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Effect of Digested Effluent of Manure on Soil Nutrient Content and Production of Dwarf Napier Grass in Southern Kyushu, Japan

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Abstract: Animal waste-derived effluents from biogas plants may potentially be used in place of inorganic chemical fertilizers and at the same time, solve the problem of animal waste disposal. Dwarf varieties of late-heading type Napier grass can supply high rates of Digested Effluent of Manure (DEM). The objective of this study was to determine the nutrient content of soils and DEM and to investigate the production of dwarf Napier grass under repeated defoliation employing three treatments comprising three rates of DEM application and a control using chemical fertilizer. The most abundant cations in the DEM solution were ammonium (NH_4^+), followed by potassium (K^+), sodium (Na^+) and calcium (Ca^{2+}), with the Ca^{2+} concentration approximately one-fifth that of NH_4^+. The pH of the soil solution ranged between 6.6-8.0 and tended to increase with increasing DEM application rate which in turn generally reflected the change in Electric Conductivity (EC) of the soil solution. However, the pH and EC of soil near the periphery of treatment plots remained essentially constant throughout the growing season. The most abundant cations in the soil solution were Ca^{2+} which accounted for 62% of cations across all treatments and seasons, followed by magnesium (Mg^{2+}) and Na^+. Cation concentrations tended to increase with increasing DEM application. Sulfate (SO_4^{2-}) accounted for 91% of anions in the soil solution. Indicators of plant growth, including plant height, tiller number, mean tiller weight and plant dry weight, increased with DEM application rate. Crop growth rate was positively and linearly correlated with leaf area index, suggesting that the increase in plant production due to application of DEM or chemical fertilizer is similarly mediated by leaf area development.

Key words: Digested effluent of manure, dwarf napier grass, plant production, mineral element

INTRODUCTION

Beef and dairy farming requires large quantities of high quality herbage production. In integrated livestock farming, the cost of supplying plant nutrients can be reduced by utilizing livestock manure and slurry (Annicchiarico *et al*., 2011). Adequate nutrient supply is essential for achieving the full potential of forage production (Barnes *et al*., 1995). One of the most promising ways to ensure this nutrient supply is to utilize Digested Effluent of Manure (DEM) produced in the operation of biogas plants (Cornes, 2006). Biogas plants use anaerobic bacteria to decompose animal manure and crop residues, resulting in a final residual material known as digested effluent (Cornes, 2006). The digested effluent from biogas plants can potentially be used as liquid fertilizer to replace inorganic chemical fertilizers. By supplying digested effluent in a timely manner, biogas plants may contribute to the maintenance of agricultural environments by reducing foul odors from livestock waste and contamination of nutrients into the underground due to animal wastes (Di *et al*., 1998; Eriksen *et al*., 1999) while, at the same time, reducing the need for livestock farmers to treat or dispose of animals wastes (Cornes, 2006).

The efficiency of nutrient cycling is affected by plant growth, fertilizer input, defoliation intensity and the rate of mineral nutrient loss from the grassland system (McFarland *et al*., 1998). In managing fertilizer application for herbage production, it is essential that high production rates of forage are combined with high rates of fertilizer uptake. Achieving optimal nutrient cycling between soil, plant, animals and fertilizer is particularly important in grassland farming (Whitehead, 2000; Eghball *et al*., 2002).

A wide range of genotypes of Napier grass (*Pennisetum purpureum* Schumach) exist in tropical and subtropical countries (Barnes *et al*., 1995), with normal Napier grass varieties achieving high production rates under intense fertilization (Muhammad *et al*., 1988;

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Sunusi *et al*., 1997, 1999; Wadi *et al*., 2003). Dwarf varieties of late-heading type (dwarf) Napier grass in Thailand and those introduced into southern Kyushu, Japan, from Thailand (Ishii *et al*., 1998; Mukhtar *et al*., 2003; Rengsirikul *et al*., 2011) were found to differ from normal varieties in terms of tiller number, mean tiller weight and percentage leaf blade (Ishii *et al*., 1998). Dwarf Napier grass can be used as a perennial grass in low-altitude areas of southern Kyushu (Utamy *et al*., 2011), resulting in high performance of beef cattle (Ishii *et al*., 2005).

The objective of this study was to determine the effect of DEM on water soluble salts in the soil and the herbage production of dwarf Napier grass under defoliation systems over the course of two years following Napier grass establishment in southern Kyushu.

MATERIALS AND METHODS

Plant culture: Dwarf Napier grass, introduced from Thailand in 1996 (Ishii *et al*., 1998), was cultivated by transplanting rooted tillers at a rate of 2 plants m^{-2} (0.5×1.0 m of spacing) in an experimental field at the University of Miyazaki in 2007 and 2008. Lime (200 g m^{-2}) and fermented cattle manure (600 g m^{-2}) were applied on May 8, 2007, as a basal dressing. Three treatments comprising four applications of DEM at three levels and a control comprising four applications of a compound chemical fertilizer as top dressing were employed in a randomized block design with three replications (blocks).

