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# Dynamics of Fertilizer Nitrogen Applied to Sweet Sorghum (*Sorghum bicolor* (L.) Moench) in the Semi-Arid Tropics

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## Abstract

Although sweet sorghum is in focus as a promising multi-purpose crop in semi-arid tropical regions, information on optimum nitrogen (N) management remains rather limited. To determine and understand the N dynamics in sweet sorghum-soil ecosystem, we evaluated the N recovery of applied fertilizer in the Alfisol field during the 2009 and 2010 rainy seasons. Sweet sorghum was grown under six N rates (0, 30, 60, 90, 120 and 150 kg N $ha^{-1}$) with split application (at 0, 30 and 60 days after sowing) and N concentration and N recovery efficiency (NRE) were measured. In addition, to determine the fate of basal and topdressed N, $^{15}N$-labeled urea was applied at each application timing within main plots of 90 kg N $ha^{-1}$. There was no significant difference in NRE calculated by the difference method between six N rates and N uptake rose with increasing N rate. The total dry weight and sugar yield increased at rates up to 90 kg N $ha^{-1}$ and higher N rates did not significantly affect sorghum productivity. As a total of basal and topdressing applications, 33% of the applied $^{15}N$-labeled urea was absorbed by the sorghum plant, while 36 and 30% remained in the soil and unaccounted for, respectively. The NRE calculated by the isotopic method was about 39 and 37% for topdressings at 30 and 60 days after sowing, respectively. The distribution ratio of absorbed N in leaves was higher for basal applications and topdressing at 30 days after sowing, while that in grains was higher for topdressing at 60 days after sowing, compared to the other application timings. In contrast to topdressings, the NRE was very low (13%) for basal N application: about 70% of the basal applied N remained in the soil at the physiological maturity. The results imply that the NRE might be increased by improving the ways in which basal N is applied.

**Discipline:** Biofuel/Soils fertilizers and plant nutrition
**Additional key words:** $^{15}N$-labeled urea, fertilizer application timing, nitrogen recovery efficiency

## Introduction

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a promising multi-purpose crop, since its grain can be used as food, the stalks to produce ethanol and bagasse and the stripped leaves for fodder and fuel. Compared to grain sorghum, which has been widely grown in semi-arid tropical regions, sweet sorghum is characterized by more biomass

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and higher sugar content in the stem juice. The wide adaptability of sweet sorghum to dry conditions as well as its multiple usages give it great potential to boost the income of smallholder farmers in the semi-arid tropics (Rao *et al.* 2009). However, information on the optimum nitrogen (N) input for sweet sorghum in semi-arid tropical regions remains scarce (Turgut *et al.* 2005, Reddy *et al.* 2008).

A few studies have been conducted to investigate the effect of N input on sweet sorghum productivity. Reddy *et al.* (2008) reported that input of 64 kg N $ha^{-1}$ (18 kg N $ha^{-1}$ as basal application and 46 kg N $ha^{-1}$ as topdressing) was sufficient to obtain the maximum sugar yield of sweet sorghum on Vertisols. In contrast, a higher amount of N input was recommended for sweet sorghum on Alfisols than Vertisols, because Alfisols, which contain a high ratio of sand, have lower soil fertility and water-holding capacity than those of Vertisols (Sahrawat 1984). In our previous study (Uchino *et al.* 2013a), for example, we investigated the effect of six rates of nitrogen inputs ranging from 0 to 150 kg N $ha^{-1}$ on sweet sorghum growth on Alfisols. Grain dry weight, juice volume and sugar yields peaked at 90 kg N $ha^{-1}$, severe lodging of the plants was observed at 150 kg N $ha^{-1}$ and it was ultimately concluded that an N rate varying from 90 to 120 kg N $ha^{-1}$ was optimum for sweet sorghum productivity.

To manage N more effectively in sweet sorghum production, it is important to understand the N dynamics of sweet sorghum-soil ecosystems in more detail, for which information regarding N uptake by sorghum and N recovery efficiency (NRE) of applied fertilizer is needed. Fertilizer N recovery by a crop can be estimated using two different methods. One is the 'difference method' and the other is the 'isotopic method' (Harmsen & Moraghan 1988). In the difference method, the amount of applied N taken up by a crop is estimated as the difference in total N uptake between fertilized and unfertilized plots (Harmsen 2003). Conversely, the isotopic method based on the use of stable isotopic $^{15}N$ allows the amount of N applied in various subsystems to be quantified, i.e. in soil and plants under field conditions. In this method, the amount of fertilizer N taken up by a crop is estimated from total N uptake and N isotope-ratio analysis of plant materials from fertilized treatments (Harmsen 2003). The difference and isotopic methods provide us with apparent and real NRE and are thus sometimes referred to as indirect and direct methods, respectively (Harmsen & Moraghan 1988).

