Science and Humanities Journal 17: 17-32 (2023)
DOI: 10.47773/shj.1998.171.2

Nitrogen Use Efficiency Evaluation in Sugarcane (*Saccharum officinarum* L.) at Early Growth Stage using ^{15}N Isotope Tracer Technique

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ABSTRACT

Sugarcane is one of the most important crops for renewable energy and biomass production which considerably exhausts the available macro-and micronutrients in the soil especially nitrogen (N). Fertilization of N has been a practice in sugarcane production to attain greater yield. However, excessive application of N fertilizer can cause harmful effects on the environment aside from the additional cost of production and lowers nitrogen use efficiency (NUE) in sugarcane plants. Thus, this study was conducted to evaluate the NUE of sugarcane at the early growth stage and determine the concentration of N in different plant parts from the applied ^{15}N-labeled urea. The experiment was laid out in a randomized complete block design with five levels of N: T_1 – control, T_2 – 40kg N ha^{-1}, T_3 – 80 kg N ha^{-1}, T_4 – 120 kg N ha^{-1} and T_5 – 160 kg N ha^{-1}. Results revealed that regardless of days after ^{15}N labeled urea application, significantly highest NUE of sugarcane plant parts was recorded when N was applied at a lower level (40 kg N ha^{-1}) which eventually translated to a significantly higher overall fertilizer NUE of the plant among other treatments. Moreover, among the different plant parts, sugarcane plant stalk tissues accumulated a significantly higher level of ^{15}N regardless of N levels.

Keywords: ^{15}N isotope, nitrogen use efficiency, sugarcane, tracer technique

INTRODUCTION

In many countries in the tropics and sub-tropics, sugarcane plays a major component in the economy which accumulates biomass as high as 550 kg ha day^{-1} and can be locally processed into added-value crops such as molasses, ethanol, energy, and sugar (Moore & Botha 2013). In the Philippines, sugar production accounts for 1.03% of the gross national product and 3.37% in terms of total agricultural revenue with production in Negros as the highest (55%), Mindanao at 21%, Luzon at 14% and 7%, and 3% in Panay and the remaining in Eastern Visayas '(Padilla-Fernandez & Nuthall 2010).

As a perennial crop, sugarcane exhausts a considerable amount of macronutrients, among which is nitrogen (N). Suman et al (2008) reported that

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that sugarcane extracts about 205 kg N for yielding a crop of 100 t ha^{-1} and for sustaining productivity, N is replenished every year through chemical fertilizer at the rate of 150 kg N ha^{-1} in the sugarcane plant crop and 200 kg N ha^{-1} for its ratoon crop. But the excess application of N fertilizer has contributed to the undesirable effects on the environment such as acidification of soil and leaching into underground water (Franco et al 2011; Ghiberto et al 2009). Aside from these, it could also result in lower nitrogen use efficiency (NUE) (Swaminathan 2018). Unlike many crop species, NUE in sugarcane has not been properly understood (Zhao et al 2014). To decrease the environmental pollution due to excessive fertilization, an improved understanding of plant physiology and NUE in sugarcane is required so that applied N is properly utilized by crops – (Whan et al 2010; Zhao et al 2014). One common tool is the use of stable isotope which reveals the nutrient requirement of plants (Lina et al 2009) and the fate of applied N into the different plant parts (Oliveira et al 2017). This study aimed to determine and investigate the NUE of sugarcane using labeled urea (^{15}N) source and the concentration of N in different parts of sugarcane from the applied ^{15}N-labeled urea at the early growth stage.

MATERIALS AND METHODS

Soil Sample Collection, Preparation and Analyses

Bulk samples from the 0-20 cm soil depth were collected from a sugarcane plantation area in Brgy. Catmon, Ormoc City, Leyte. The soils were air-dried for 2-3 days, pulverized, and sieved using a 2 mm wire screen to remove further big soil clods and were used for potting. The subsample of about one kg was subsequently taken for chemical analyses and the rest was prepared for bagging. The subsamples for chemical analyses passed through a 2-mm and 0.425-mm sieve and were stored in a plastic bag until use for the initial and final determination of soil chemical properties. Soil pH was determined potentiometrically using distilled water at a soil-solution ratio of 1:2.5 (ISRIC 1995); total N, total carbon (C) and ^{15}N were analyzed using elemental analyzer coupled with isotope ratio mass spectrometer following the method of Gremaud and Hilkert (2008). Available phosphorus (P) was extracted using the Bray No. 2 method of Bray and Kurtz (1945) and Murphy and Riley (1962) for color development and quantified by measuring the percent absorbance at 880 nm using Spectronic 20 and exchangeable potassium (K) was extracted using sodium cobalt nitrite (Peech & English 1945). Except for total N, C and ^{15}N which was analyzed at the Philippine Nuclear Research Institute (PNRI), the rest of the soil parameters were quantified at Central Analytical Services Laboratory (CASL) and Soil Research Testing and Plant Analysis Laboratory (SRTPAL), Visayas State University, Visca, Baybay City, Leyte.