Application of DEM and chemical fertilizer, chemical analysis of DEM: The DEM, generated by a biogas plant located on the campus of the University of Miyazaki, was heat-sterilized at approximately 60-70°C for 1 h and applied twice at 27 and 63 days after Napier grass establishment and again at 96 and 126 days after establishment, immediately following the 1st and 2nd cuttings, respectively. DEM treatments comprised three levels of application (2.4, 1.2 and 0.6 L m^{-2} per application equivalent to 5.04, 2.52 and 1.26 g m^{-2} Nitrogen (N) per application) which constituted High (H), Medium (M) and Low (L) application rates, respectively. The control involved application of a compound chemical fertilizer at 36 g m^{-2} per application (5.04 g N m^{-2} per application), equivalent to the N rate of the H treatment, on the same days as the DEM applications. Concentrations of major cations and anions in the DEM were determined using an ion analyzer (Model: IA-300, Toa-DKK Co. Ltd., Tokyo, Japan).

Plant collection and sampling: Three plant samples per plot were collected per sampling period after the third and fourth defoliations in 2007 and 2008, respectively. In 2007, plants were cut at 10 cm above ground level for the first cutting and at 15 cm above ground level for the second and third cuttings. In 2008, cutting heights were 20 and 30 cm above ground level for the first and second cuttings, respectively and 35 cm above ground level for the third and fourth cuttings.

After harvest, plants were divided into Leaf Blade (LB), stem with leaf sheath (ST) and dead parts (D) and dried at 70°C for 3-4 days. Indicators of plant growth including plant height, tiller number and leaf area were recorded at each harvest and leaf area was measured using an automatic area meter (AAM-8, Hayashi Denko Co. Ltd., Tokyo, Japan).

Soil and soil solution sampling: Three replicate soil samples were collected three times in May, August and November in both 2007 and 2008, from the periphery of the blocks at 0-5, 15-20, 30-35 and 45-50 cm below the soil surface. Three replicate soil solution samples were collected five times monthly from early June to late October in both years using a porous cup which was set at 1-1.2 m below the soil surface in each plot.

Chemical analysis of soil and soil solution: The pH and Electric Conductivity (EC) of soil and soil solution were measured every month during the experimental period (June-October) using a twin compact pH meter (B-212, Horiba Co. Ltd., Tokyo, Japan) and twin conductivity meter (B-173, Horiba Co. Ltd, Tokyo, Japan). Anion and cation concentrations in the soil solution were determined using an ion analyzer (Model: IA-300, Toa-DKK Co. Ltd., Tokyo, Japan).

Statistical analysis: Data were analyzed in accordance with the randomized block design. The least significant difference between mean values was used to identify statistical differences at the 0.05 level.

RESULTS

DEM ion concentrations: Analysis of the change in concentration of major ions in DEM over the seasons revealed that the concentrations of ammonium (NH_4^+) and other major ions tended to decline after the second application in mid-July 2007 and after the first application in early June 2008. In both years, NH_4^+ was observed to be the most abundant ion, followed by potassium (K^+), sodium (Na^+) and calcium (Ca^{2+}) which had a concentration of approximately 10-20% that of NH_4^+. Phosphoric acid (PO_4^-) was found to be the least abundant ion (Fig. 1).

pH, EC and ion concentrations in soil solution and plot-border soil: Soil pH and EC at different depths near the periphery of the plots were measured at the beginning and the end of the growing seasons in 2007 and 2008 (Fig. 2). In May of 2007, soil pH ranged from 7.4-7.7, due to the application of lime to adjust pH at establishment. In both years, pH returned to the normal range of 6.6-7.4 after application of DEM. Soil EC tended to decrease with soil depth for each sampling date in both years (Fig. 2).

Seasonal changes in cation and anion concentrations of the plot-border soil at four depths in 2007 and 2008 are shown in Fig. 3. The concentrations of almost all cations decreased with soil depth across all seasons in both years, with the most abundant cation being Ca^{2+}, followed by K^+, Na^+ and Mg^{2+} and NH_4^+ being the least abundant. Soil anion concentration tended to increase with soil depth and over the course of the seasons. With the exception of the most abundant anions in the top soil layer-nitrate (NO_3^-) and chloride (Cl^-) immediately following establishment on May 9, 2007, the trend for anions was the opposite of that observed for cations. This trend suggests that leached anions accumulate in the deepest clay soil layer above the bedrock. The most