Some studies investigated the NRE of grain sorghum. For example, Yamaguchi (1991) reported, in his review, that the NRE calculated by the difference method was 10% while the NRE by the isotopic method was 21%. Adu-Gyamfi *et al.* (1996) investigated the effects of N application timing on the NRE of grain sorghum; reporting that the NRE by difference method was 15.0% when fertilizer was applied as a basal application, but increased to 32.2% when fertilizer was applied at 40 days after sowing (DAS). Venkateswarlu *et al.* (1978) also studied the effects of fertilizer application methods on the NRE of the grain sorghum hybrid 'CSH1' and revealed that the NRE improved markedly when N was applied in two or three splits, compared to the single application in basal form.

Compared to grain sorghum, there is little information on the NRE of sweet sorghum, particularly in the semi-arid tropical regions on light-textured Alfisols. The objective of the present study is 1) to analyze the effect of a variation in N input with three split applications (basal and two topdressings) on N uptake by sweet sorghum using the difference method and 2) to evaluate the N dynamics in sweet sorghum grown under an optimum N input of 90 kg N $ha^{-1}$ in Alfisol fields in the semi-arid tropics by quantifying the N uptake and measuring the residual N in 0-90 cm soil layers using $^{15}N$-labeled urea by the isotopic method.

## Materials and methods

### 1. Study site

The study was carried out at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) at Patancheru (17.53 °N, 78.27 °E) in India during the 2009 and 2010 rainy seasons (June-October). The soil type was Alfisols with 1.11 and 0.76 g $kg^{-1}$ of N (by the Kjeldahl method) in the 0-15 cm soil layer during the 2009 and 2010 seasons, respectively. Detailed information on the other soil chemical properties is reported in Uchino *et al.* (2013a).

### 2. Un-labeled urea study

Detailed experimental procedures were described by Uchino *et al.* (2013a). Briefly, the promising sweet sorghum hybrid in India 'CSH22SS' was sown on 16 June, 2009 and 21 June, 2010 during the two seasons, using spacing of 60 cm between rows and 15 cm between plants. The experiment was arranged as a randomized complete block design with three replications and each plot measuring 6 × 10 m and 6 × 13 m in 2009 and 2010, respectively. Six rates of N (0, 30, 60, 90, 120 and 150 kg N $ha^{-1}$) were applied as un-labeled urea fertilizer (46% N) and these six treatments are hereafter referred to as 0N, 30N, 60N, 90N, 120N and 150N, respectively. For each N treatment except for 0N, 15 kg N $ha^{-1}$ was band-applied as basal and half the remaining N was band-applied as first and second topdressings at around 30 and 60 DAS, respectively. Other nutrients (phosphorus: 40 kg $P_2O_5$ $ha^{-1}$, sulfur: 200 kg gypsum $ha^{-1}$, boron: 0.475 kg B $ha^{-1}$, zinc: 50 kg $ZnSO_4$ $ha^{-1}$) were uniformly applied as basal dressing to all treatments in both growing seasons. Irrigation was given as furrow irrigation at 2, 10 and 42 DAS in the 2009 season and at 3 DAS in the 2010 season respectively.

Sorghum plants were harvested at 119 (90N, 120N, 150N) and 126 DAS (0N, 30N, 60N) in 2009 and at 115 (90N, 120N, 150N) and 122 DAS (0N, 30N, 60N) in 2010, because the date of the physiological maturity stage (i.e. when a seed black layer in 50% of the plants was visible) was delayed more in lower than higher N inputs (Uchino *et al.* 2013a). The sampling areas were 9.18 and 6.48 $m^2$ in 2009 and 2010, respectively. Twelve plants were randomly selected from the harvested samples, oven-dried at 60°C for more than 120 h, weighed, then ground (< 250 micron) using a cyclone sample mill (Udy Corporation, USA). Organic N + ammonia N (hereafter referred to as 'Kjeldahl N') concentration in each plant part was determined by the Kjeldahl method and N uptake (kg $ha^{-1}$) was calculated by multiplying the dry weight by the Kjeldahl N concentration. The juice of the remaining harvested samples was then extracted by using a common sugar cane crusher, whereupon the brix was measured using a hand-held refractometer (Master-α, Atago, Japan). Sugar yield was estimated according to the following equation, as reported by Reddy *et al.* (2005):