Pot Experiment

Establishment of the ^{15}N fertilizer, experimental design and layout

For the pot experiment, one tissue-cultured seedling of sugarcane (Phil sugarcane variety 99-1793) obtained from Sugar Regulatory Administration in Bacolod, Negros Occidental was planted in polyethylene pots with five kg of soil. Fertilizer treatments consisted of five N amounts (0, 40, 80, 120, and 160 kg N ha^{-1}). ^{15}N-labeled urea at 1.28 atom percent excess (APE) was used to supply the needed amount of N per pot. The ^{15}N-labeled urea was applied in split (the first half was applied 2 weeks after replanting and the other half was applied 1 month after planting) at 10 cm from the seedling and was evenly distributed at a depth of 5 cm around the seedling. Treatment pots were laid out in a randomized complete block design (RCBD) with three replicates and three treatment pots per replicate. Selective sampling was done at 1, 3, and 5 months after planting, hence, 45 pots were harvested per sampling and a total of 135 pots were prepared. The same amount of $P_2O_5$and K_2O (105 and 520 kg ha^{-1}) were applied in T_2-T_5 treatments using ordinary superphosphate (14.58 g pot^{-1}) and muriate of potash (21.69 g pot^{-1}) as recommended by Sarimong (2017).

Growth Parameters of Sugarcane

The plant height (cm) was determined monthly by measuring the height of the primary tillers of each plant from the base to the tip of the last dewlap. The leaf area index (LAI) was recorded monthly using a leaf area meter. The total number of tillers was recorded at monthly intervals by counting the total number of tillers per plant at one month after the ^{15}N application and was continued for five months. The number of leaves was taken each month by counting the number of fully expanded leaves of each primary tiller up to harvest. Chlorophyll reading was measured using a chlorophyll meter. Three individual plants from each treatment's replicate were destructively sampled at 1, 3, and 5 months after the ^{15}N applications. Each sugarcane plant was carefully excavated with shovel and knife to capture all the roots. Above-ground biomass was recorded separately from the below-ground biomass (roots).

Nitrogen Uptake by Sugarcane

Plant parts such as leaves, stalk, and roots were sampled at the 1, 3, and 5 months after ^{15}N application. As a result, a new sugarcane plant with a different size will be harvested every 1, 3, and 5 months. Collected plant samples were washed with distilled water, oven-dried for 3 days, ground and ^{15}N, total N and C were analyzed at PNRI, Diliman, Quezon City, Metro Manila. Fertilizer nitrogen use efficiency (FNUE) of sugarcane from the applied labeled urea was calculated using the following formula from IAEA (2001):

$$\text{Equation 1 } FNUE\ (\%) = \frac{FNY}{Rate\ of\ N\ application} \times 100$$

where:

FNUE = fertilizer nitrogen use efficiency
FNY = fertilizer nitrogen yield

$$\text{Equation 2 } FNUE\ \left(\frac{ha}{kg}\right) = \frac{N\ yield\ \left(\frac{ha}{kg}\right) \text{ x } \%\ N\ d\ f\ f}{100}$$

$$\text{Equation 3 } N \text{ yield } \left(\frac{ha}{kg}\right) = \frac{\text{DM of each plant part x } \%\ N}{100}$$

$$\text{Equation 4 } \%\ N\ d\ f\ f = \frac{atom\ \%\ N - 15\ excess\ of\ plant}{atom\ \%\ N - excess\ of\ fertilizer}$$

where:

FW = fresh weight
DM = dry matter
Ndff= nitrogen derived from fertilizer
FNY = fertilizer nitrogen yield

Statistical Analysis

The analysis of variance (ANOVA) for all parameters was carried out using Statistical Tool for Agricultural Research (STAR) v. 2. 0. 1. If found significant, the treatment means were compared using Least Significant Difference (LSD) test at 5% level of significance.

RESULTS AND DISCUSSION

Table 1 presents the initial chemical properties of soil used in the study. The soil shows a moderately acidic, very low amount of total C, low amount in total N with high available P and exchangeable K (Landon, 1991). The observed soil acidity and low amounts of C, - and N of the soil could be due to the continuous planting of sugarcane and the use of acid-releasing inorganic fertilizers such as urea. On the other hand, high amounts of available P and K is due to the residual contents of applied fertilizers from the soil which should be considered for the next cropping season to reduce fertilizer requirement.