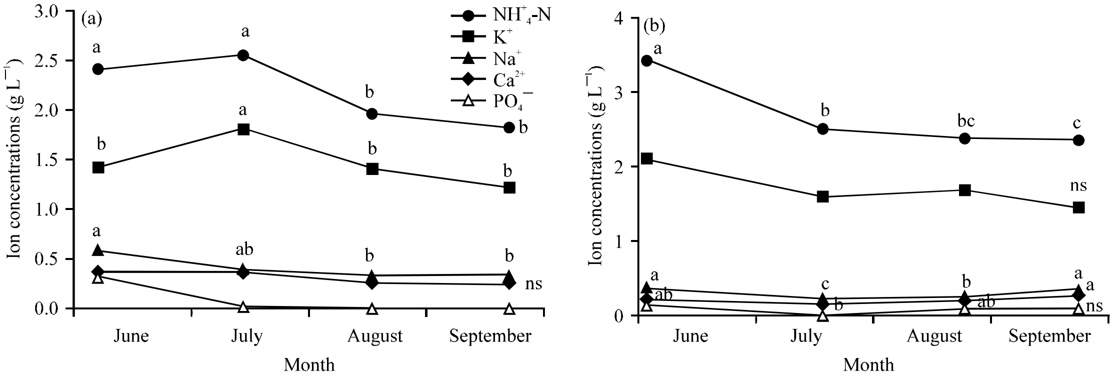

Fig. 1(a-b): Change in ion content of digested effluent of manure (DEM) at each application point in (a) 2007 and (b) 2008. Symbols with different letters denote significant differences among dates at the $p<0.05$ level

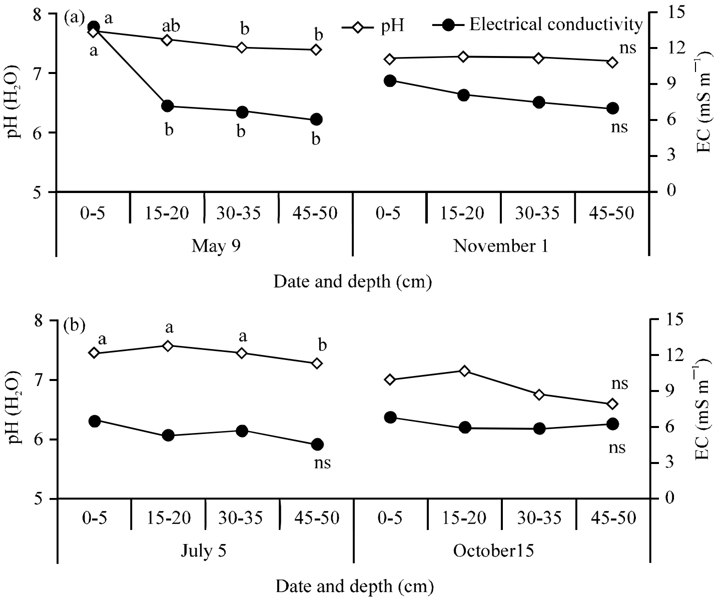

Fig. 2(a-b): Changes in pH and electric conductivity of soil at different depths in the border area between treatment plots in (a) 2007 and (b) 2008. Symbols with different letters denote significant differences among samples taken at different depths on the same date at the $p<0.05$ level