Sugar yield (t $ha^{-1}$) = {(brix (%) × 0.8746) + 0.1516}/100 × juice volume (kl $ha^{-1}$)

## 3. Labeled urea study

To evaluate the fate of applied N, three micro-plot treatments (basal $^{15}N$, 1st-topdress $^{15}N$ and 2nd-topdress $^{15}N$, which are referred to as B-$^{15}N$, $TP_1$-$^{15}N$ and $TP_2$-$^{15}N$ respectively) receiving $^{15}N$-labeled urea (10 atom% enrichment, Sigma-Aldrich, USA) were established within each main plot of 90N treatment of the un-labeled study in 2010 (Table 1). Each micro-plot (60 × 120 cm) containing eight plants in the central portion was enclosed with a metal frame extending to a depth of 10 cm below the soil surface, while granules of labeled urea were applied along the sorghum rows at a depth of 5 cm and other nutrients applied as basal dressing at the same rate as outside the micro plots.

All plants in the micro plots were harvested at the physiological maturity stage (115 DAS) and separated into their constituent parts (leaf, stem and grain). The dry weight was recorded after drying at 60°C for more than 120 h, whereupon the plants were ground (< 250 micron). Two soil samples from 0-15, 15-30, 30-60 and 60-90 cm depths were also collected from inside and outside the micro plots at 116 DAS. Roots, plant residues and gravel were removed from soil samples after air drying and the soil samples were ground. Inorganic N + organic N (hereafter referred to as 'total N') concentration in the plant and soil samples was determined using an NC analyzer (Sumigraph NC-220F, Sumika Chemical Analysis Service, Osaka, Japan). The powdered plant and soil samples were sealed into small tin capsules and introduced into an element analyzer (Flash EA-1112, Carlo Erba, Milan, Italy) connected to a continuous-flow isotopic ratio mass spectrometer (Delta XPplus, Thermo Finnigan, Hamburg, Germany) to determine $^{15}N$ abundance. All the analysis for total N concentration and $^{15}N$ abundance were performed at JIRCAS, Tsukuba, Japan.

## 4. Data processing and statistical analysis

Fertilizer NRE was computed by the 'difference method' ($NRE_{difference}$) and the 'isotopic method' ($NRE_{isotopic}$) (Hauck & Bremner 1976, Varvel & Peterson 1990). $NRE_{difference}$ was calculated by the following equation 1 in the un-labeled urea study.

$$NRE_{difference}\ (\%) = 100 \times (NF_{plant} - NC_{plant}) / N_{fertilizer} \quad \text{------ (Equation 1)}$$

where the $NF_{plant}$ (kg N $ha^{-1}$) constitutes the total N uptake by plants in the N fertilized plot, $NC_{plant}$ (kg N $ha^{-1}$) is the total N uptake by plants in the unfertilized plot (i.e. 0N) and $N_{fertilizer}$ (kg N $ha^{-1}$) is the rate of fertilizer N applied.

The $NRE_{isotopic}$ values of the plant and soil were calculated by the following equations 2 and 3, respectively, in the labeled urea study.

$$NRE_{isotopic}\ \text{of plant}\ (\%) = 100 \times \{NF_{plant} \times (AF_{plant} - AB_{plant}) / (A_{fertilizer} - AB_{plant})\} / N_{fertilizer} \quad \text{------ (Equation 2)}$$

where $AF_{plant}$ is atom% $^{15}N$ in plants grown with labeled urea, $AB_{plant}$ is atom% $^{15}N$ in plants grown with un-labeled urea and $A_{fertilizer}$ is atom% $^{15}N$ in fertilizer. The $AB_{plant}$ varied from 0.3684 to 0.3898% according to the constituent parts of plants and replications.

$$NRE_{isotopic}\ \text{of soil}\ (\%) = 10000 \times [\{NFsoil \times (AF_{soil} - AB_{soil}) / (A_{fertilizer} - AB_{soil})\} \times \text{soil depth (cm)} \times \text{bulk density}] / N_{fertilizer} \quad \text{------ (Equation 3)}$$

where $NF_{soil}$ (g N $kg^{-1}$) is the N concentration in soil in N fertilized plot, $AF_{soil}$ is atom% $^{15}N$ in soil grown with labeled