Table 1. Initial chemical properties of soil in Catmon, Ormoc City

PROPERTY	VALUE	Critical Value Landon (1991)
pH (1:2.5 soil to H_2O)	5.70	<6.50 ->7.40
Total N (%)	0.12	<0.10 - >0.50
TOC (%)	1.23	<2.0 - >10
Available P (mg kg^{-1} soil)	82.80	<5.00 - >50.00
Exchangeable K (meq$100g^{-1}$ soil)	1.02	<0.15 - >0.50
^{15}N natural abundance (%)	0.37	

The study showed significant differences in soil chemical properties with the fertilized pots such as lower pH, total C (%), total N (%), and ^{15}N (%) content (Fig. 1). The decreased values in soil pH may be attributed to the application of urea-based fertilizer which might be the source of soil C but have caused an acidifying effect to the soil through the combined reaction of urea hydrolysis and nitrification (Chen et al & Hartemink 2008). This result agrees with the findings of Hati et al (2008) who also reported a continuous decrease in soil pH with an increased application level of N and a reduced NUE in soil-nutrient transport. Furthermore, the study also showed an evident decrease in soil total N and ^{15}N as the month progresses which is expected as it is being the only source of nitrogen for the crop's us e for its physiological processes during its growth. N uptake of sugarcane during the early growth stage is critical to sustain its needs in producing biomass and to attain a sustainable level of productivity (Franco 2011). This result conforms to the studies of Coale et al (1993) and Meyer (2013) where the early growth stage of sugarcane is generally characterized as the most rapid nutrient uptake stage for its biomass accumulation which can deplete an average of 1.16 kg N t^{-1}.

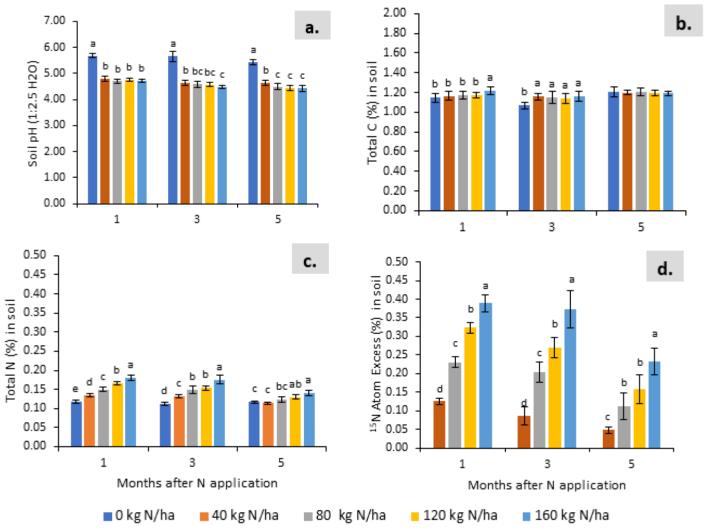

Figure 1. Mean of a) Soil pH (1:2.5 H_2O), b) Total C (%), c) Total N (%) and d) ^{15}N Atom Excess (%) in soil planted with Phil sugarcane variety 99-1793 at 1, 3 and 5 months after ^{15}N-labeled urea application

Influence of Fertilization on the Morphological and Physiological Parameters of Sugarcane

Application of N fertilizer to plants is vital for its growth and development which significantly increases and enhances yield by playing a very important role in biochemical and physiological functions (Leghari et al 2016). In sugarcane, N is a restrictive factor for continuous sugarcane output where the proper amount can remarkably increase plant productivity (Mariano et al 2016; Yang et al 2019). The results of the study with the application of ^{15}N-labeled urea showed a significant effect on the growth parameters of sugarcane plants during the 1, 3, and 5 months of the conduct of the experiment (Table 2). Increases in plant height, LAI, and the number of leaves were recorded at one month after ^{15}N application and were significantly pronounced when applied with 40 kg N ha^{-1} (T_2). In contrast, the lowest values were observed when applied with 160 kg N ha^{-1} (T_5). This result conforms with Dinh et al. (2017) where plant height and total number of sugarcane leaves were significantly higher in lower N (90 kg N ha^{-1}) compared with sugarcane applied with higher N levels (180 and 270 kg N ha^{-1}). In the third month of the experiment, despite not being significantly affected by N levels, T_2 has taller plants while T_5 has the shortest. Furthermore, significantly better tiller count and chlorophyll reading were recorded from fertilized sugarcane plants compared to the control. Previous study by Zeng et al (2020) indicated that chlorophyll content in leaves is closely related to the growth and yield of the plant. In this study, high N fertilization led to a significant increase in chlorophyll reading and tiller count, suggesting that N application primarily affects the plant's capacity for photosynthetic energy storage. This capacity is then jointly determined by the number of tillers, which may then determine the number of millable canes of sugarcane at a later stage of growth. On the fifth month, among the morphological and physiological parameters of sugarcane, the chlorophyll reading, and above-ground biomass were significantly affected by the application of ^{15}N-labeled urea. The fertilized pots recorded comparable values of chlorophyll reading but are significantly different from the control. Moreover, T_2 attained a greater weight in the above-ground biomass among the different treatments with control as the lowest. Although not significantly different from the previous months, T_2 has maintained much greater values in terms of tiller count, LAI, above-ground, and below-ground biomass despite receiving the lowest amount of fertilizer. This result could indicate that sugarcane has a lower N requirement during the early stages of growth and should be taken into account to avoid excessive fertilization. Low N need of sugarcane was documented by Muchow et al (1996) where application of 55 kg N ha^{-1} in sugarcane maximized stalk biomass, cane yield and sucrose for early harvest. In addition, although not significantly different, Lofton and Tubaña (2015) observed a much greater values in stalk weight and cane tonnage with the application of 45 kg N ha^{-1} compared to plants applied with 135 kg N ha^{-1}.

