d after application of MBS in the field.

Incorporation of MBS increased mineralization by 22%. When MBS were incorporated, organic N mineralization was 374 g organic N mineralized kg^{-1} versus 304 g organic N kg^{-1} for surface-applied MBS.

Decomposition of MBS (mass remaining) followed the same pattern of response during spring and summer 2007 and summer 2008 at RCEC, and when MBS were incorporated in summer 2008 at BRU. Approximately 200 g $kg^{-1}$of mass was lost during a nearly 1-yr incubation period at RCREC. In spring 2008 at RCREC and following surface application of MBS at BRU, MBS decomposition was reduced. Less decomposition was associated with limited rainfall and low temperatures during the first 48 d of incubation at RCREC and during the latter part of the incubation period at BRU. Thus, when the models were fit to these data, decomposition of MBS approximated a linear response with k (rate constant from the model) values that approached zero.

Results suggest that season of application of MBS can have great influence on organic N mineralization rates of MBS. This can have particular implications with regard to meeting the nutrient requirements of short-cycle crops especially when grown during winter or early spring. Both the elephantgrass DM yield in Chapter 3, and the litter bag study in Experiment 2 of this chapter support incorporation of MBS in order to maximize availability of nutrients to plants. Dry matter yield and N removal were increased by 33 and 30% when MBS were incorporated vs. surface applied. The litter bag study suggests that incorporation of MBS increased organic N mineralization by 22%.

CHAPTER 5
SUMMARY AND CONCLUSIONS

Elephantgrass (*Pennisetum purpureum* Schum.) represents a potentially important species for cellulosic biomass production in the southeastern USA. Increasing N fertilization has been documented to affect establishment, dry matter (DM) production, and forage nutritive value of elephantgrass. Municipal biosolids (MBS) represent an alternative source of nutrients for biomass production, not only due to lower prices compared to commercial fertilizers, but also because the material already exists as a by-product of municipal waste water treatment plants. However, concerns exist relative to long-term application of MBS, including excessive accumulation of soil P above concentrations needed for optimum crop yield and possible water quality impairment leading to eutrophication.

Despite the potential as a nutrient source for biomass production in renewable energy systems, the use of MBS requires site-specific management strategies to produce high biomass yields while maximizing environmental benefits and lowering production cost. Thus, the objectives of these studies were: 1) to quantify elephantgrass DM yield and N and P concentrations and removals when N is supplied in differing proportions from MBS and ammonium nitrate fertilizer (Chapter 3); 2) to evaluate the effect of soil incorporation vs. surface application of MBS and a double rate of MBS surface applied on elephantgrass DM yield, N and P concentrations, and N and P removal (Chapter 3); 3) to quantify changes in soil C and P concentrations when MBS are applied as a nutrient source (Chapter 3); and 4) to evaluate the effect of incorporation and season of application on patterns of organic N mineralization and DM decomposition of the Ocala MBS using polyester litter bags incubated under field conditions in Florida (Chapter 4).

The objectives were accomplished in experiments conducted in 2007 and 2008 at the Range Cattle Research and Education Center (RCREC), Ona, FL and in an experiment conducted in 2008 at the Beef Research Unit (BRU), Gainesville, FL. Experiment 1 at RCREC was designed to evaluate plant responses and monitor soil C and P concentrations as a function of the proportion of total N applied that came from Ocala class AA MBS vs. ammonium nitrate. Two elephantgrass entries, Chinese Cross and Merkeron, received four different proportions (0, 33, 66, and 100%) of N from MBS and ammonium nitrate. The MBS and ammonium nitrate were surface-applied, to supply a total annual rate of 350 kg N ha^{-1} yr^{-1}. One half of the N was applied at the beginning of the growing season and the other half after the first harvest of each year. In the treatment where all N was from ammonium nitrate, P was supplied using triple superphosphate fertilizer at a rate of 60 kg ha^{-1} yr^{-1}. Two additional treatments, applied to Chinese Cross only, included plots where all N was applied as MBS, but it was soil incorporated (100% MBS-INC) by slightly disturbing the top 5 cm of soil, and plots where MBS were surface applied but at twice the rate of the 100% MBS treatment (2x-MBS surface).

In Experiment 2 at RCREC, 75-µm mesh polyester bags (15 × 20 cm) containing 25 g of Ocala MBS were field incubated on the soil surface in experimental plots that were established within existing plots of elephantgrass PI 300086. The treatments were the season of the year when bags were placed in the field (spring vs. summer). Dates corresponded to the two application dates of MBS and ammonium nitrate in Experiment 1 and also represented periods of typical spring drought and summer high temperature and rainfall. The incubation times (3, 6, 12, 24, 48, 96, 144, 192, 240, and 336 d) provided information on organic N mineralization and DM decomposition of MBS over the course of nearly 1 yr after placement in the field. In Experiment 3 conducted at BRU, polyester bags were used to measure organic N mineralization and DM

decomposition of MBS incorporated to a depth of 5 cm below the soil surface and surface-applied. Methods were the same as for Experiment 2.

Plant Responses

Elephantgrass DM yield decreased linearly as the percentage of N supplied by MBS increased. The decrease in yield from 0 to 100% MBS treatments was 7.4 Mg ha^{-1} for Chinese Cross but only 4.1 Mg ha^{-1} for Merkeron. Nitrogen removal in harvested biomass decreased linearly as the percentage of N supplied from MBS increased, which was primarily a function of DM yield. For treatments receiving MBS, P removal increased as the percent of N supplied by MBS increased. The latter response was associated with greater tissue P concentration and greater amounts of P applied. The difference between P application and removal was least for the 33% MBS treatment and increased rapidly as percentage N supplied by MBS increased above 33%.