**Table 1. Details of each experimental treatment of $^{15}N$-labeled urea application timing**

| $^{15}N$-labeled urea application timing | Treatment code | Basal urea (15 kg N $ha^{-1}$) | 1st topdressing urea (37.5 kg N $ha^{-1}$) | 2nd topdressing urea (37.5 kg N $ha^{-1}$) |
|---|---|---|---|---|
| Basal $^{15}N$ | B-$^{15}N$ | Labeled | Un-labeled | Un-labeled |
| 1st-topdress $^{15}N$ | $TP_1$-$^{15}N$ | Un-labeled | Labeled | Un-labeled |
| 2nd-topdress $^{15}N$ | $TP_2$-$^{15}N$ | Un-labeled | Un-labeled | Labeled |

urea and $AB_{soil}$ is atom% $^{15}N$ in soil grown with un-labeled urea. The $AB_{soil}$ varied from 0.3687 to 0.3695% according to the soil depth and replications. The bulk density in each soil depth was 1.50 g $cm^{-3}$ (0-15 cm), 1.58 g $cm^{-3}$ (15-30 cm), 1.59 g $cm^{-3}$ (30-60 cm) and 1.46 g $cm^{-3}$ (60-90 cm), as reported by Pathak *et al.* (2013a), who investigated the soil physical properties of the same Alfisol field as our study.

Statistical analysis was conducted using the SPSS software (version 14.0J, SPSS Japan). Analysis of variance (ANOVA) was also performed via a combined model for the un-labeled urea study, where the year and N treatment were treated as fixed factors and replication as a random factor (McIntosh 1983). Differences between treatments were tested by the Tukey-Kramer method when ANOVA was significant.

## Results

### 1. Meteorological data

Figure 1 shows the daily mean air temperature and precipitation during growing seasons in 2009 and 2010. The air temperature often exceeded 30°C in the early growth stages and gradually decreased in the run up to the late growth stage, while precipitation was very low from 0 to 60 DAS in 2009. During this dry spell, the sorghum plants exhibited drought stress symptoms such as leaf rolling. Well-distributed rainfall was received and no drought stress symptoms were observed during the 2010 growing season. In particular, the rainfall was relatively high around the date of the second topdressing.

### 2. Un-labeled urea study

The main effect of the treatment was significant for the dry weight of the whole plant: as mean values of two years, although the dry weight rose with increasing N input, it did not differ significantly among 90N, 120N and 150N (Table 2). A significant interaction of year x treatment was also observed for the dry weight of the whole plant, but this interaction was of the non-crossover type, because the ranking of treatment was similar for both years (detailed data were shown in Uchino *et al.* (2013a)). Traits relating to sugar productivity (brix, juice volume and sugar yield) tended to increase at rates up to 90 kg N $ha^{-1}$ (Fig. 2: detailed data shown in Uchino *et al.* (2013a)).

Kjeldahl N concentration, Kjeldahl N uptake and $NRE_{difference}$ are shown in Table 2. Although the dry weight tended to be higher in 2010 than in 2009, the Kjeldahl N concentration was significantly lower in 2010 than in 2009 (Table 2), which revealed no significant difference in Kjeldahl N uptake (= computed by multiplying the dry weight by Kjeldahl N concentration) between two years. The N input treatment significantly affected Kjeldahl N concentrations in the leaf, stem and whole plant. The Kjeldahl N concentrations rose with increasing N input, but were unaffected by N input rates exceeding 120 kg N $ha^{-1}$. When comparing the effect of N input on N concentrations among the constituent parts of sorghum, it was larger in leaf than in the stem and grain: the N concentration in leaves rose by 121% when increasing N inputs from 0 to 150 kg N $ha^{-1}$, but those in stem and grain only increased by 36 and 4%, respectively.