Table 2. Growth parameters of Phil sugarcane variety 99-1793 at 1, 3, and 5 months after ^{15}N-labeled urea application

TREATMENT	PLANT HEIGHT (CM)	TILLER COUNT	LAI (M^2/M^2)	NUM. OF LEAVES	STALK DIAM. (MM)	CHLOROPHYLL READING (SU)	ABOVE GROUND BIOMASS (G)	BELOW GROUND BIOMASS (G)
First Month								
T_1 - 0 kg N ha^{-1}	133.83^{bc}	2.33	1.78^{bc}	8.00^{a}	13.24	6.79	15.71	9.07
T_2 - 40 kg N ha^{-1}	138.71^{a}	3.67	1.97^{a}	7.67^{ab}	10.95	7.54	20.03	6.43
T_3 - 80 kg N ha^{-1}	136.30^{ab}	4.33	1.81^{abc}	7.00^{ab}	10.35	7.50	18.34	7.15
T_4 - 120 kg N ha^{-1}	134.11^{abc}	3.33	1.92^{ab}	6.67^{bc}	10.94	7.52	19.66	6.01
T_5 - 160 kg N ha^{-1}	129.99^{c}	3.67	1.69^{c}	5.67^{c}	9.26	6.40	15.80	7.49

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MEAN	134.59	3.47	1.83	7.00	10.95	7.15	17.91	7.23
CV (%)	1.84	30.25	5.18	9.58	15.94	23.31	10.59	21.57
Third Month								
T_1 - 0 kg N ha $^{-1}$	141.53	1.50^b	2.64	5.56	14.46	21.30^b	16.53	17.19
T_2 - 40 kg N ha $^{-1}$	147.00	5.89^a	3.53	5.11	15.79	39.99^a	32.13	17.34
T_3 - 80 kg N ha $^{-1}$	138.88	5.33^a	3.28	5.56	15.16	38.94^a	25.44	14.63
T_4 - 120 kg N ha $^{-1}$	142.88	7.56^a	3.47	5.17	15.92	40.51^a	25.89	16.08
T_5 - 160 kg N ha $^{-1}$	137.33	5.13^a	2.93	4.51	15.73	40.79^a	16.10	11.89
MEAN	141.52	5.08	3.17	5.18	15.41	36.31	23.22	15.43
CV (%)	6.53	28.14	15.84	11.85	3.84	11.92	32.3	17.71
Fifth Month								
T_1 - 0 kg N ha $^{-1}$	152.40	1.33	2.33	7.44	15.87	19.55^b	36.01^d	19.43
T_2 - 40 kg N ha $^{-1}$	138.87	8.33	2.46	6.00	17.72	38.15^a	85.17^a	32.95
T_3 - 80 kg N ha $^{-1}$	137.93	7.56	2.35	6.67	18.00	42.06^a	68.58^{bc}	19.86
T_4 - 120 kg N ha $^{-1}$	135.60	7.89	2.27	6.11	18.00	43.17^a	76.00^{ab}	30.45
T_5 - 160 kg N ha $^{-1}$	126.42	6.56	2.45	5.89	18.74	40.80^a	56.46^c	28.53
MEAN	138.24	6.33	2.35	6.42	17.67	36.75	64.44	26.25
CV (%)	10.03	54.57	13.2	9.61	5.66	9.21	11.22	34.98

Treatments within a column having the same letter/s are not significantly different from each other at 5% level of significance *LAI – Leaf Area Index; SU – SPAD Units*

Influence of ^{15}N fertilization on N Uptake of Sugarcane

The application of ^{15}N-labeled urea showed a significant difference in the atom percent excess in leaf, stalk, and root parts in sugarcane (Table 3). During the first and third month after fertilizer application, T_5 recovered a significantly higher ^{15}N content in leaves compared to T_3 and T_2 but is only comparable to T_4. Up until the fifth month, significantly higher ^{15}N content was observed in T_5 compared to other treatments which are expected because T_5 received the highest level of ^{15}N at the start of the study (4.00 ^{15}N g pot^{-1}). Aside from ^{15}N content, a significant difference in nitrogen derived from fertilizer (Ndff) and soil nitrogen yield (SNY) in sugarcane leaves was also observed. Ndff (%) in T_5 and T_4 is mostly comparable but significantly higher compared to the remaining treatments. Additionally, SNY (g pot^{-1}) under T_4 and T_5 are significantly lower towards the end of the study compared to T_2. This means that N uptake is mostly derived from the applied ^{15}N fertilizer and not from the soil N. This observation was similar to the study of Franco et al (2007) where the highest accumulation of N was noted on the plants with a higher level of fertilizer applied.

