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Effect of Repeated Application of Manure on Herbage Yield, Quality and Wintering Ability during Cropping of Dwarf Napiergrass with Italian Ryegrass in Hilly Southern Kyushu, Japan

Renny Fatmyah Utamy [1], Yasuyuki Ishii [2,*], Sachiko Idota [2] and Lizah Khairani [3]

[1] Faculty of Animal Husbandry, University of Hasanuddin, Makassar 90245, Indonesia; rfusat@yahoo.com
[2] Faculty of Agriculture, University of Miyazaki, Miyazaki 899-2192, Japan; sidota@cc.miyazaki-u.ac.jp
[3] Faculty of Animal Husbandry, University of Padjadjaran, Bandung, WestJava 40123, Indonesia; lizahkh@yahoo.com
* Correspondence: yishii@cc.miyazaki-u.ac.jp; Tel.: +81-985-58-7251

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Abstract: The effects of two levels of manure application (184 and 275 kg N ha^{-1} year^{-1}) on herbage yield, quality, and wintering ability during the cropping of a dwarf genotype of late-heading (DL) Napiergrass (*Pennisetum purpureum* Schumach) oversown with Italian ryegrass (IR; *Lolium multiflorum* Lam.) were examined and compared with chemical fertilizer application (234 kg N ha^{-1} year^{-1}) for 4 years to determine a sustainable and environmentally harmonized herbage production in a hilly area (340 m above sea level). No significant ($p > 0.05$) differences in growth attributes of plant height, tiller density, percentage of leaf blade, or dry matter yield appeared in either DL Napiergrass or IR among moderate levels (184–275 kg N ha^{-1} year^{-1}) of manure and chemical fertilizer treatments. IR exhibited no significant detrimental effect on spring regrowth of DL Napiergrass, which showed a high wintering ability in all treatments. In vitro dry matter digestibility of DL Napiergrass tended to increase with increasing manure application, especially at the first defoliation in the first three years. Manure application improved soil chemical properties and total nitrogen and carbon content. The results suggested that the lower rate of manure application of 184 kg nitrogen ha^{-1} year^{-1} would be suitable, which would be a good substitute for chemical fertilizer application with an equilibrium nitrogen budget for sustainable DL Napiergrass and IR cropping in the hilly region of southern Kyushu.

Keywords: chemical fertilizer; herbage production and quality; nitrogen budget; organic fertilizer; temperate annual grass; tropical perennial grass

1. Introduction

The dwarf genotype of late-heading type (DL) Napiergrass (*Pennisetum purpureum* Schumach) harvested as a summer crop plays an important role in providing high biomass, vigor, palatability, and digestibility for livestock feed in southern Kyushu, Japan [1–4]. Temperate species of Italian ryegrass (IR; *Lolium multiflorum* Lam.) harvested as a winter crop can be oversown into dwarf Napiergrass pasture [1,5] since IR has the opposite seasonal growth pattern to DL Napiergrass. The cropping combination of DL Napiergrass with annual IR needs to be determined for the sustainable production in several years at the elevated area (>300 m above sea level) of southern Kyushu, where dairy and beef cow farming operations are concentrated.

Dry matter (DM) production of Napiergrass including a DL genotype is enhanced by high chemical fertilizer input [4,6,7], but this increases the cost of forage production. Chemical fertilizer is widely used in agriculture. However, in recent years, serious concern has arisen about long-term adverse effects of continuous and indiscriminate use of chemical fertilizer in intensive agriculture on the deterioration of soil structure and function and environmental pollution [8,9]. The depletion of soil nutrients is brought about by an imbalance of nutrients due to an outflow higher than the compensation level for agricultural land with increasing pressure on land resources [10]. The excessive application of chemical fertilizer increases soil acidity since the mineral components not utilized by the crops can react with water in the soil to form acidic compounds. Soil acidity is accelerated by rainfall [11], and the average rainfall in Kobayashi City, southern Kyushu for a recent decade (2000–2010) was quite high, above 2500 mm. Other factors promoting soil acidity are thought to be the decomposition of plant residues, intensive agricultural production, and poor drainage. The former 2008 study revealed that soil pH ranged from 5.7 to 6.9, regarded as weakly acidic, though this pH range had no significant effect on the DM yield of DL Napiergrass in the present volcanic ash soils [4].

Livestock manure is an organic fertilizer that plays a key role in chemical and biological soil functions of intensively cropping fields under sustainable and environmentally harmonized herbage production. Prompt management of manure application should be a top priority for increasing herbage production in grassland agriculture to prevent environmental pollution. Since manure has a high concentration of organic matter, its application as a fertilizer helps decelerate depletion of organic matter in arable land, especially when there is a high frequency of heavy erosion [12–14]. It also increases the soil levels of the macroelements of nitrogen (N), phosphorus (P), and potassium (K) [15–19], improves soil physical properties [20,21], enhances DM yield [22–25], and improves the crude protein concentration of herbages [26].

However, excessive manure application can lead to several problems, such as pollution of soil and ground water by leaching and runoff of nutrients [27–29], increased emission of nitrous oxide as a global warming potential [30], and excess accumulation of nutrients in soils [15,31]. Therefore, the proper rate of manure application that prevented runoff losses from leaching was determined to be an N rate of 150–250 kg ha^{-1} year^{-1} [22,32], which was adopted as the application rate in the present study.

In our previous studies [4,7,33], DL Napiergrass required high chemical fertilizer input for high DM production; it is also costly, making the required level of application unaffordable for smallholder farmers, and risks environmental pollution by rapid nutrient leaching under heavy rainfall. However, organic manure application has lower risk of nutrient leaching by mineralization when compared with chemical fertilizer input. Therefore, the objectives of this study were to compare the effects of consecutive manure application with chemical fertilizer application on growth, yield, and quality attributes, and the sustainability of DL Napiergrass intersown with IR, to determine sustainable and environmentally harmonized herbage production by manure application in a hilly area of southern Kyushu, Japan over four years (2007–2010).

2. Results

2.1. Soil Nutrient Status

At the beginning of the trial in May 2007, no significant difference in the soil mineral status of total nitrogen (TN) and total carbon (TC) values nor carbon-to-nitrogen ratio (CN) appeared among treatment plots. The experiments covered a low (LM) and a high rate of manure application (HM). Soil total nitrogen and total carbon concentrations were significantly ($p < 0.05$) higher for LM than for HM or the chemical fertilizer application (CF), and the increase in TN and TC was largest in the LM plot after four years of cropping DL Napiergrass with IR. However, the carbon-to-nitrogen ratio in October 2010 was the highest in CF, followed by the LM and HM plots with a range of 10.25–10.37 among treatments, and decreased similarly from the initial range of 10.77–10.85 in May 2007 (Table 1).

Table 1. Total nitrogen (TN) and total carbon (TC) content, and carbon-to-nitrogen ratio (CN) in soils at the start and end of trials (May 2007 and October 2010, respectively), as affected by fertilizer treatments.

Month and Year	Treatment ‡	TN (% DM)	TC (% DM)	CN Ratio
May 2007	CF	0.48 ± 0.01	5.16 ± 0.17	10.85 ± 0.08
	LM	0.50 ± 0.01	5.30 ± 0.10	10.81 ± 0.10
	HM	0.48 ± 0.04	5.17 ± 0.43	10.77 ± 0.05
	Significance ‡‡	NS	NS	NS
October 2010	CF	0.57 ± 0.04b	5.93 ± 0.47b	10.37 ±0.16a
	LM	0.65 ± 0.04a	6.72 ± 0.53a	10.32 ± 0.16b
	HM	0.59 ± 0.03b	6.07 ± 0.27b	10.25 ± 0.07c
	Significance‡‡	*	*	*

Data are presented as means ± standard deviation. ‡ CF, chemical fertilizer application; DM: dry matter; LM: low rate of manure application; HM: high rate of manure application. ‡‡ * significant at $p < 0.05$; NS: not significant.