$NRE_{difference}$ did not differ significantly between either years or treatments (Table 2). Regardless of the N input rates, about 34-48% (as mean values of the two years) of the applied N was apparently absorbed by sweet sorghum, which suggests that the amount of N not absorbed by the sorghum rose with the increase in the applied N rate: 17, 40, 49, 63 and 82 kg $ha^{-1}$ of N was not apparently absorbed

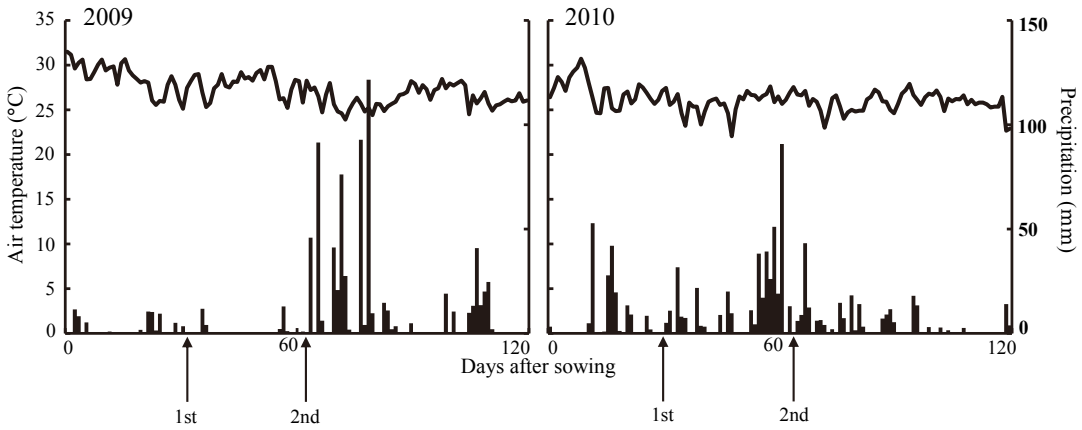


**Fig. 1. Daily average air temperature and precipitation during the growth periods in 2009 (left) and 2010 (right). Line: air temperature; Bar: precipitation. Arrows denote the timings of first and second topdressings.**

**Table 2. Dry weight, Kjeldahl nitrogen (N) concentration, N uptake and N recovery efficiency calculated by the difference method ($NRE_{difference}$) of sweet sorghum at the physiological maturity stage in un-labeled urea studies in 2009 and 2010.**

| | Dry weight (t ha$^{-1}$) [a] | Kjeldahl N concentration (%) | | | | Kjeldahl N uptake (kg ha$^{-1}$) | | | | $NRE_{difference}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | Whole | Leaf | Stem | Grain | Whole | Leaf | Stem | Grain | Whole | (%) |
| Year (Y) [b] | | | | | | | | | | |
| 2009 | 15.5 | 0.97 | 0.18 | 1.46 | 0.48 | 25.1 | 19.1 | 29.5 | 73.7 | 35.4 |
| 2010 | 18.3 | 0.87 | 0.14 | 1.14 | 0.41 | 24.4 | 17.2 | 33.6 | 75.2 | 51.0 |
| Treatment (T) [c] | | | | | | | | | | |
| 0N | 12.9 d | 0.58 c | 0.14 ab | 1.26 | 0.32 d | 12.8 c | 13.4 c | 15.0 d | 41.2 d | – |
| 30N | 14.9 cd | 0.67 c | 0.13 b | 1.14 | 0.36 d | 16.9 c | 13.2 c | 24.0 cd | 54.2 cd | 43.3 |
| 60N | 15.9 bc | 0.71 c | 0.14 ab | 1.22 | 0.39 cd | 17.6 c | 15.2 c | 28.5 bc | 61.4 c | 33.8 |
| 90N | 18.2 ab | 0.90 b | 0.15 ab | 1.32 | 0.45 bc | 25.9 b | 18.5 bc | 37.7 ab | 82.2 b | 45.6 |
| 120N | 19.3 a | 1.17 a | 0.17 ab | 1.33 | 0.51 ab | 34.7 a | 22.9 ab | 41.1 ab | 98.7 ab | 47.9 |
| 150N | 20.3 a | 1.28 a | 0.19 a | 1.31 | 0.54 a | 40.5 a | 25.7 a | 42.9 a | 109.1 a | 45.3 |
| ANOVA | | | | | | | | | | |
| Y | NS | * | *** | *** | ** | NS | NS | NS | NS | NS |
| T | *** | *** | ** | NS | *** | *** | *** | *** | *** | NS |
| Y x T | * | NS | NS | NS | NS | NS | NS | NS | NS | NS |

[a] Data of dry weight were modified from Uchino *et al.* (2013a).
[b] Mean values of six N treatments and three replications.
[c] Mean values of two years and three replications.
[d] Mean values with a different letter indicate significant differences among treatments by the Tukey–Kramer method ($P<0.05$).
NS: Not significant, *: Significant at a 5% level of probability, **: Significant at a 1% level of probability, ***: Significant at a 0.1% level of probability.

by sweet sorghum for 30N, 60N, 90N, 120N and 150N, respectively.