The total N content in sugarcane leaves applied with ^{15}N-labeled urea in 1, 3, and 5 months showed a significantly greater N concentration than in unfertilized pots (Figure 2a). During each investigated month, significantly lower N content in leaves was recorded from control plants and a comparable N content in the remaining treatments. Regardless of levels of N in ^{15}N applied pots, remarkably decreasing N content in sugarcane leaves from the first month to the fifth month was observed although not statistically different as the month progresses. Decreasing N content in leaves may be attributed to redistribution to other organs such as shoots, roots, and rhizomes since N is a highly mobile nutrient in plants (Vieira-Megda et al

2015). This nutrient translocation is possibly a mechanism that ensures a vigor root system for the ratoon crop (Trivelin et al., 2002). In contrast, increased total N yield in sugarcane leaves as the month progresses was significantly affected by the application of ^{15}N-labeled urea (Figure 2d).

Total N yield is the dry matter yield of each plant part multiplied by the N concentration in plants (as shown in Eq. 2). In the whole duration of the experiment, among the different levels of N, T_2 despite receiving the lowest amount of ^{15}N fertilizer exhibited a significantly higher total N yield in sugarcane leaves compared to untreated pots and a quantity comparable to T_3 and T_4, while T_5 exhibited the lowest total N yield despite receiving the highest amount of ^{15}N.

Table 3. ^{15}N Atom Excess (%), Ndff (%), FNY (g pot^{-1}) and SNY (g pot^{-1}) in different plant parts of Phil sugarcane variety 99-1793 at 1, 3, and 5 months after ^{15}N-labeled urea application

TREATMENT	^{15}N ATOM EXCESS (%)			Ndff (%)			FNY (g pot^{-1})			SNY (g pot^{-1})		
	First	Third	Fifth	First	Third	Fifth	First	Third	Fifth	First	Third	Fifth
	Leaf											
T_2 - 40 kg N ha^{-1}	0.42^{c}	0.56^{c}	0.61^{d}	32.94^{c}	43.72^{c}	47.81^{d}	0.09	0.11	0.17	0.18	0.14^{a}	0.19^{a}
T_3 - 80 kg N ha^{-1}	0.51^{b}	0.76^{b}	0.81^{c}	40.09^{b}	59.48^{b}	63.07^{c}	0.09	0.15	0.23	0.14	0.10^{ab}	0.13^{b}
T_4 - 120 kg N ha^{-1}	0.64^{a}	0.84^{a}	0.89^{b}	49.79^{a}	65.75^{a}	68.99^{b}	0.13	0.15	0.25	0.13	0.08^{b}	0.11^{bc}
T_5 - 160 kg N ha^{-1}	0.65^{a}	0.88^{a}	0.96^{a}	50.26^{a}	68.17^{a}	74.98^{a}	0.12	0.13	0.25	0.12	0.06^{b}	0.08^{c}
MEAN	0.56	0.76	0.82	43.27	59.28	63.71	0.11	0.14	0.23	0.14	0.10	0.13
CV (%)	3.43	2.62	1.47	3.27	2.45	1.54	14.74	21.23	17.47	10.32	23.58	16.48
	Stalk											
T_2 - 40 kg N ha^{-1}	0.53^{c}	0.52^{d}	0.60^{d}	41.10^{c}	40.01^{d}	46.97^{d}	0.07	0.14	0.23^{c}	0.10^{a}	0.21^{a}	0.26^{a}
T_3 - 80 kg N ha^{-1}	0.69^{b}	0.72^{c}	0.80^{c}	54.03^{b}	55.95^{c}	62.14^{c}	0.09	0.15	0.28^{bc}	0.08^{b}	0.12^{b}	0.17^{b}
T_4 - 120 kg N ha^{-1}	0.72^{b}	0.81^{b}	0.89^{b}	56.08^{b}	63.27^{b}	69.07^{b}	0.10	0.22	0.41^{a}	0.08^{b}	0.13^{b}	0.18^{b}
T_5 - 160 kg N ha^{-1}	0.78^{a}	0.85^{a}	0.97^{a}	60.42^{a}	66.27^{a}	75.20^{a}	0.10	0.16	0.38^{ab}	0.06^{c}	0.08^{b}	0.13^{b}
MEAN	0.68	0.72	0.81	52.91	56.37	63.44	0.10	0.17	0.33	0.08	0.14	0.18
CV (%)	3.58	2.22	1.72	3.46	2.34	1.71	17.93	26.75	19.9	7.45	27.02	19.42
	Roots											
T_2 - 40 kg N ha^{-1}	0.45^{c}	0.45^{c}	0.54^{d}	34.57^{c}	35.15^{c}	42.36^{d}	0.04	0.08^{c}	0.15	0.08	0.15^{a}	0.21
T_3 - 80 kg N ha^{-1}	0.51^{bc}	0.63^{b}	0.76^{c}	39.01^{bc}	49.23^{b}	59.06^{c}	0.06	0.12^{b}	0.20	0.09	0.13^{b}	0.14
T_4 - 120 kg N ha^{-1}	0.56^{b}	0.72^{a}	0.82^{b}	43.46^{b}	55.73^{a}	63.88^{b}	0.06	0.17^{a}	0.31	0.07	0.13^{b}	0.17
T_5 - 160 kg N ha^{-1}	0.65^{a}	0.73^{a}	0.89^{a}	49.47^{a}	56.71^{a}	69.18^{a}	0.07	0.14^{b}	0.35	0.07	0.10^{c}	0.15
MEAN	0.54	0.63	0.75	41.71	49.21	58.62	0.06	0.13	0.25	0.08	0.13	0.17
CV (%)	7.58	5.17	2.32	7.36	5.15	2.20	26.53	9.45	32.14	12.49	4.47	28.58