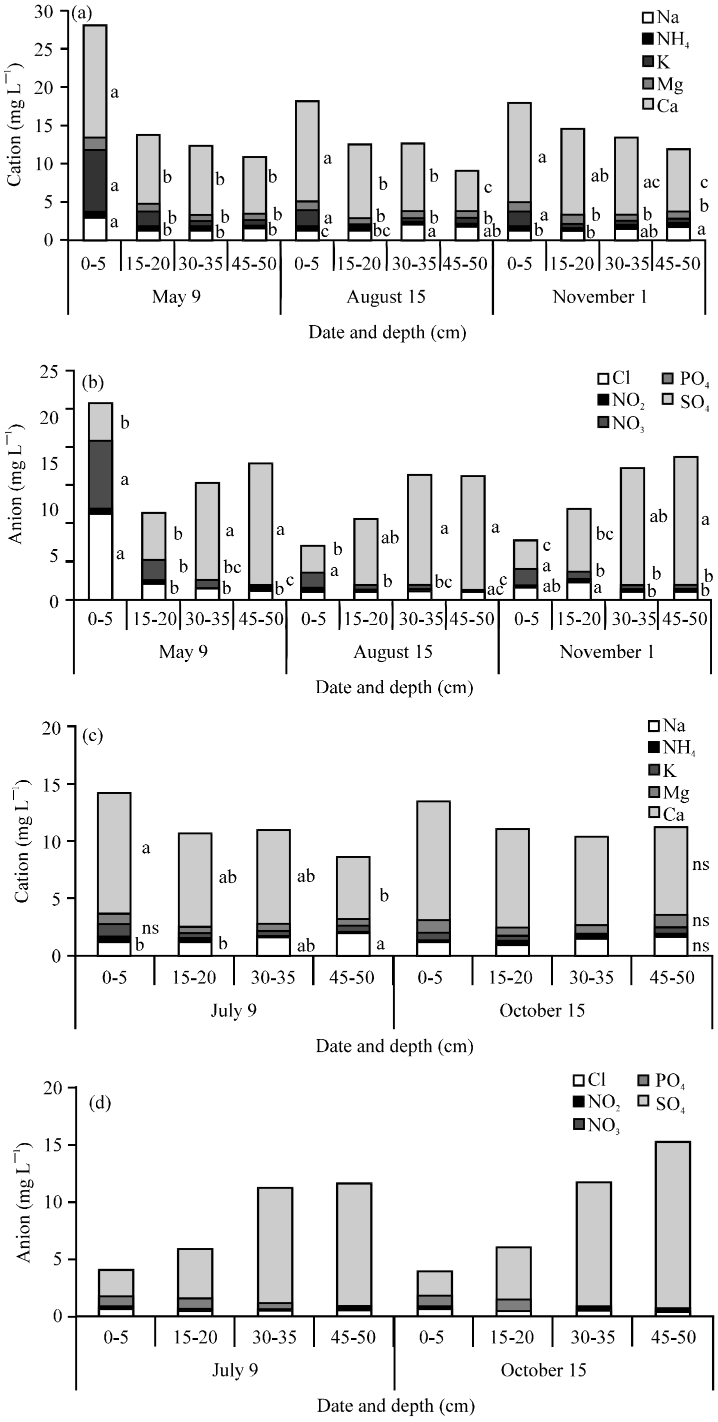

Fig. 3(a-d): Changes in cation (a, c) and anion (b, d) concentrations in soil at different depths in the border area between treatment plots in (a, b) 2007 and (c, d) 2008. Symbols with different letters denote significant differences among samples taken at different depths on the same date at the $p<0.05$ level

abundant anion was sulfate (SO_4^{2-}) across all seasons in both years, followed by Cl^- and NO_3^- while nitrite (NO_2^-) and PO_4^- concentrations were very low (Fig. 3).

Monthly changes in the pH and EC of soil solution collected from plots receiving chemical fertilizer or different rates of DEM in 2007 and 2008 are shown in

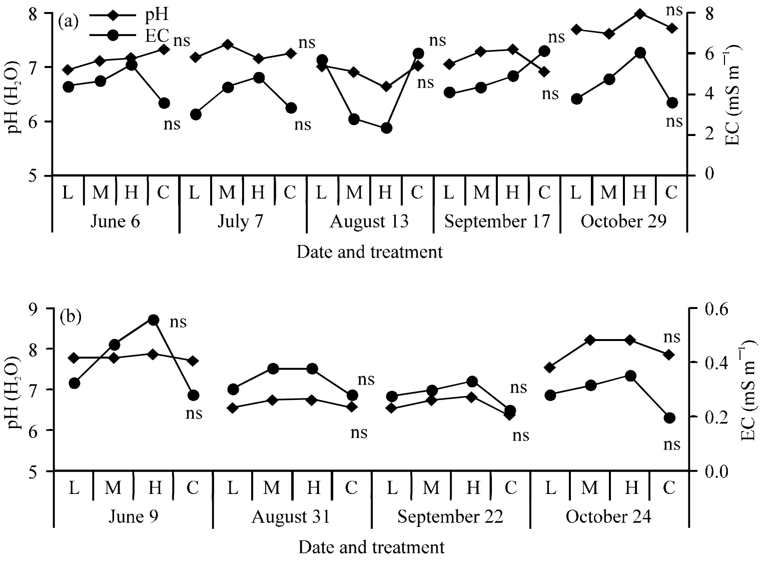

Fig. 4(a-b): Changes in pH and electric conductivity of soil solution from plots receiving chemical fertilizer or different rates of DEM in (a) 2007 and (b) 2008. Treatments: Chemical fertilizer control (C); low, medium and high rate of DEM (L, M, H). Symbols with different letters denote significant differences among treatments on the same date at the $p<0.05$ level

Fig. 4. Data for July 2008 do not exist as soil solution could not be collected in all plots due to drought conditions. In the majority of soil solution samples, pH in all DEM and control plots ranged from neutral to basic (pH 6.4-8.2) during the experimental period, tending to decrease up to August and then increase until late October in both years. The EC of the soil solution tended to increase with an increase in DEM application rate, a trend that was particularly evident during the first and final sampling months in both years (Fig. 4).

Seasonal changes in cation and anion concentrations in the soil solution for all DEM and control plots were monitored monthly in 2007 and 2008, with the exception of July 2008, for the reason stated above (Fig. 5). Cation concentrations in the soil solution tended to increase with increasing DEM application rate and were lowest in chemical fertilizer (control) plots across all sampling months in both years. The most abundant cation was Ca^{2+}, followed by Mg^{2+}, Na^+ and K^+, with the NH_4^+ concentration being extremely low. Change over the seasons in anion concentration differed between DEM and control plots in both years. The most abundant anion was SO_4^{2-}, accounting for an average of 91% of total anion concentration across all sampling months in both years (Fig. 5).