To evaluate the effect of N status in the sorghum plant on sugar productivity, correlations of Kjeldahl N concentrations in the leaf, stem and grain with brix, juice volume and sugar yield were analyzed. There were significant correlations of the N concentration in leaves with brix, juice volume and sugar yield ($p < 0.05$, $n = 6$) (Fig. 2). In addition, the relationship flattened off at a rate of 90 kg N ha$^{-1}$, particularly for juice volume and sugar yield (Figs. 2B and C), because N input significantly increased the N concentration in the leaf at rates of up to 120 kg N ha$^{-1}$ (Table 2), but up to only 90 kg N ha$^{-1}$ for juice volume and sugar yield (detailed data shown in Uchino *et al.* (2013a)). This means the effect of N inputs was more pronounced for the N concentration in leaves than for traits relating to sugar productivity. Contrary to the N concentration in leaves, N concentrations in the stem and grain did not correlate significantly with any traits relating to sugar productivity (data not shown).

### 3. Labeled urea study

Since total dry weight and total N uptake of sweet sorghum did not differ significantly between the un-labeled treatment (90N) and labeled treatments (B-$^{15}$N, TP$_1$-$^{15}$N and TP$_2$-$^{15}$N) (data not shown), it is considered that $^{15}$N-labeling did not influence dry weight and N content.

The distribution ratio of absorbed basal and topdressing N to leaf, stem and grain is shown in Table 3 and was similar between B-$^{15}$N and TP$_1$-$^{15}$N: peaking in grain (41-42%), followed by leaf (32-33%) and stem (25-26%). The ratio to grain was higher in TP$_2$-$^{15}$N than in B-$^{15}$N and TP$_1$-$^{15}$N: 55% of the absorbed N was distributed to grain, as opposed to just 22 and 23% to the leaf and stem, respectively, in TP$_2$-$^{15}$N.

The residual N remaining in the 0-90 cm soil profile at the physiological maturity of sweet sorghum is shown in Table 3. Throughout all timings of N application, the residual N in the soil peaked (exceeding 80%) in the shallowest layer (i.e. 0-15 cm) and decreased with soil depth.

The fate of applied $^{15}$N-labeled urea, including N recovery in the sorghum plant and soil and the unaccounted for N losses, with basal and two topdressings, is shown in Fig. 3. $NRE_{isotopic}$ in the plant (i.e. the sum of NRE in the grain, stem and leaf) was markedly higher in TP$_1$-$^{15}$N (38.8%) and TP$_2$-$^{15}$N (36.3%) than in B-$^{15}$N (12.9%). In contrast, the residual N in the soil profile peaked in B-$^{15}$N (68.0%), followed by TP$_1$-$^{15}$N (41.0%) and TP$_2$-$^{15}$N (19.3%). The unaccounted for N loss tended to be higher in TP$_2$-$^{15}$N