Treatments within a column having the same letter/s are not significantly different from each other at 5% level of significance

Ndff – Nitrogen derived from fertilizer; FNY – Fertilizer Nitrogen Yield; SNY – Soil Nitrogen Yield

In stalk tissues of sugarcane, nitrogen is very much needed accounting for about 0.3 to 2% of dry matter (Moore & Botha 2013). Wood (1990) reported that the application of N fertilizer can stimulate the growth of sugarcane stalks but could also decrease their quality. In this study, significant differences in ^{15}N content, Ndff, FNY, and SNY were observed in the stalk part during the early growth stage of sugarcane (Table 3). Pots under T_5 recovered significantly higher ^{15}N content and Ndff from the first month until the termination of the experiment compared to the other ^{15}N-fertilized pots. Regardless of treatments, ^{15}N and Ndff were observed to from the first month until the termination of the experiment compared to the other ^{15}N-fertilized pots. Regardless of treatments, ^{15}N and Ndff were observed to increase as the month progresses. Moreover, FNY in sugarcane stalk was only affected significantly towards the end of the study with T_4 as the highest. For the SNY in the stalk part, T_2 recovered a significantly higher amount of nitrogen from the soil which is anticipated since it received the lowest amount of ^{15}N-labeled urea, utilizing the native mineral nitrogen in the soil. As reported by Vieira-Megda et al (2015), sugarcane efficiently utilizes soil N for long growth cycle even without fertilization. Franco et al (2011) added that many studies using ^{15}N-labeled fertilizers show that most N absorbed by the plants comes from the soil since the contribution of N from fertilizers is low. A study conducted by Dourado-Neto et al (2010) revealed that despite the application of 300 g N ha^{-1} of ^{15}N-labeled inorganic fertilizer to sugarcane, 79% of unlabeled N (presumed to come from the mineralization of soil organic matter) was accounted of the total N in the crop.

Although total nitrogen in the stalk did not differ among treatments in the first month after fertilizer application, significant differences among treatments were observed in the third and fifth months with the greatest N uptake in treatments T_4 and T_5(Figure 2b). Irrespective of the levels of N, a surge of N content in sugarcane stalk was observed in the third month but declines towards the fifth month after ^{15}N application. The decreasing N content in stalk could possibly due to remobilization or translocation of N from stalks to leaves or roots as the total N yield is still increasing towards the fifth month (Franco et al 2011). This observation with decreasing N with time was also observed by the study of Meier et al (2006) on sugarcane grown in the wet tropics of Australia. As for the total N yield in sugarcane stalk (Figure 2e), a significant difference was observed after ^{15}N fertilization with T_4 as the highest compared to the unfertilized pots but only comparable to the rest of the treatments. The increasing value of total N yield was also depicted until the fifth month regardless of levels of N which is expected since the plants were in vegetative growth (development of harvestable parts). This was previously reported by Boschiero et al (2018) where N yield in stalk parts increased as days after fertilization progresses irrespective with fertilizer sources.