Change in plant growth indicators and dry matter weight: Effects of different treatments on plant growth indicators at the third and fourth cuttings in 2007 and 2008, respectively, are shown in Fig. 6. With the exception of the last and first cuttings in 2007 and 2008, respectively, when no significant differences in tiller number were observed, both plant height and tiller number increased with increasing in DEM application at each cutting in both years.

Dry Matter Weight (DMW), Mean Tiller Weight (MTW) and the leaf blade to stem (including leaf sheath) ratio (LB/ST) were measured at each cutting in 2007 and 2008 (Fig. 7). The DMW of whole plants increased significantly with increasing DEM application in both years. With the exception of the October 29, 2007 harvest, the DMW for the highest DEM amendment rate was not significantly different from that of the control plot. LB/ST was lowest for the first cutting in 2007 and tended to decrease with increasing DEM application across seasons in both years. Although, MTW tended to increase with an increase in DEM application rate at the first cutting, MTW did not appear to be affected by the DEM application rate in subsequent samplings in either year.

The relationships between Crop Growth Rate (CGR) and Leaf Area Index (LAI) in 2007 and 2008 are plotted in Fig. 8. Positive, linear relationships were observed for all three treatments and the control at each cutting in both years. In 2007, the regression coefficient increased from the first to the second cutting and decreased in the third

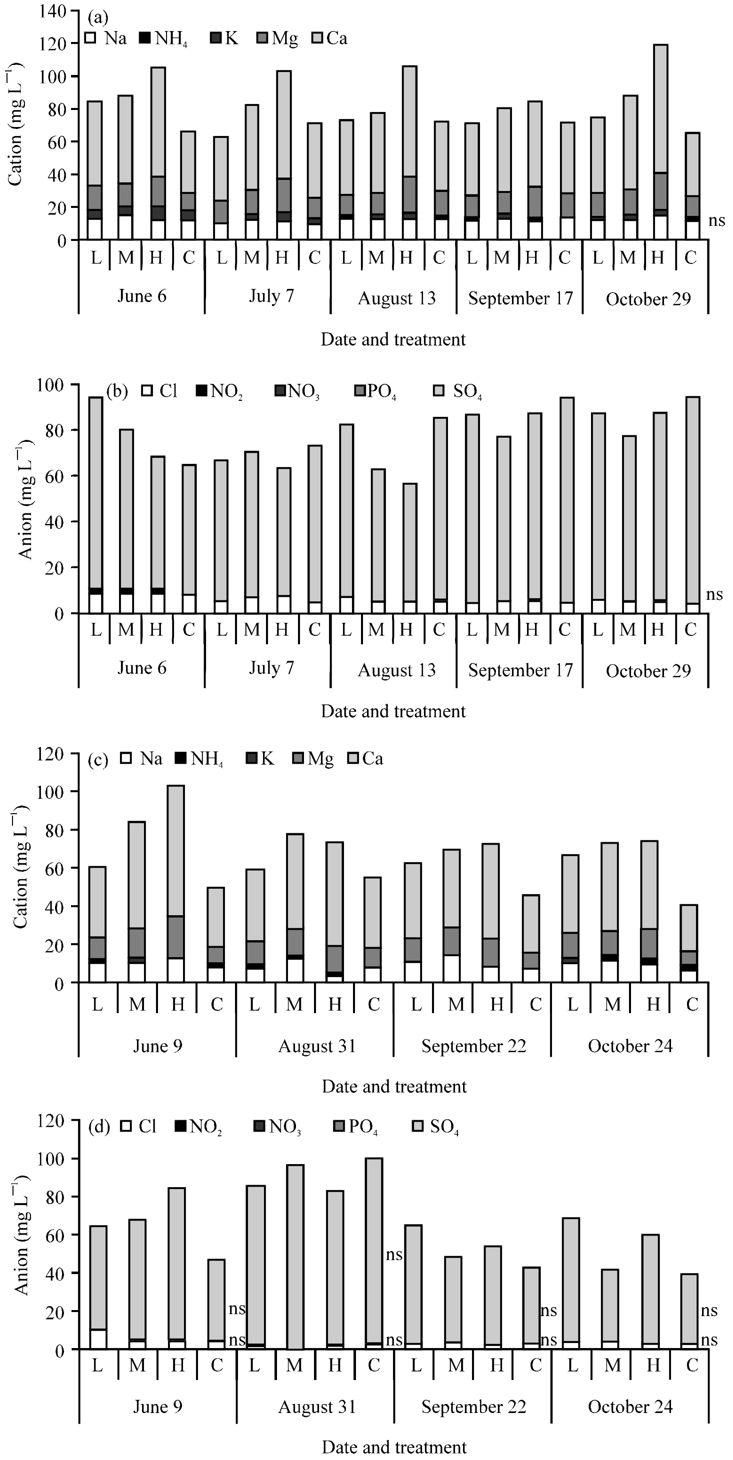

Fig. 5(a-d): Changes in cation (a, c) and anion (b, d) concentrations in soil solution from plots receiving chemical fertilizer or different rates of DEM in (a, b) 2007 and (c, d) 2008. Treatments: Chemical fertilizer control (C); low, medium and high rates of DEM application (L, M, H). Symbols with different letters denote significant differences among treatments on the same date at the $p<0.05$ level