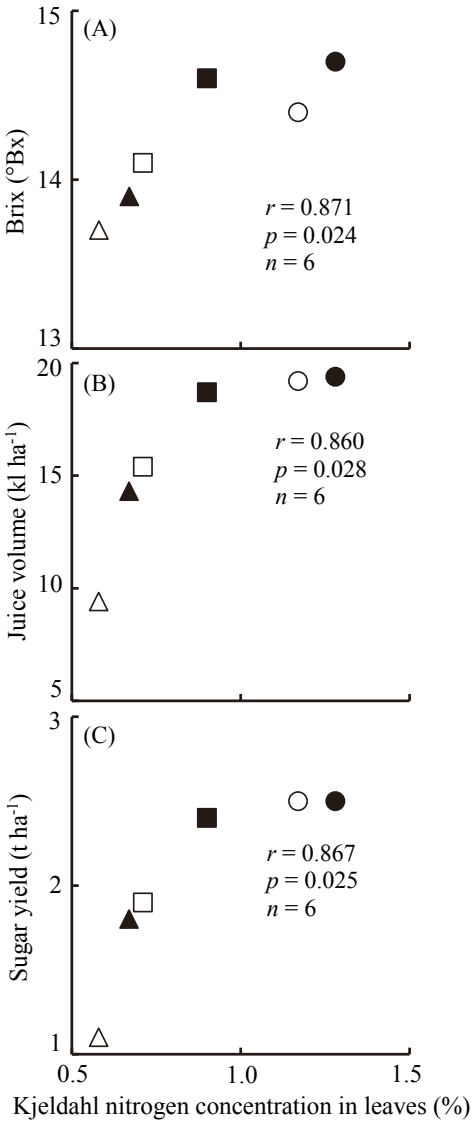


**Fig. 2. Correlations of Kjeldahl nitrogen (N) concentration in leaves with (A) brix, (B) juice volume and (C) sugar yield. Data of brix, juice volume and sugar yield were modified from Uchino *et al.* (2013a). Δ: 0 kg N ha$^{-1}$, ▲: 30 kg N ha$^{-1}$, □: 60 kg N ha$^{-1}$, ■: 90 kg N ha$^{-1}$, ○: 120 kg N ha$^{-1}$, ●: 150 kg N ha$^{-1}$.**

(44.4%) than in the other two treatments. As a total of basal and topdressing applications, 33.4% of the applied $^{15}$N-labeled urea was absorbed by the sorghum plant, while 36.5 and 30.1% were in soil and unaccounted for, respectively.

## Discussion

### 1. Nitrogen concentration in the sorghum plant

Tobita *et al.* (1994) investigated total N concentration in the sorghum aboveground part in the semi-arid tropics and revealed that it ranged from 0.60 to 0.77% under N inputs from 0 to 100 kg N ha$^{-1}$. Blümmel *et al.* (2009) reported that total N concentration in the stalk (= leaf + stem) of 34 sweet sorghum cultivars ranged from 0.44 to 0.89% under 80 kg N ha$^{-1}$ input in the semi-arid tropics. Han *et al.* (2011) also reported that N concentration in the aboveground part of five sweet sorghum cultivars at maturity stage was 0.75-1.02% under 120 kg N ha$^{-1}$ input. Compared to these studies, the Kjeldahl N concentration in our study was very low (Table 2): the Kjeldahl N concentration in the whole plant was 0.32-0.54% and that in the stalk was 0.22-0.39%, as calculated from the dry weight and the Kjeldahl N concentration.

Nitrate N, which cannot be measured by the Kjeldahl method, might be one of the reasons for the lower N concentration in the present study than in previous studies. It is known that sorghum plants accumulated high concentrations of the nitrate N in the stem (Harada *et al.* 2000). In the present study, both total N (including nitrate N) and Kjeldahl N concentrations were measured by an NC analyzer and the Kjeldah method, respectively in 2010 and the total N concentration (0.44%) significantly exceeded the Kjeldahl N concentration (0.39%) ($p < 0.05$, $n = 3$). However, the difference between these concentrations was very small: total N concentration in the present study was still much lower than previous studies, which means nitrate N concentration in the stem cannot explain the very low stem N concentration in the present study.

A similarly low N concentration in stem as our study was only reported in sugar cane (*Saccharum officinarum* L.) (Thorburn *et al.* 2007), but we could not find any studies reporting similar low stem N concentrations in sweet sorghum. Further investigation is required to clarify whether or not the low N concentration found in the present study constitutes an inherent characteristic of the sweet sorghum hybrid, CSH22SS.

### 2. Fate of N absorbed by sweet sorghum

$NRE_{isotopic}$ was reportedly higher in topdress-$^{15}$N than in basal-$^{15}$N for wheat (*Triticum aestivum* L.) (Jia *et al.* 2011) and maize (*Zea mays* L.) (Yang *et al.* 2011). In the present study, $NRE_{isotopic}$ was markedly lower in basal N application than in topdressings (Fig. 3). In addition, it was reported that basal N did not significantly affect the SPAD reading of sweet sorghum, which correlated positively with leaf N content (Uchino *et al.* 2013a, b). Our results and those of earlier studies therefore suggest that the effect of basal N on sweet sorghum growth and N uptake was low. These results match those of Adu-Gyamfi *et al.* (1997) who reported a relatively lower effect of the applied N through basal application than by topdressing on sorghum yield.

Jia *et al.* (2011) and Yang *et al.* (2011) reported that the distribution ratio of $^{15}$N to grain was higher in topdressing than in basal applications for wheat and maize, respectively.