Roots enable the intake of water and nutrients from the soil, and it serves as anchorage. With fertilization of N, it promotes root growth contributing to the absorption of nutrients by sugarcane (Oliveira et al 2017). In this experiment, significant differences were observed in ^{15}N content, Ndff, FNY, and SNY of sugarcane roots with the application of ^{15}N-labeled urea (Table 3). Significantly, T_5 recovered the highest amount of ^{15}N in sugarcane roots compared to other treatments while T_2 recovered the lowest from the first month until termination. Significantly, higher Ndff was observed in T_5 and T_4 in the third month of the experiment and with much higher in T_5 at the termination of the experiment. The application of ^{15}N fertilizer significantly affected the FNY of sugarcane roots in the third month, with T_5 as the highest and T_2 as the lowest in value. In contrast, T_2 recovered the significantly highest SNY in the third month of the experiment while T_5 recovered the lowest among the rest of the treatments. Total N accumulation in sugarcane roots showed that in the first month of the experiment, although not significantly different with T_4, T_3 had a significant higher total N content compared to the other treatments (Figure 2c). An evident increase in levels of total N from the first month up to the third month of the study was observed in treatments T_3, T_4, and T_5 while T_2 and control values were decreasing as the month progresses. Upon termination, only T_3 and T_5 maintained the increasing level of total N accumulated in the root part of the sugarcane. As for the total N yield in sugarcane roots, values continually increase from the first month up to the fifth month of the study with control having constantly the lowest values of total N yield (Figure 2f). Increasing total N yield of sugarcane roots conforms to the study of Robinson et al (2009) where rapid N capture is likely for the efficient vigorous early growth of sugarcane from the first month up to the third month of its development. Meanwhile, remobilization of captured N from sugarcane stalk to roots was also observed in the previous research of De Oliveira et al (2013). Effective N remobilization from the sugarcane stalk and leaves to the belowground stool at harvest was detected which implies a decrease in N removal from the field and provides an N source for the ratoon crop (Oliveira et al 2017).

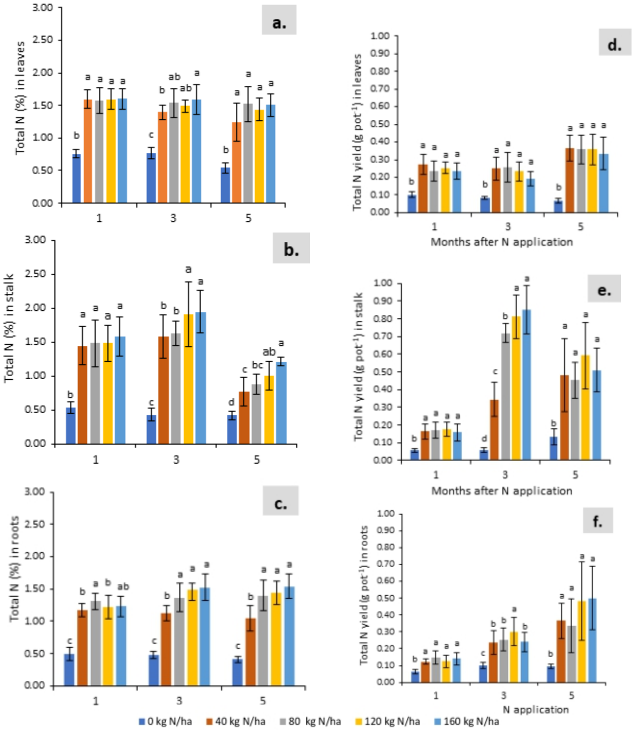

Figure 2. Total N (%) and Total N Yield (g pot^{-1}) in leaves, stalk and roots from ^{15}N and soil N of Phil sugarcane variety 99-1793 at 1, 3, and 5 months after ^{15}N-labeled urea application

Fertilizer Nitrogen Use Efficiency of Sugarcane

NUE is described as the efficiency of N fertilizer utilization in crop production (Brentrup & Palliere 2010). Higher values of NUE mean a crop produce more harvestable biomass per unit of N supplied (Yang et al 2019; Zeng et al 2020). In the present study, the FNUE was quantified using the ^{15}N stable isotope technique. Among the fertilized pots, lowest FNUE in sugarcane leaves, stalks and roots was observed in T_5 (Figure 3). Results revealed that T_5 produced the lowest number of leaves, above-ground, and below-ground biomass (Table 2) despite receiving the highest amount of N indicating luxury in N uptake. In contrast, T_2 which received the lowest amount of N had a higher FNUE compared to the other treatments throughout the conduct of the study which translated to significantly higher number in leaves, LAI and above-ground biomass. These findings revealed that the application of 40 kg N ha^{-1} resulted in better N uptake, greater biomass production and N use efficiency in different plant parts of sugarcane compared to pots with higher N fertilizer. Significant increase in FNUE in the different plant parts of sugarcane as time progresses regardless of the levels of N means that nitrogen needed by plants with time also increases (Bell 2014).