2.2. Growth Attributes

Growth attributes of plant height, tiller density, and percentage of leaf blade (PLB) did not differ significantly in either DL Napiergrass or IR among treatments, except for tiller density of IR in the first year, tiller density of DL Napiergrass at the first defoliation in the second year, and PLB of DL Napiergrass at the third defoliation in both the second and third years. In DL Napiergrass, plant height decreased between the second and third defoliation, whereas PLB and tiller density increased from the first to the third defoliation in the second and third years, which was consistent with the seasonal changes in these attributes with the progression of cutting practice [4,7]. In the fourth year, when no defoliation was imposed until October, DL Napiergrass plant height increased and PLB decreased more than the first three years (Table 2).

Table 2. Growth attributes of plant height, tiller density, and percentage of leaf blade in dwarf genotype of late-heading type (DL) Napiergrass and Italian ryegrass (IR), as affected by fertilizer treatments for four consecutive years (2007–2010).

Attribute	Treatment †	First Year ‡			Second Year				Third Year				Fourth Year
		DL Napiergrass		IR	DL Napiergrass			IR	DL Napiergrass			IR	DL Napier-Grass
		1st Defoliation	2nd Defoliation		1st Defoliation	2nd Defoliation	3rd Defoliation		1st Defoliation	2nd Defoliation	3rd Defoliation		First Defoliation
Plant height (cm)	CF	121	155	80	130	158	57	92	130	137	70	54	199
	LM	127	153	61	134	161	53	90	137	126	74	50	207
	HM	126	154	69	131	161	52	88	140	124	70	57	205
	Significance ††	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Tiller density (No m^{-2})	CF	62.9	80.0	404.7a	56.0c	88.2	226.7	265.7	69.9	173.0	264.7	536.3	63.9
	LM	57.9	78.0	288.7b	61.1a	79.2	218.4	254.3	72.2	174.1	242.0	586.0	57.6
	HM	61.1	76.2	240.0b	60.7b	82. 9	209.0	312.0	75.1	174.1	236.3	556.7	73.8
	Significance	NS	NS	*	*	NS	NS	NS	NS	NS	NS	NS	NS
Percentage of leaf blade	CF	77.0	54.1	— **	76.9	58.2	89.3c	—	74.2	71.2	90.5b	—	32.1
	LM	80.3	52.6	—	73.6	60.8	92.0a	—	72.4	71.2	90.5b	—	31.7
	HM	82.9	54.2	—	72.2	62.7	91.7b	—	67.4	74.2	92.9a	—	25.7
	Significance	NS	NS		NS	NS	*		NS	NS	*		NS

† CF: chemical fertilizer application at 234 kg N ha^{-1} $year^{-1}$; LM: low rate of manure application at 184 kg N ha^{-1} $year^{-1}$; HM: high rate of manure application at 275 kg N ha^{-1} $year^{-1}$. †† *, $p < 0.05$; NS: not significant. ‡ First year: 2007–2008; Second year: 2008–2009; Third year: 2009–2010; Fourth year: 2010. ** Not determined. Symbols with different letters were significantly different among treatments in each year by the least significant difference (LSD) method at the 5% level.

2.3. DM Yield

No significant effect of treatment on annual total DM yield appeared when cropping DL Napiergrass with IR for four years. Annual DM yield increased from the first to the second year, while the lowest annual yield occurred uniformly across treatments in the third year, which correlated with the lowest annual precipitation. The DM yield of DL Napiergrass increased from the first to the second defoliation and then decreased sharply by the third defoliation in the first three years. The CF plot tended to have slightly higher DM yield of DL Napiergrass than the two manure plots at the first defoliation in the first year, whereas the DM yield in the CF plot tended to be lower thereafter. The DM yield of DL Napiergrass in the HM plot was significantly higher at the first defoliation in the third year, while the DM yield in the CF plot was significantly higher at the third defoliation in the second year. The DM yield of IR was significantly higher in the CF plot the first year; however, there were no significant differences among treatments thereafter (Figure 1).

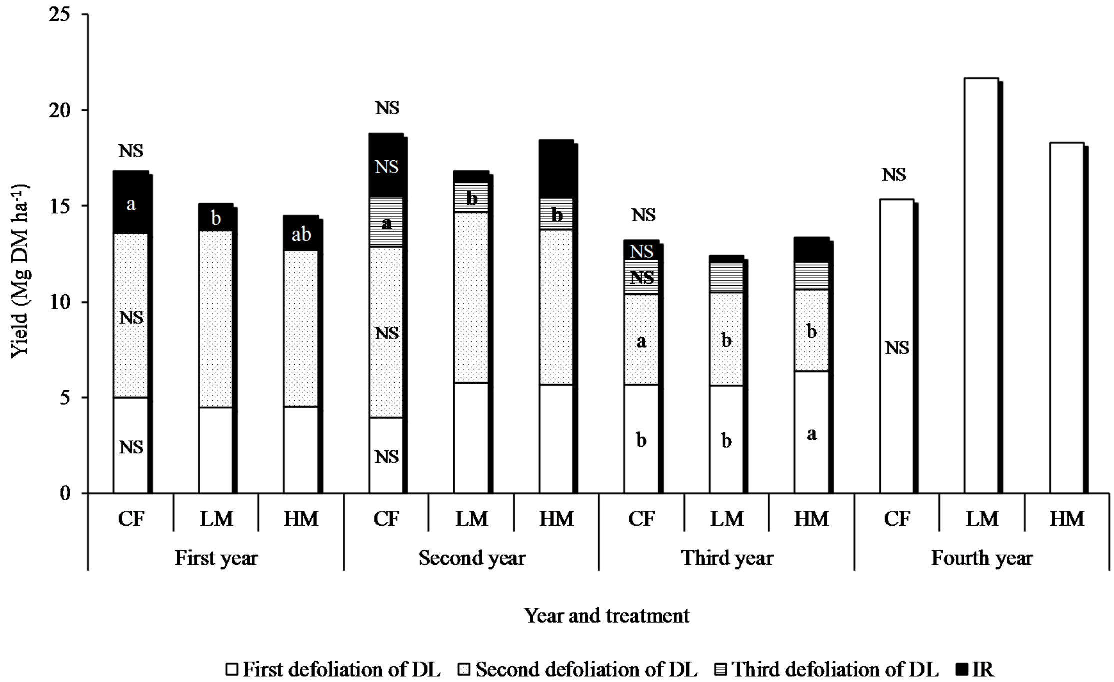


Figure 1. Dry matter (DM) yield of dwarf genotype of late-heading type (DL) Napiergrass at the first, second, and third defoliation and of Italian ryegrass (IR), as affected by fertilizer treatments in 2007–2010. Symbols with different letters were significantly different among treatments in each year by the least significant difference (LSD) method at the 5% level. Non-significant (NS): $p > 0.05$. First year (2007–2008), Second year (2008–2009), Third year (2009–2010), Fourth year (2010). CF: chemical fertilizer application at 234 kg N ha^{-1} year^{-1}; LM: low rate of manure application at 184 kg N ha^{-1} year^{-1}; HM: high rate of manure application at 275 kg N ha^{-1} year^{-1}.

2.4. Overwintering Ability

Overwintering ability, measured as the percentage of overwintering plants (POP), was above 93% across all treatments in the first two years, with no significant effect of fertilizer treatment on either POP or regrown tiller number (RTN) of DL Napiergrass. The RTN of DL Napiergrass was close to satisfactory for spring growth with a range of 18–30 tillers m^{-2} in both late April 2008 and June 2009 (Table 3).