Incorporation of MBS had a positive effect on DM yield and N and P removal. When MBS were incorporated, DM yields were not different than the treatment where N was supplied solely as ammonium nitrate fertilizer or from the treatment where MBS were applied at a double rate. Yields of all three of those treatments were greater than when MBS were surfaced applied. The DM yield data suggest that mineralization of MBS is enhanced when MBS are incorporated. This result was supported by the litter bag study described in Chapter 4. Nitrogen and P removals increased approximately 39 and 15 kg ha^{-1} yr^{-1}, respectively, when MBS were incorporated compared to surface-applied; thus, there are potential environmental benefits to MBS incorporation in addition to agronomic benefits through yield increases.

Soil Carbon and Phosphorus

There was no effect of addition of MBS on water extractable P, Mehlich-1 P, total soil P, and total soil C concentrations in the Ap soil horizon over the 2-yr period during which

treatments were imposed. Water extractable P concentrations were greater in 2007 than 2008, and total soil P concentration was greater in 2008 than 2007 for both entries. Results suggest a gradual accumulation of total P in the Ap soil horizon (0 to 20 cm) across treatments when comparing 2007 versus 2008; nevertheless, no differences were detected among treatments. Given that the 100% MBS treatment supplied approximately three times more P than the 33% MBS treatment, lack of response was probably due to natural soil variability and the size of the total P pool.

Incorporation of MBS had no effect on water extractable P, Mehlich-1 P, total soil P, and total soil C concentrations compared to surface application. Total soil P concentration in the treatment where the double rate of MBS was surface-applied was the highest compared to the other treatments, reflecting the much greater amounts of P applied, but total soil P was not different for the 0% MBS treatment compared to the 100% MBS surface applied and 100% MBS incorporated treatments.

Organic N Mineralization and Dry Matter Decomposition Patterns

Lower temperatures and rainfall were associated with lower rates of N mineralization and DM decomposition for spring- than summer-applied MBS (Chapter 4). When MBS were applied in summer, organic N mineralization stabilized approximately 48 d after application during 2007 and 2008, with total N mineralization of 353 and 380 g kg^{-1} in the 2 yr, respectively. In contrast, when MBS were applied in spring, organic N mineralization did not stabilize until approximately 150 d after incubation was initiated in 2007 and 200 d in 2008. Greatest differences in organic N mineralization between seasons occurred mainly in the first 50 d after application of MBS in the field.

Incorporation of MBS increased organic N mineralization by 22% (374 versus 304 g organic N kg^{-1} for soil-incorporated and surface-applied MBS, respectively). A similar

proportional increase was observed for elephantgrass DM yield and N removal when MBS were soil incorporated (Experiment 1).

Implications of the Research

These studies have shown that MBS represent an alternative to commercial fertilizers as sources of nutrients to inorganic fertilizers for bioenergy crop systems. Further, season and method of application of MBS can have a significant impact on rate and extent of organic N mineralization, which will ultimately influence crop response. Although there are agronomic and potential environmental benefits of incorporation of MBS, the extent of environmental risk associated with gradual accumulation of P due to addition of MBS will require longer-term studies and site-specific assessment.

In this research, the yield decrease in elephantgrass with increasing amounts of N from MBS was not as great proportionally as the decrease in plant-available N. Specifically, the 33, 66, and 100% MBS treatments provided 79, 58, and 37% as much plant-available N, respectively, as did the 100% ammonium nitrate treatment. These numbers were based on organic N mineralization estimates from Experiments 2 and 3. Elephantgrass DM yields for the three MBS treatments, expressed as a percentage of that observed for the ammonium nitrate control, were 79, 76, and 70%, respectively, for Chinese Cross, and 100, 88, and 83%, respectively, for Merkeron. The responses suggest a possible interaction of MBS and ammonium nitrate such that values of plant-available N determined by incubating MBS alone may underestimate organic N mineralization when the two N sources were applied together. This issue is of considerable importance because it affects choice of MBS application rates and potential agronomic and environmental impact.

Future Research Needs

Because organic N mineralization can be affected by a number of dynamic and site-specific factors, a challenge remains to accurately establish correlations between organic N mineralization rates determined in field incubation studies and plant responses (DM yields, N and P concentration, and N and P removal). This applies to situations when MBS are applied on a total-N basis or even when N rates from MBS are adjusted to plant-available N, taking into account mineralization values determined in incubation studies. Additionally, the effect on MBS organic N mineralization when MBS is applied in association with inorganic N sources versus when it is applied alone merits further research.

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BIOGRAPHICAL SKETCH

Miguel S. Castillo was born in 1984 in Loja, Ecuador. His interest in agriculture developed at early stages of his life on a family farm. He received a B.S. degree in agricultural science and production (2006) from Zamorano University-Honduras, CA. His first experience at the University of Florida was in spring 2006, when he came to the Everglades Research and Education Center, as a short-term scholar. Miguel joined the Agronomy Department at the University of Florida in spring 2007, where he began his graduate studies under the supervision of Dr. Lynn E. Sollenberger. Miguel completed the master's degree program in summer 2009 and began a Ph.D. program.