Significant differences in the overall FNUE at the early growth stage of sugarcane were also recorded after the application of labeled urea (Figure 4). Results showed that in the first month of the conduct of the study, plants under T_2 attained the highest FNUE of 20.70% on all of the plant parts compared to T_5 attaining a value of 7.17% only. A similar trend of the result was also observed in the third month with T_2 having the highest FNUE among the different treatments. At the end of the study, T_2 attained a significantly highest overall FNUE of 55.46%, comparable values in T_3 and $T_{4,}$ and significantly lowest overall FNUE in T_5 with 24.57%. Regardless of N rates applied, data revealed that in the early stage of growth, the overall FNUE of sugarcane, N uptake and biomass production increased linearly with time. Moreover, this study revealed that with increasing levels of N, N use efficiency of sugarcane plants decreases. This is similar to the findings of Thorburn et al (2017), where high values of NUE occurred at low N rates and decreasing N use efficiency was recorded with increasing N levels of fertilizer. The study of Hajari et al. (2015) also supported this claim where the significantly highest NUE was obtained at 4 mM NH4+-N compared to plants applied with 20 mM NH4+-N. Moore and Botha (2013) explained that excessive N application in sugarcane plants will only results to increase N uptake than the accumulation of biomass indicating that sugarcane has a capacity for luxury N uptake. Luxury consumption or yield plateau is well known to occur in sugarcane where increased N fertilization results in increased N uptake but not necessarily in biomass (Malavolta 1994). This phenomenon was described by Uribe et al (2013) and Mariano et al (2016) in sugarcane where plants take up more N than they actually need which causes production to remain constant and therefore, a waste of fertility or fertilizer.

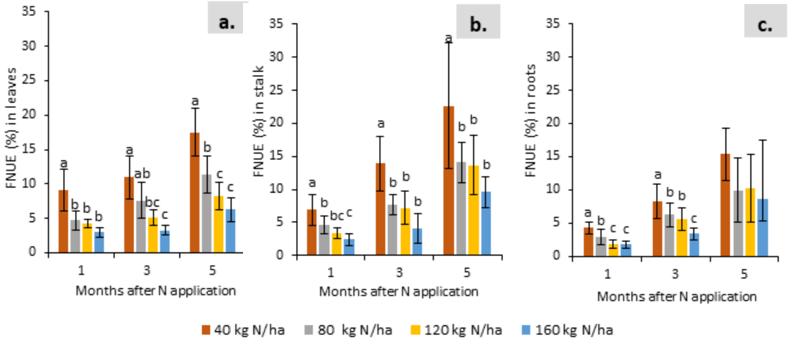

Figure 3. FNUE (%) in a) leaves b) stalk and c) roots of Phil sugarcane variety 99-1793 at 1, 3, and 5months after ^{15}N-labeled urea application

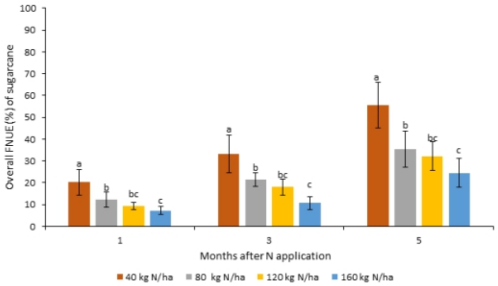

Figure 4. Overall FNUE (%) of Phil sugarcane variety 99-1793 at 1, 3, and 5 months after ^{15}N-labeled urea application

CONCLUSION AND RECOMMENDATION

Results of this study showed that application of fertilizer had evidently resulted in much acidic soil but with increased values in total N, total C, exchangeable P and K. Regardless of days after ^{15}N-labeled urea application, among treatments, T_5 (160 kg N ha^{-1}) plants attained a superior value of accumulated N in its plant tissues, however, it did not translate to higher production of biomass which indicates that sugarcane has a capacity for luxury N uptake. Moreover, the application of 40 kg N ha^{-1} resulted in significantly higher overall FNUE compared to other treatments. Among the different plant parts, stalk tissues accumulated higher levels of N regardless of N levels. Since the study was conducted in a protected area and was planted in pots, further work is needed in an open-field area using the same amount of fertilizer until the maturity stage of sugarcane. In addition, the quantification of N losses in the environment such as leaching, denitrification and volatilization should be considered to further investigate the FNUE of the sugarcane plants.

ACKNOWLEDGEMENT

The first author is grateful for the Graduate Scholarship from the Department of Science and Technology-Accelerated Science and Technology Human Resource Development Program (DOST-ASTHRD) and likewise to the Graduate Advisory Committee, Dr. Victor B. Asio and Dr. Marlito Jose M. Bande for their constructive comments. The author would also like to thank Philippine Nuclear Research Institute and the Sugar Regulatory Administration for funding this research endeavor.

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