Table 3. Overwintering attributes of percentage of overwintering plants (POP) and regrown tiller density (RTN) of DL Napiergrass in the first and second years.

Month and Year	Attribute	Treatment †		
		CF	LM	HM
29 April 2008	POP (%)	96.7 ± 5.8 NS ††	100.0 ± 0.0	100.0 ± 0.0
	RTN (No. m^{-2})	18.2 ± 3.5 NS	25.0 ± 3.4	27.6 ± 3.9
18 June 2009	POP (%)	93.3 ± 5.8 NS	100.0 ± 0.0	96.7 ± 5.8
	RTN (No. m^{-2})	30.3± 3.4 NS	23.9 ± 8.3	25.6 ± 4.5

Data are presented as means ± standard deviation. † As for treatment, CF: chemical fertilizer application; LM: low rate of manure application; HM: high rate of manure application. †† As for significance, NS: not significant at $p > 0.05$.

2.5. Herbage Quality Attributes

In vitro dry matter digestibility (IVDMD) of leaf blade (LB) and stem inclusive of leaf sheath (ST) of DL Napiergrass and of whole IR plants showed no significant differences among treatments, except for higher LB IVDMD in the HM plot at the first defoliation in the first year and at the second defoliation in the second year, with conversely lower LB IVDMD at the second defoliation in the first year (Figure 2a). No significant effect of fertilizing treatment on crude protein (CP) concentration was observed in either DL Napiergrass or IR (Figure 2b).

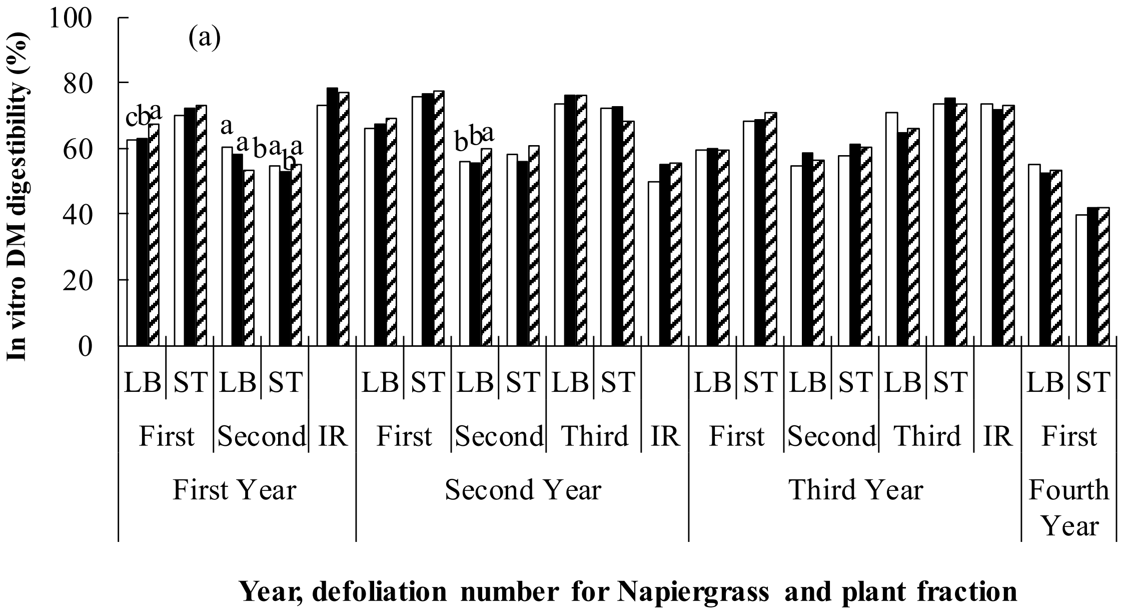


Figure 2. *Cont.*

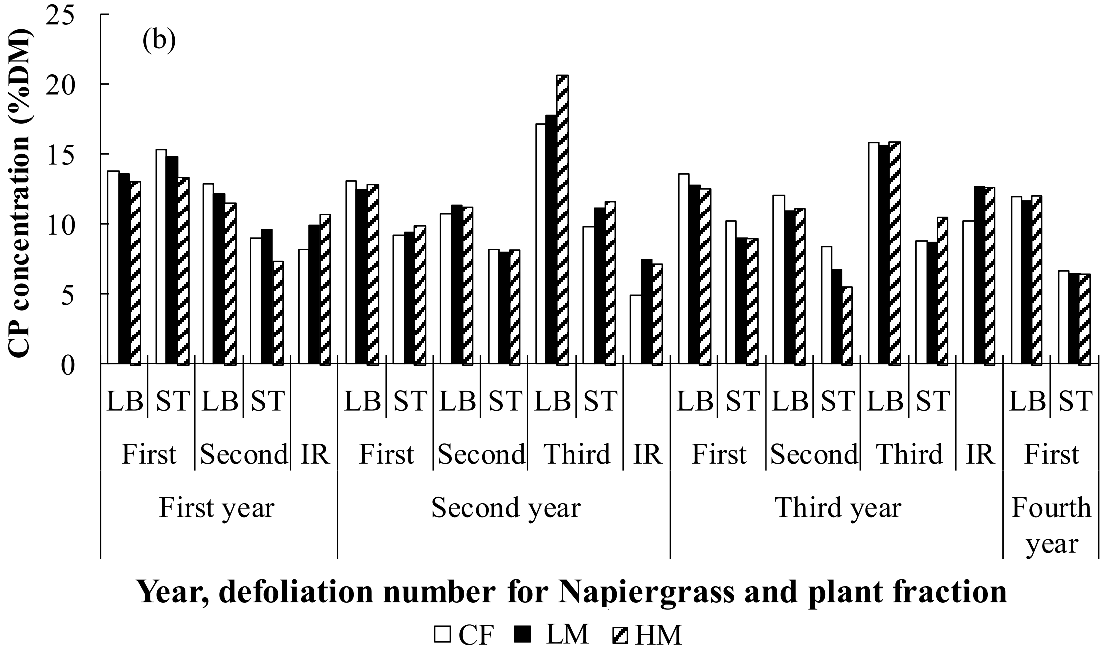
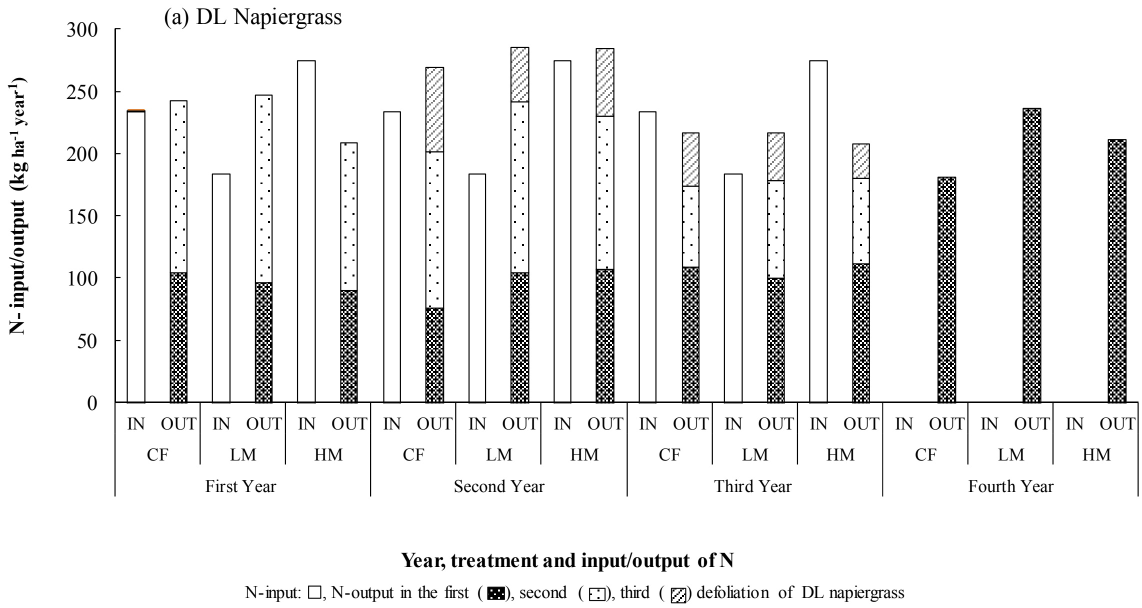