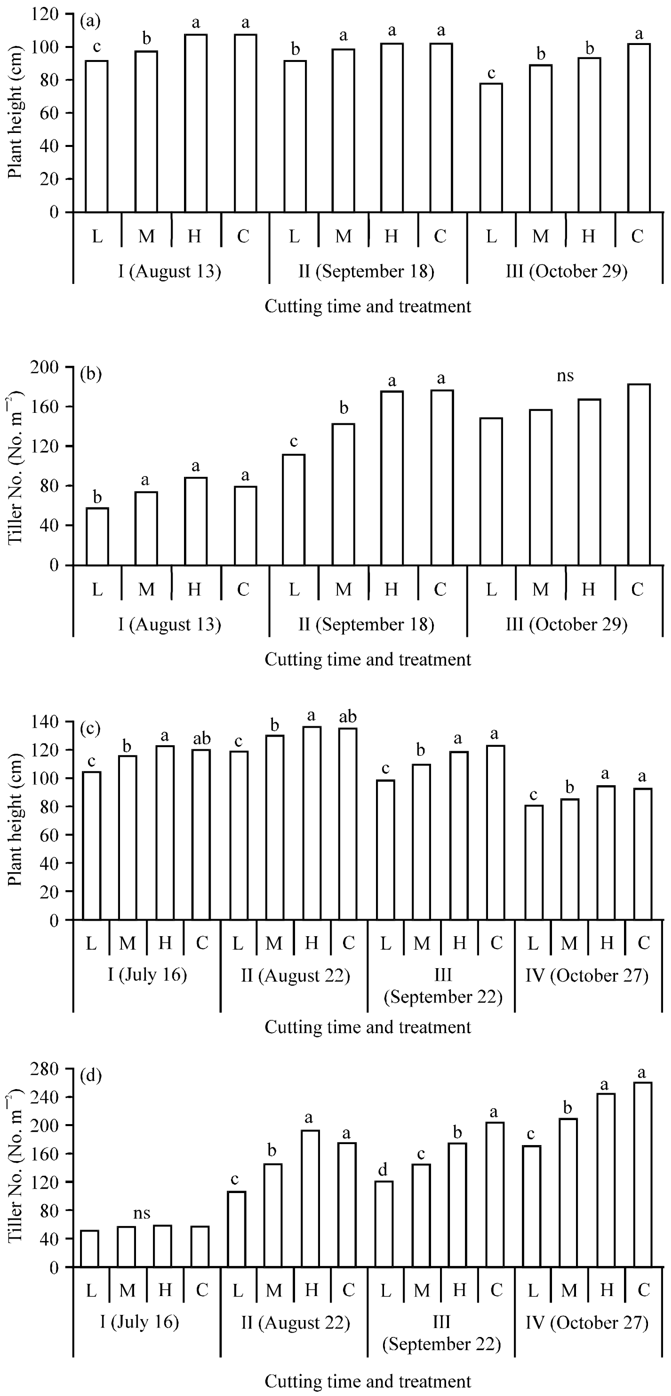

Fig. 6(a-d): Changes in plant height (a, c) and tiller No. (b, d) in dwarf napier grass under different treatments in (a, b) 2007 and (c, d) 2008. Symbols with different letters denote significant differences among treatments on the same date at the $p<0.05$ level

cutting. In 2008, the regression coefficient was almost the same for the first to the third cuttings while the regression coefficient decreased at the fourth cutting (Fig. 8).

DISCUSSION

Consistent with other research, the most common N form in the DEM used in this study was NH_4^+ which is