Figure 2. (**a**) In vitro DM digestibility and (**b**) Crude protein (CP) concentration of dwarf genotype of late-heading (DL) Napiergrass and Italian ryegrass (IR), as affected by fertilizer treatments in 2007–2010. Symbols with different letters were significantly different among treatments at each defoliation by the LSD method at the 5% level. NS: not significant at $p > 0.05$. For abbreviations of treatments and years, refer to Figure 1. Plant fractions: leaf blade (LB), stem inclusive of leaf sheath (ST).

2.6. Input and Output of Total N

The N budget in the cropping of DL Napiergrass with IR was assessed from N input from fertilizer application and N output assessed by harvested DM yield combined with total N concentration for the four consecutive years, as shown in Table 4. No significant effect of fertilizer treatment was observed on the output either from DL Napiergrass or IR (Figure 3). Total N output across treatments was inconsistent from the first to the third year, reflected mainly by the change in DM yield. However, the effect of treatment on total N output consistently tended to be highest in the LM, followed by the CF and HM plots in the first and second years, with no trend among treatments in the third year. In the fourth year, when no fertilizer was applied, N output tended to be highest in the LM plot, followed by the HM and CF plots (Figure 3a). In contrast, output of total N in IR plants tended to be higher, with an increase in manure application in both the second and third years. However, an imbalance between input and output of N occurred in IR plants for the CF plot (Figure 3b).



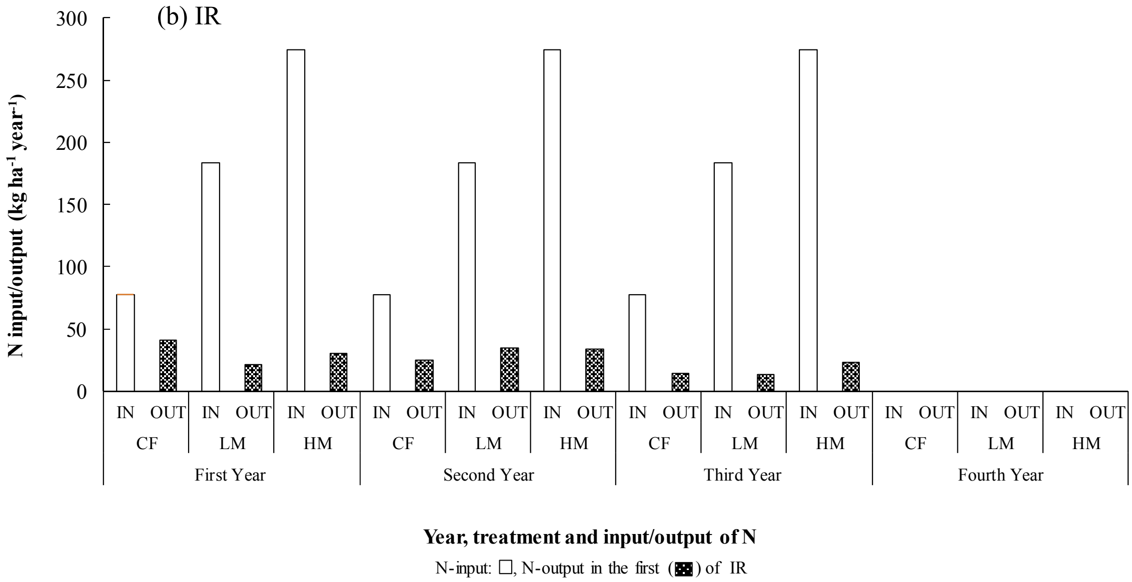


Figure 3. Input (IN) and output (OUT) of nitrogen (N) from defoliation of (**a**) DL Napiergrass and (**b**) Italian ryegrass (IR). For abbreviations of treatments and years, refer to Figure 1.

3. Discussion

DL Napiergrass growth characters of plant height, tiller density, and PLB showed almost no significant differences under the examined fertilizer treatments over four years in a hilly area of southern Kyushu. Changes in growth characters with the defoliation practice showed a synchronized trend of increasing plant height and tiller density and decreasing PLB from the first to the second defoliation and increasing tiller density and PLB and decreasing plant height from the second to the third defoliation across treatments. Climatic factors of air temperature and precipitation presumably affected these changes in the highest DM yield at the second defoliation due to high temperature promoting the growth of DL Napiergrass, which has C_4 photosynthetic metabolism [2] and lowest yield at the third defoliation due to a decline in air temperature, as shown in Figure 1. Similarly, the lowest annual yield of DL Napiergrass in the third year might reflect the lowest precipitation among the four years (Figure 4). Since the cutting interval should affect the yield of Napiergrass, showing a higher yield with longer intervals [34–36], DM yield in the fourth year, with no midway defoliation, tended to be higher than the preceding three years, even though no fertilizer was applied (Figure 1).

Manure's advantages as a slow-releasing fertilizer lead to higher DM yield of DL Napiergrass [37]. In the present study, even though fertilizer treatments were not imposed in the last year, higher yield was achieved in the organic fertilizer plots—especially in the LM plot, which achieved the highest DM yield at 22 Mg ha^{-1} across all plots and years (Figure 1). High yield was presumably achieved due to increased nutrient absorption capacity of a high density of roots due to improved soil physical properties [38] and continuous nutrient absorption from earlier manure input [39]. Moreover, the LM plot had higher TN and TC content at the end of the study than the other two plots (Table 1). The expected improvement of soil physical properties by organic matter application from fermented cattle manure [20,21] was evidenced by comparison with pre-established DL Napiergrass plots. Organic matter components usually encourage the formation of stable soil crumb structure, thus improving soil internal drainage, infiltration, aeration and microbial activity and root development.

The DM yield of Napiergrass increases under a high rate of fertilizer input in both normal [24,25] and dwarf genotypes [4,7]. However, in the present study, DM yield reached a plateau in the LM and HM plots, and in the CF plot in the third year. Humphreys [40] and Crowder [41] found a 30–50% increase in herbage DM yield at doses increasing from 200 to 600 kg N ha^{-1}; thus, N use efficiency in terms of DM yield per kg of applied N decreased sharply with increasing N application rate. Therefore, as also supported by Mohammad et al. [42], it can be assumed that yield of DL Napiergrass should be the most responsive to organic manure supply up to the moderate rate (i.e., the LM plot), which was verified by higher DM yield in the moderate manure application than the heaviest application.

IR intersown with rows of DL Napiergrass at the last defoliation gave an additional crop the following spring. IR might have other advantages in reducing N leaching effectively in manure-applied systems [29] and suppressing weed growth in the spring before the regrowth of DL Napiergrass starts [43].