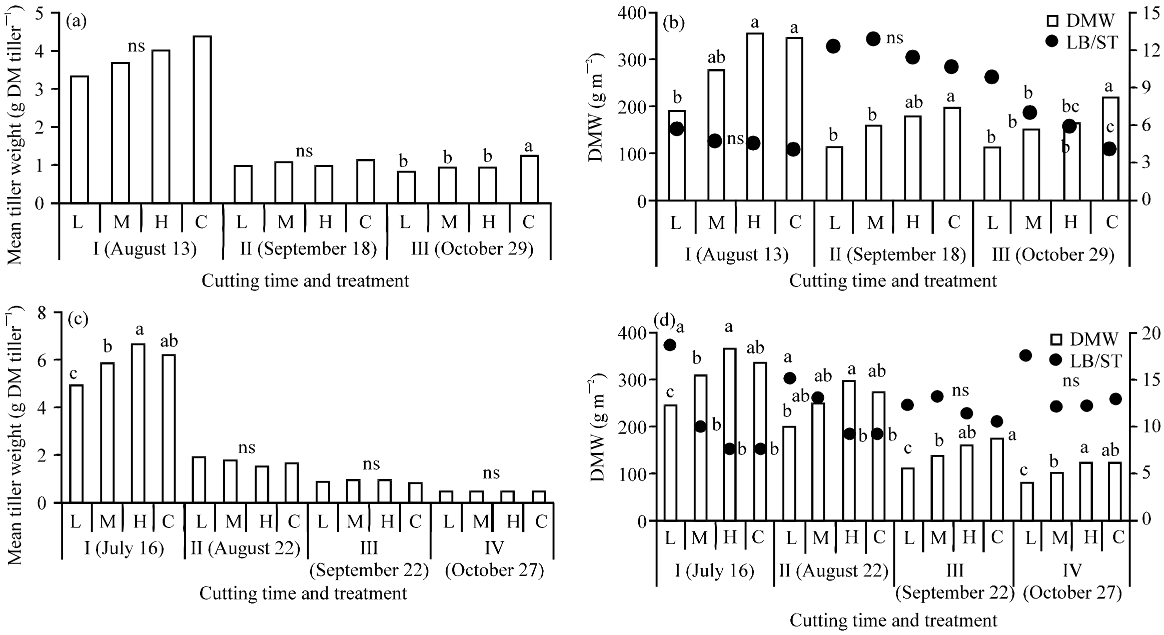

Fig. 7(a-d): Changes in mean tiller weight (a, c), dry matter weight of whole plant (DMW) and ratio of leaf blade to stem inclusive of leaf sheath (LB/ST; b, d) in dwarf napier grass under different treatments in (a, b) 2007 and (c, d) 2008. Symbols with different letters denote significant differences among treatments on the same date at the p<0.05 level

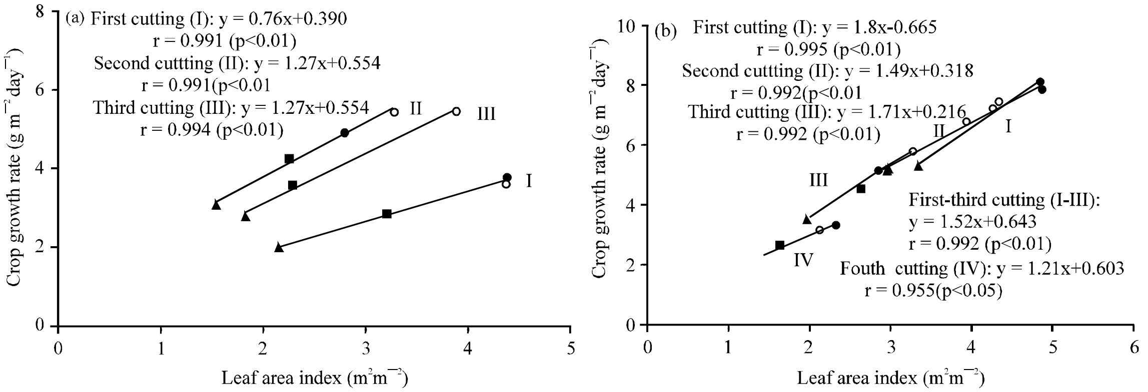

Fig. 8(a-b): Relationships between leaf area index (LAI) and crop growth rate (CGR) in dwarf napier grass under different treatments in (a) 2007 and (b) 2008

readily available for use by plants in the first year of application (Di *et al*., 1998). Son *et al*. (2005) reported N concentrations and NH_4^+ proportions of bio-digester effluent in the range of 889 to 1690 mg N L^{-1} and 0.40-0.60, respectively which were both slightly lower than those observed in the present study. High rates of N fertilization usually increase the concentration of other major elements, such as Phosphorus (P) and potassium (K) in forage plants while NH_4^+ applied to grasses reduces concentrations of calcium (Ca), K and magnesium (Mg) in plants (Barnes *et al*., 1995). In general, about 25% of the N that is applied in a slurry is lost to the groundwater, likely due mineralization of N from the organic matter, resulting in a linear increase in N concentration of groundwater with increasing slurry application (Jarvis *et al*., 1987). It is expected that this loss of N to the environment will influence biomass yield. For example, Guinea grass (*Panicum maximum*) yield was found to be higher after fertilization with bio-digester effluent compared to equivalent fertilization in terms of N (120 kg N ha^{-1}) using raw manure and urea (Son *et al*., 2005). In the present study, N levels for the high rate

DEM treatment was 5.04 g N m^{-2} per application (averaged across seasons) while NH_4^+ concentration varied across seasons from 1.82-2.55 g L^{-1} and from 2.34-3.41 g L^{-1} in 2007 and 2008, respectively. The seasonal decrease in ion concentrations, especially those of NH_4^+ after mid-July, may have resulted in reduced growth potential of plants in the second and third cuttings. At same time, the negative effects of decreased ion concentration on plant production may have been masked by the decrease in air temperatures after mid-August. These results demonstrate that the efficiency of nutrient cycling is affected by grass production potential, fertilizer input, defoliation intensity and loss of elemental nutrients from grassland systems (Whitehead, 2000).

In terms of soil chemical properties, pH and EC are fundamental factors that must be considered in order to optimize fertilizer management of field crops. Soil pH influences plant production by mediating nutrient availability and the activity of soil microorganisms (Whitehead, 2000). In the present study, soil pH and pH of the soil solution following DEM or chemical fertilizer application remained in the neutral range, with the exception of weakly acidic soil solution (pH 6.4) measured in H treatment plots in mid-August 2007. The EC of the soil solution from both M and H treatments was lower than during other sampling periods. We speculate that the vigorous growth observed in H and M treatments in mid-summer may result in greater uptake of applied nutrients relative to the other treatments, resulting in lower abundance of such nutrients in the soil solution. Soil solution pH approached pH 8 in late October both years. This may have resulted from the cumulative application of alkaline DEM.

Fertilization had the primary effect of increasing cation and anion concentrations in the soil solution, as indicated by the change in EC. It is expected that the increase in ion concentration of the soil solution would result in increased plant uptake of not only N, but also Ca, Mg and K in order to maintain the electrical neutrality of the soil solution (Yanai *et al*., 1995). However, since high concentrations of Na, N and P have the potential to negatively impact soil and water resources (Di *et al*., 1998), it is important to match the levels of nutrient input with the nutrient requirements of crops. While any increase in fertilizer application rate should enhance plant production, care should be taken to prevent any negative impacts on the soil and ground water environments (Weslien *et al*., 1998). Nitrogen exists in soil environments in many forms, changing form as it moves through the nitrogen cycle. In general, differences in the NO_3^--N content of different soil types might be caused by differences in drainage, manure mineralization rates, denitrification rates and inputs with precipitation. In the present study, average soil NO_3^- concentrations across all depths and NO_3^- concentrations of the soil water across all treatments ranged from 1.39-0.63 mg L^{-1} and from 0.54 and 0.73 mg L^{-1} in 2007 and 2008, respectively. Although, it is difficult to designate a "safe level" of nitrogen in water, we adopted the maximum allowable contamination levels for regulated public water systems of 10 and 1 mg L^{-1} for nitrate and nitrite-nitrogen, respectively (Oram, 2010). Based on these standards, DEM application rates of up to the H level in the present study would be deemed safe for the soil and groundwater environments.

In terms of plant production, the variety of dwarf Napier grass used in the study exhibited increasing plant height and tiller number with an increase in the DEM application rate. This response is consistent with chemical fertilizer rate-growth relationships reported for this variety (Mukhtar *et al*., 2003) and for manure rate-growth relationships reported for normal varieties of Napier grass (Hasyim *et al*., 2007). It is recommended that tropical grass pastures be fertilized with at least 200 kg N ha^{-1} over the growing season and with P and K according to plant requirements (Moss, 2009). Consistent with observations by Mukhtar *et al*. (2003), in the present study, tiller number of dwarf Napier grass increased with repeated defoliation in all DEM and control treatments.

It has been reported that the increase in dry matter yield due to heavy fertilization is accompanied with an increase in total N in normal (Sunusi *et al*., 1999) and semi-dwarf Napier grass (Williams and Hanna, 1995) varieties. In the present study, annual total dry matter yield was linearly correlated with DEM application rate, ranging from 4.1-7.6 and 6.2-9.4 t ha^{-1} in 2007 and 2008, respectively. In a Danish study in which herbage production was 4.6 t DMW ha^{-1}, approximately 25% of the 114 kg N ha^{-1} applied in a slurry was leached (Eriksen *et al*., 1999). In contrast, in a New Zealand study in which herbage production was 15 t DM ha^{-1}, only 2.5-3.7% of 400 kg N ha^{-1} applied as dairy-shed effluent was leached (Di *et al*., 1998). It has also been reported, however that the amount of N leached decreases as the application date approaches the start of grass or crop growth (Weslien *et al*., 1998). DEM generated by a biogas plant was demonstrated to be as effective in promoting herbage production of dwarf Napier grass as high rates of organic fertilizer (up to 96 KL (m^3) ha^{-1}), without also contaminating the soil and soil solution environments.

CONCLUSION

In this study, except for the LB/ST ratio, all of the indicators of dwarf Napier grass growth increased with an increase in the DEM application rate in both years. These results suggest that dwarf Napier grass has potential as a highly productive, high quality herbage for fodder and that the desirable range of DEM application rates do not negatively impact the soil and water environments. Thus, DEM generated from biogas plants could potentially be used as a novel "organic" fertilizer for the cultivation of dwarf Napier grass as it is rapidly utilized by the plants without negatively effecting the environment.

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