The slow-release capability of organic manure might lead to the retention of nutrients in the soil; therefore, roots of DL Napiergrass in the manure plots might have taken up more nutrients than in the inorganic CF plot to store as food reserves. It is well known that a high concentration of nonstructural carbohydrate reserves can be beneficial to the viability of tiller buds under low temperature, and can promote the spring regrowth in tropical grass species [44], which correlates with the observed higher tendency in the percentage of overwintered plants in the LM and HM plots compared with the CF plot (Table 3).

Herbage quality attributes of IVDMD and CP concentration did not show any negative effects of manure application compared to chemical fertilizer application, and was combined with a high level of IVDMD and a CP concentration above 10% in almost all DL Napiergrass and IR plots, with the exception of the delayed defoliation of DL Napiergrass in the fourth year (Figure 3). The mean IVDMD value was above 60%, which is categorized as medium-quality forage and high enough for beef-cattle feeding. The CP concentration was also high enough for quality feeding above the minimum level required for rumen functions [45].

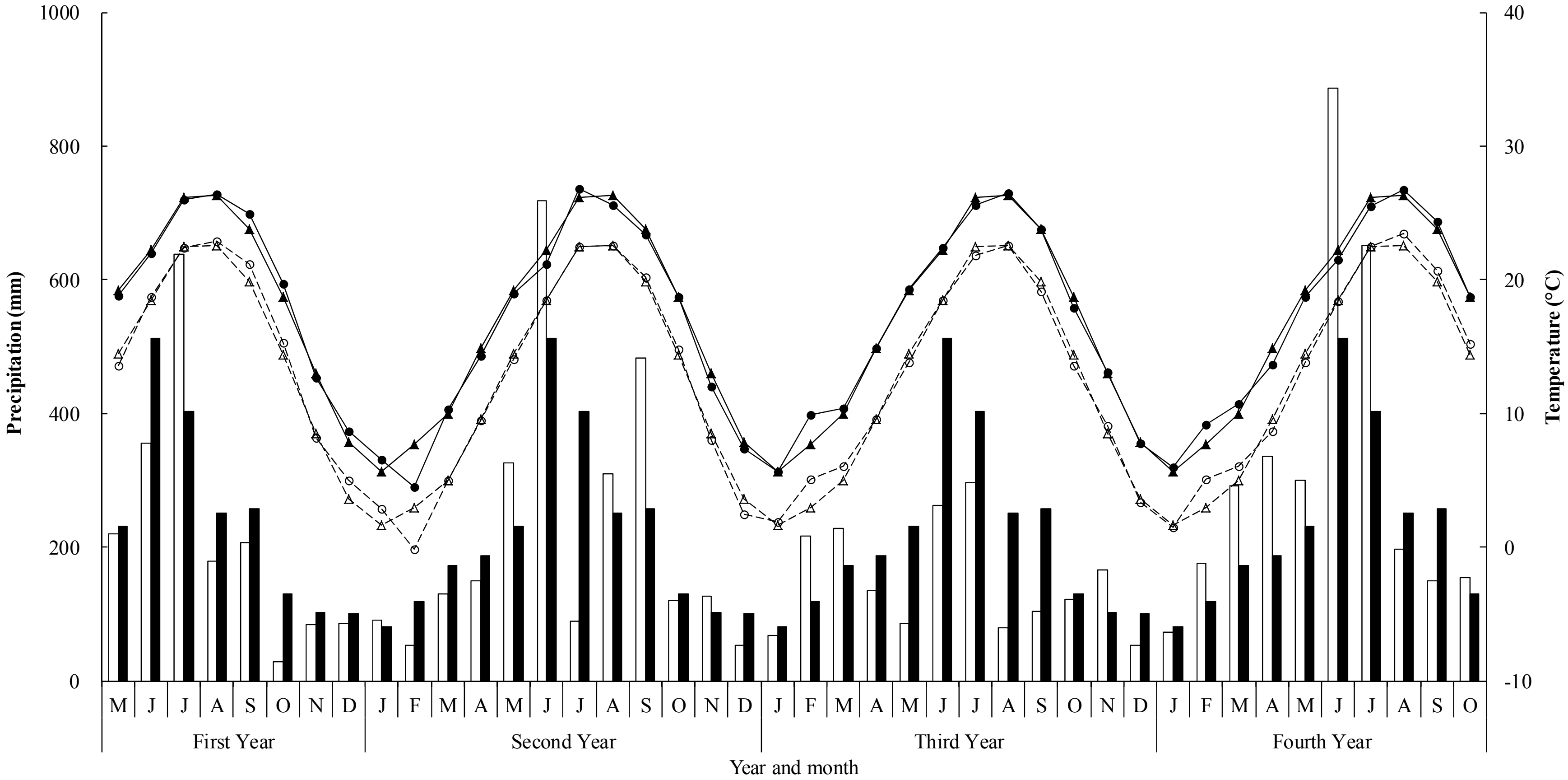


Figure 4. Monthly precipitation in the experimental period (□) and long-term average (■), monthly mean of daily mean temperature in the experimental period (●) and long-term average (▲), and monthly mean of minimum temperature in the experimental period (○) and long-term average (Δ). The experimental period was 2007–2010; long-term period was 2000–2010. As for the abbreviation of the month, M, J, J, A, S, O, N, D, J, F, M and A are May, June, July, August, September, October, November, December, January, Februay, March, and April, respectively.

Table 4. N uptake (g m^{-2}) by DL Napiergrass and IR under different fertilizer treatments in 2007–2010.

Species	Treatment †	First Year ‡		Second Year			Third Year			Fourth Year	Pooled
		First Defoliation	Second Defoliation	First Defoliation	Second Defoliation	Third Defoliation	First Defoliation	Second Defoliation	Third Defoliation	First Defoliation	
DL Napiergrass	CF	105.2 ± 14.9	137.3 ± 29.9	76.5 ± 5.3	125.1±11.7	68.0 ± 9.5	109.5 ± 21.4	64.6	42.6	181.2 ± 20.3	110.5 ± 11.2
	LM	97.0 ± 12.0	150.4 ± 26.2	104.7 ± 15.4	137.2 ± 3.4	43.5 ± 8.9	100.3 ± 11.4	78.4	37.9	236.3 ± 44.9	118.6 ± 2.5
	HM	90.8 ± 23.2	117.7 ± 9.6	107.5 ± 20.3	122.6 ± 17.1	54.3 ± 18.0	111.9 ± 8.0	68.6	27.8	211.7 ± 52.6	110.7 ± 12.2
	Significance ††	NS	NS	NS	NS	NS	NS			NS	NS
IR	CF	41.5 ± 12.0		25.9 ± 1.6			15.4 ± 6.3				27.6 ± 5.0
	LM	22.0 ± 4.8		35.5 ± 13.6			14.4 ± 4.0				24.0 ± 5.2
	HM	31.0 ± 10.8		34.5 ± 11.8			24.5 ± 2.8				30.0 ± 8.3
	Significance	NS		NS			NS				NS

Data are presented as means ± standard deviation. † As for treatment, CF: chemical fertilizer application; LM: low rate of manure application; HM: high rate of manure application. †† As for significance, NS: not significant at $p > 0.05$. ‡ First year: 2007–2008; Second year: 2008–2009; Third year: 2009–2010; Fourth year: 2010.

The present study revealed that herbage quality, influenced by temperature and cutting period, had a negative correlation with DM yield of DL Napiergrass, with the highest yield for the second defoliation with the lowest IVDMD and CP concentration across the first three years. These phenomena were normal for the tendency of herbage quality in tropical pastures, where high temperature stimulates lignification of plant cell walls, resulting in decreasing IVDMD [45]. Additionally, IVDMD and CP concentration decreased with the delay of defoliation in the fourth year—the same tendency observed with normal Napiergrass [23]. Numerous changes occur as forage plants mature, and are concurrent with decreasing herbage quality. An increase in N supply reduces the structural carbohydrate content, thus diluting the proportion of DM present as cell wall and increasing digestibility [45]. Dormaar et al. [12] found that plots receiving manure showed a greater increase in total N concentration. In the present study, it was difficult to detect a positive effect of manure application on herbage quality attributes in comparison with the CF plot. These data support the conclusion that the range of fertilizer application was high enough to maintain herbage yield and quality.

The stem fraction of DL Napiergrass had higher IVDMD than LB at the first defoliation in the first three years across all treatments. Van Soest [45] found that not all leaves are more digestible than the stem fraction, since the function of the stem as a storage organ gives them a higher nutritive value than leaves. In DL Napiergrass, as in most tropical grasses, herbage digestibility decreased as maturity progressed from the first to the second defoliation, while the digestibility tended to be higher in ST than in LB at the juvenile stage of the first defoliation. The digestibility of ST generally declines as plants mature [46,47], such as between the first and second defoliation in the present study.

Furthermore, the results confirmed that a low rate of organic manure application would be a good substitute for chemical fertilizer application due to the low energy intensity of organic fertilizer [48] and equivalent N input and N output—a key issue for environmentally-friendly and healthy soil conditions with less impact on global warming potential.

4. Materials and Methods

4.1. Experimental Site, Climatic Conditions, and Grass Species

The experiments were conducted during four consecutive years in 2007–2010 (i.e., first year: 2007–2008; second year: 2008–2009; third year: 2009–2010; and fourth year: 2010) at Miyazaki Ranch, National Livestock Breeding Center (31°57′ N, 130°57′ E, 340 m a.s.l.). In this area, the mean annual rainfall was 2569 mm and the mean annual temperature was 16.3 °C for the recent decade of 2000–2010 [49].

The change in monthly precipitation was almost synchronized with air temperature in all four years from 2007 to 2010, with monthly precipitation highest in June or July and the lowest in October or December (Figure 4). Annual precipitation was the highest at 3398 mm in the fourth year and the lowest at 1822 mm in the third year; the long-term average over the previous 10 years (2000–2010) was 2569 mm in the region. Annual temperature in the four years averaged 15.8–16.6 °C, which was similar to the long-term average of 16.3 °C. The lowest winter temperature was lower in the first than in the second and third years (Figure 4).

The grass species used were a dwarf genotype of late-heading (DL) Napiergrass (*Pennisetum purpureum* Schumach) as a summer crop and Italian ryegrass (*Lolium multiflorum* Lam. cv. Ace, IR) as a winter crop. The soil is the volcanic ash soil of Andosols (Kuroboku), having a pH of 6.6, electrical conductivity of 0.100 dS m^{-2}, soil nutrient concentrations of 0.5% TN and 5.3% TC, and a soil CN ratio of 10.7 at the start of the experiments [4].

4.2. Experimental Design and Treatments

The experiments were arranged in a randomized complete block design with three replications, each containing three treatments; that is, chemical fertilizer (CF), low rate of manure application (LM), and high rate of manure application (HM). Each treatment consisted of 28 plants at 2 plants m^{-2} for DL Napiergrass (1 and 0.5 m for inter- and intra-row spacing, respectively) with 3 m × 3 m (9 m^2). The spacing between plots and between blocks was the same (1 m).

Chemical compound fertilizer (containing 14%, 14%, and 14% of N, P_2O_5, and K_2O, respectively) and commercial fermented cattle manure (Sun Green, containing 1.18, 2.19, and 2.09% of N, P_2O_5, and K_2O, respectively) were supplied as a top-dressing; the amount supplied was 1500, 14,000, and 21,000 g $year^{-1}$ (resulting in 234, 184, and 275 kg N ha^{-1} $year^{-1}$) for the CF, LM, and HM plots, respectively. In the first year, the chemical fertilizer was split and supplied to DL Napiergrass three times (78 kg N ha^{-1} $time^{-1}$) on 21 June, 28 July, and 17 August 2007 for the CF plot, while the organic manure was supplied once on 21 June 2007 for both the LM and HM plots. In the second and third years, the chemical fertilizer for the CF plot was split-supplied to DL Napiergrass three times on observation of regrowth on 29 April in 2008 and 18 June in 2009, and at the first and second defoliation as well, as for the first year, while fermented manure for both the LM and HM plots was supplied once per year at observation of regrowth. However, in the fourth year, no fertilizer was applied to any plot in order to assess the after-effect of fertilizer treatments in the preceding three years. The fertilizer treatment for IR was the same as for the LM and HM plots, while the rate for the CF plot was 78 kg N ha^{-1} $year^{-1}$—one-third the total supplied to DL Napiergrass.

4.3. Planting and Management Practices

Plots were cultivated by hand tractor once, and were established on 24 May 2007 by a single rooted tiller from overwintered stubble of DL Napiergrass without basal fertilizer application. IR was sown into the inter-row space at the rate of 20 kg ha^{-1} by hand at the third defoliation of DL Napiergrass every autumn. The plots received no irrigation.

4.4. Data Collection and Analytical Procedures

4.4.1. Soil Sampling and Chemical Analysis

Soils were sampled at a 0–10 cm depth [32] by a soil core sampler (volume: approximate 100 mL) with three replications before planting on 24 May 2007 and with three replications per plot at the last defoliation of DL Napiergrass on 29 October 2010. Soil samples were dried at room temperature for 4 days and were passed through a 2 mm sieve as a pretreatment before chemical analysis. Soil chemical properties were determined in duplicate by pH meter (Model: F-51, Horiba, Ltd., Kyoto, Japan) for pH (H_2O) and by conductivity meter (Model: CM-40S, DKK-TOA Corporation, Tokyo, Japan) for electrical conductivity only in 2007. TN and TC concentrations were determined in duplicate for each sample by an N and C determination unit (Model: Sumigraph NC-220F, Sumika Chemical Analysis Service, Ltd., Osaka, Japan).

4.4.2. Growth Attributes and DM Yield

Growth characteristics of DL Napiergrass of plant height and tiller density were determined for 10 plants per replicated plot, while for IR they were determined in three random areas by a 0.5 × 0.5 m quadrat (0.25 m^2) per replicated plot. DM yield of DL Napiergrass was determined randomly for two plants per replicated plot by defoliating plants using a hand sickle at 10 cm above the ground [1]. Above-ground samples were hand-separated by scissors into leaf blade (LB), stem inclusive of leaf sheath (ST), and dead parts, and oven-dried at 70 °C for 4 days to determine DM yield. Fresh weight (FW) of whole IR plants defoliated at 5 cm above ground using a hand sickle and a subsample of around 250 g FW were dried at 70 °C for 4 days to determine percentage DM. The DM yield was calculated according to Tarawali et al. [50] as DM yield (Mg ha^{-1}) = (Total FW × (DWss/FWss) × 10^{-2}), where Total FW = total fresh weight (g m^{-2}), DWss = dry weight of the subsample in grams, and FWss = fresh weight of the subsample in grams.

4.4.3. Overwintering Ability

Overwintering ability of DL Napiergrass plants was determined by assessing the percentage of overwintering plants (POP) and number of regrown tillers (RTN) per plant for 10 plants per replicated plot on 29 April 2008 and 18 June 2009.

4.4.4. Determination of Herbage Quality

After dried samples of DL Napiergrass and IR were ground by mill to pass through a 2 mm mesh, IVDMD of the herbage parts (LB and ST in DL Napiergrass) and whole herbage in IR were measured in duplicate by a pepsin–cellulase digestion method [51] using an in vitro incubator (Model: ANKOM DAISY II, ANKOM Technology, Macedon, NY, USA). The TN and TC concentrations of herbage parts and IR were determined in duplicate by an N and C determination unit (Sumigraph NC-220F, Sumika Chemical Analysis Service, Ltd.), and crude protein (CP) concentration was calculated by N concentration multiplied by 6.25.

4.5. Statistical Analysis

Analysis of variance was carried out for the single year's effect of fertilizer application on the growth and quality attributes using SPSS software for Windows ver. 16.0, Chicago, IL, USA. Differences in mean values were tested at the 5% level using the least significant difference. Proportional data were arcsine-transformed [52] to meet the assumption of normality and homogeneous variance prior to analysis.

5. Conclusions

Manure application at the low rate in the present study tended to lead to higher DM yield of DL Napiergrass and higher sustainability of growth than other fertilizer treatments in a hilly area in southern Kyushu. The availability of N and C components remaining in the soil below a depth of 0–10 cm after manure application showed that this field should still have enough nutrients for subsequent cultivation and might avoid N leaching. Furthermore, the results confirmed that a low rate of organic manure application would be a good substitute for chemical fertilizer application to maintain the equivalent N input and N output—{2,10}a key issue for environmentally friendly and healthy soil conditions.

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References

1. Ishii, Y.; Mukhtar, M.; Idota, S.; Fukuyama, K. Rotational grazing system for beef cows on dwarf napiergrass pasture oversown with Italian ryegrass for 2 years after establishment. *Grassl. Sci.* **2005**, *51*, 223–234. [CrossRef]
2. Ishii, Y.; Hamano, K.; Kang, D.J.; Rengsirikul, K.; Idota, S.; Nishiwaki, A. C_4-Napier grass cultivation for cadmium phytoremediation activity and organic livestock farming in Kyushu, Japan. *J. Agric. Sci. Technol. A* **2013**, *3*, 321–330.

3. Ishii, Y.; Dong-Jing, K.; Yamano, A.; Idota, S.; Fukuyama, K. Adaptability and extension activity of dwarf napiergrass in southern Kyushu and elsewhere since its introduction to Japan 15 years ago. In *Development and Impact of Sown Temperate Species, Proceedings of the 22nd International Grassland Congress, Sydney, Australia, 15–19 September 2013*; New South Wales Department of Primary Industry: Orange New South Wales, Australia, 2013.
4. Utamy, R.F.; Ishii, Y.; Idota, S.; Harada, N.; Fukuyama, K. Adaptability of dwarf napiergrass under cut-and-carry and grazing systems for smallholder beef farmers in southern Kyushu, Japan. *J. Warm Reg. Soc. Anim. Sci. Jpn.* **2011**, *54*, 65–76. [CrossRef]
5. Hasyim, H.; Wadi, A.; Ishii, Y.; Idota, S.; Fukuyama, K. Production and quality in dwarf napier grass pasture fertilized by digested effluent of manure under two-years of dairy cow-grazing in warm regions of Japan. *Am. J. Appl. Sci.* **2016**, *13*, 479–489. [CrossRef]
6. Jones, C.A. *C_4 Grasses and Cereals*; John Wiley and Sons: New York, NY, USA, 1985; p. 1189.
7. Hasyim, H.; Ishii, Y.; Wadi, A.; Idota, S. Effect of digested effluent of manure on soil nutrient content and production of dwarf Napiergrass in southern Kyushu, Japan. *J. Agron.* **2014**, *13*, 1–11. [CrossRef]
8. Singh, R.B. Environmental consequences of agricultural development: A case study from the Green Revolution state of Haryana, India. *Agric. Ecosyst. Environ.* **2000**, *82*, 97–103. [CrossRef]
9. Prasad, P.V.V.; Satyanarayana, V.; Murthy, V.R.K.; Boote, K.J. Maximizing yields in rice-groundnut cropping sequence through integrated nutrient management. *Field Crops Res.* **2002**, *75*, 9–21. [CrossRef]
10. Wopereis, M.C.S.; Tamélokpo, A.; Ezui, K.; Gnakpénou, D.; Fofana, B.; Breman, H. Mineral fertilizer management of maize on farmer fields differing in organic inputs in the West African savanna. *Field Crops Res.* **2006**, *96*, 355–362. [CrossRef]
11. Fageria, N.K.; Baligar, V.C.; Edwards, D.G. Soil-plant-nutrient relationship at low pH stress. In *Growth and Mineral Nutrition of Field Crops*, 3rd ed.; CRC Press, Taylor and Francis Group: New York, NY, USA, 1990; pp. 125–174.
12. Dormaar, J.F.; Lindwall, C.W.; Kozub, G.C. Effectiveness of manure and commercial fertilizer in restoring productivity of an artificially eroded dark brown Chernozemic soil under dryland conditions. *Can. J. Soil Sci.* **1988**, *68*, 669–679. [CrossRef]
13. Larney, F.J.; Janzen, H.H. Restoration of productivity to a desurfaced soil with livestock manure, crop residue and fertilizer amendments. *Agron. J.* **1996**, *88*, 921–927. [CrossRef]
14. Larney, F.J.; Olson, B.M.; Janzen, H.H.; Lindwall, C.W. Early impact of topsoil removal and soil amendments on crop productivity. *Agron. J.* **2000**, *92*, 948–956. [CrossRef]
15. Chang, C.; Sommerfeldt, T.G.; Entz, T. Soil chemistry after eleven annual applications of cattle feedlot manure. *J. Environ. Qual.* **1991**, *20*, 475–480. [CrossRef]
16. Kingery, W.L.; Wood, C.W.; Delaney, D.P.; Williams, J.C.; Mullins, G.L. Impact of long-term land application of broiler litter on environmentally related soil properties. *J. Environ. Qual.* **1994**, *23*, 139–147. [CrossRef]
17. Dormaar, J.F.; Chang, C. Effect of 20 annual applications of excess feedlot manure on labile soil phosphorus. *Can. J. Soil Sci.* **1995**, *75*, 507–512. [CrossRef]
18. Eghball, B.; Power, J.F. Phosphorus- and nitrogen-based manure and compost applications corn production and soil phosphorus. *Soil Sci. Soc. Am. J.* **1999**, *63*, 895–901. [CrossRef]
19. Schmidt, L.; Warnstorff, K.; Dörfel, H.; Leinweber, P.; Lange, H.; Merbach, W. The influence of fertilization and rotation on soil organic matter and plant yields in the long-term *Eternal Rye* trial in Halle (Saale), Germany. *J. Plant Nutr. Soil Sci.* **2000**, *163*, 639–648. [CrossRef]
20. Sommerfeldt, T.G.; Chang, C. Changes in soil properties under annual applications of feedlot manure and different tillage practices. *Soil Sci. Soc. Am. J.* **1985**, *49*, 983–987. [CrossRef]
21. Benbi, D.K.; Biswas, C.R.; Bawa, S.S.; Kumar, K. Influence of farmyard manure, inorganic fertilizers and weed control practices on some soil physical properties in a long-term experiment. *Soil Use Manag.* **1998**, *14*, 52–54. [CrossRef]
22. Pieterse, P.A.; Rethman, N.F.G. The influence of nitrogen fertilisation and soil pH on the dry matter yield and forage quality of *Pennisetum purpureum* and *P. purpureum* × *P. glaucum* hybrids. *Trop. Grassl.* **2002**, *36*, 83–89.
23. Sunusi, A.A.; Ito, K.; Tanaka, S.; Ishii, Y.; Ueno, M.; Miyagi, E. Yield and digestibility of napiergrass (*Pennisetum purpureum* Schumach) as affected by the level of manure input and the cutting interval. *Jpn. J. Grassl. Sci.* **1997**, *43*, 209–217. [CrossRef]

24. Tessema, Z.; Baars, R.M.T.; Yami, A. Effect of plant height at cutting and fertilizer on growth of Napier grass (*Pennisetum purpureum*). *Trop. Sci.* **2003**, *42*, 57–61.
25. Wadi, A.; Ishii, Y.; Idota, S. Effects of cutting interval and cutting height on dry matter yield and overwintering ability at the established year in *Pennisetum* species. *Plant Prod. Sci.* **2004**, *7*, 88–96. [CrossRef]
26. Ahmad, T.; Butt, N.M. Effect of precipitation and nitrogen fertilizer on napiergrass. In Proceedings of the 15th International Grassland Congress, Kyoto, Japan, 24 August–7 September 1985.
27. Chang, C.; Entz, T. Nitrate leaching losses under repeated cattle feedlot manure applications in southern Alberta. *J. Environ. Qual.* **1996**, *25*, 145–153. [CrossRef]
28. Sweeten, J.M. Cattle feedlot manure and wastewater management practices. In *Animal Waste Utilization: Effective Use of Manure as a Soil Resource*; Hatfield, J.L., Stewart, B.A., Eds.; CRC Press LLC: Boca Raton, FL, USA, 1998; p. 125155.
29. Torstensson, G.; Aronsson, H. Nitrogen leaching and crop availability in manured catch crop systems in Sweden. *Nutr. Cycl. Agroecosyt.* **2000**, *56*, 139–152. [CrossRef]
30. Chang, C.; Janzen, H.H.; Cho, C.M. Nitrous oxide emission from long-term manured soils. *Soil Sci. Soc. Am. J.* **1998**, *62*, 677–682. [CrossRef]
31. King, L.D.; Burns, J.C.; Westerman, P.W. Long-term swine lagoon effluent applications on 'Coastal' Bermudagrass: II. Effect on nutrient accumulation in soil. *J. Environ. Qual.* **1990**, *19*, 756–760. [CrossRef]
32. Snyder, C.S. Fertilization. In *Forages Volume II*, 6th ed.; Barnes, R.F., Nelson, C.J., Moore, K.J., Collins, M., Eds.; Blackwell Publishing: Ames, IA, USA, 2007; pp. 355–377.
33. Rahman, M.M.; Ishii, Y.; Niimi, M.; Kawamura, O. Effects of levels of nitrogen fertilizer on oxalate and some mineral contents in napiergrass (*Pennisetum purpureum* Schumach). *Grassl. Sci.* **2008**, *54*, 146–150. [CrossRef]
34. Nyaata, O.Z.; O'Neill, M.K.; Dorward, P.T.; Keatinge, J.D.H. Harvesting strategies for improved mixtures of Calliandra and Napier Grass in the central Kenyan highlands. *J. Sustain. Agric.* **2002**, *19*, 77–95. [CrossRef]
35. Tekletsadik, T.; Tudsri, S.; Juntakool, S.; Prasanpanich, S. Effect of dry season cutting management on subsequent forage yield and quality of ruzi (*Brachiaria ruziziensis*) and dwarf napier (*Pennisetum purpureum* L.) in Thailand. *Kasetsart J. (Nat. Sci.)* **2004**, *38*, 457–467.
36. Jørgensen, S.T.; Pookpakdi, A.; Tudsri, S.; Stölen, O.; Ortiz, R.; Christiansen, J.L. Cultivar-by-cutting height interactions in Napier grass (*Pennisetum purpureum* Schumach) grown in a tropical rain-fed environment. *Acta Agric. Scand. Sect. B—Plant Soil Sci.* **2010**, *60*, 199–210. [CrossRef]
37. Banik, P.; Ghosal, P.K.; Sasmal, T.K.; Bhattacharya, S.; Sarkar, B.K.; Bagchi, D.K. Effect of organic and inorganic nutrients for soil quality conservation and yield of rainfed low land rice in sub-tropical plateau region. *J. Agron. Crop Sci.* **2006**, *192*, 331–343. [CrossRef]
38. Boparai, B.S.; Singh, Y.; Sharma, B.D. Effect of green manure (*Sesbania aculeate*) on physical properties of soil and growth of rice-wheat and maize-wheat cropping system. *Int. Agrophys.* **1992**, *6*, 95–101.
39. Hasyim, H.; Ishii, Y.; Wadi, A.; Sususi, A.A.; Fukagawa, S.; Idota, S. Residual effects of composted digested effluent on growth of dwarf Napier grass in warm regions of Japan. *J. Exp. Biol. Agric. Sci.* **2016**, 74–84. [CrossRef]
40. Humphreys, L.R. *Tropical Pastures and Fodder Crops*, 2nd ed.; Longman Scientific & Techinical: London, UK, 1987; pp. 1–154.
41. Crowder, L.V. Potential of tropical zone cultivated forages. In *Potential of the World Forages for Ruminant Animal Productions*; Winrock Intern Livestock Research and Training Centre: Morrilton, AR, USA, 1977.
42. Mohammad, N.; Butt, N.M.; Qamar, I.A. Effect of nitrogen fertilization and harvesting intervals on the yield and nutritional value of napier grass. *Pakistan J. Agric. Res.* **1988**, *9*, 478–482.
43. Utamy, R.F.; Ishii, Y.; Iwamura, K.; Idota, S. Effect of weed control on establishment and herbage production in dwarf Napiergrass. *J. Life Sci.* **2014**, *8*, 46–50.
44. Kobayashi, T.; Nishimura, S. Winter hardiness and carbohydrate reserve of some tropical and subtropical grasses as affected by the final cutting date in autumn. *J. Jpn. Grassl. Sci.* **1978**, *24*, 27–33.
45. Van Soest, P.J. *Nutritional Ecology of the Ruminants*, 2nd ed.; Comstock Publishing Associates, Cornell University Press: Ithaca, NY, USA, 1994; pp. 1–476.
46. Ishii, Y.; Ito, K.; Numaguchi, H. Seasonal changes in the dry matter digestibility of individual tillers of napiergrass at two sites of different altitudes. In Proceedings of the 17th International Grassland Congress, Rockhampton, Australia, 18–21 February 1993; pp. 2010–2011.

47. Fukagawa, S.; Ito, K.; Ishii, Y. Changes in respiratory activity and dry matter disappearance with aging in napiergrass (*Pennisetum purpureum* Schumach). *Jpn. J. Grassl. Sci.* **2000**, *46*, 167–174. (In Japanese) [CrossRef]
48. Fadare, D.A.; Bamiro, O.A.; Oni, A.O. Energy and cost analysis of organic fertilizer production in Nigeria. *Energy* **2010**, *35*, 332–340. [CrossRef]
49. Japan Meteorology Agency. Available online: http://www.data.jma.go.jp/obd/stats/etrn/ (accessed on 2 November 2010).
50. Tarawali, S.A.; Tarawali, G.; Larbi, A.; Hanson, J. *Evaluation of Forage Legumes, Grasses and Fodder Trees for Use as Livestock Feed*; International Livestock Research Institute: Nairobi, Kenya, 1995; Available online: http://www.plantpath.cornell.edu/mba_project/ciepca/exmats/forage.pdf (accessed on 27 May 2011).
51. Goto, I.; Minson, D.J. Prediction of the dry matter digestibility of tropical grasses using pepsin-cellulase assay. *Anim. Feed Sci. Technol.* **1977**, *2*, 247–253. [CrossRef]
52. McDonald, J.H. *Handbook of Biological Statistics*, 2nd ed.; Sparky House Publishing: Baltimore, MD, USA, 2009; Available online: http://udel.edu/~mcdonald/stattansform.html (accessed on 2 November 2010).