**Table 3. Amount of basal and topdressing 15N in crop and soil at the physiological maturity stage**

| Treatment code | Labelled N in crop (kg N ha$^{-1}$) | | | | Labelled N in the soil (kg N ha$^{-1}$) | | | | | Labelled N unaccounted for (kg N ha$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Leaf | Stem | Grain | Whole | 0-15 | 15-30 | 30-60 | 60-90 | 0-90 | |
| B-$^{15}$N | 0.6 (32) | 0.5 (26) | 0.8 (42) | 1.9 (100) | 8.3 (81) | 1.1 (10) | 0.6 (6) | 0.3 (2) | 10.2 (100) | 2.9 |
| TP$_1$-$^{15}$N | 4.9 (33) | 3.7 (25) | 6.0 (41) | 14.5 (100) | 12.8 (83) | 1.5 (10) | 0.7 (4) | 0.3 (2) | 15.4 (100) | 7.6 |
| TP$_2$-$^{15}$N | 3.0 (22) | 3.2 (23) | 7.4 (55) | 13.6 (100) | 6.0 (83) | 0.7 (10) | 0.4 (5) | 0.2 (3) | 7.2 (100) | 16.7 |
| Total | 8.5 (28) | 7.3 (24) | 14.2 (47) | 30.1 (100) | 27.1 (82) | 3.3 (10) | 1.7 (5) | 0.8 (2) | 32.8 (100) | 27.1 |

Values in parentheses are the percentages of each constituent plant part to the whole plant or each soil layer to the 0-90 cm soil depth.

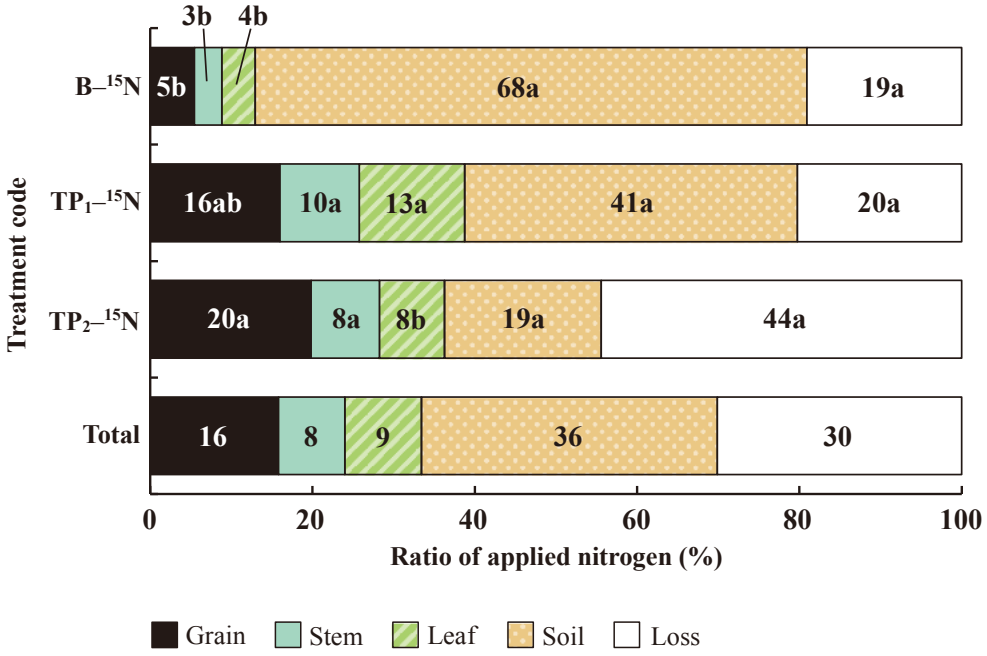


**Fig. 3. The fate of $^{15}$N-urea (%), including N recovery in the grain, stem and leaf, in the soil (0-90 cm depth) and as unaccounted for as affected by the time of N application (basal, first topdressing and second topdressing). Values with different letters indicate significant differences among the three timings of N application, according to the Tukey-Kramer method (P<0.05).**

Our study also found that the distribution ratio of absorbed $^{15}$N to grain was higher in TP$_2$-$^{15}$N than N applied at the other timings (Fig. 3), indicating that topdressed N, which was applied at 60 DAS, could increase N accumulation in grain as compared to other N application timings.

Conversely, the distribution ratio of absorbed $^{15}$N to leaf was higher in TP$_1$-$^{15}$N than in TP$_2$-$^{15}$N. It is known that leaf N concentration is highly correlated with extended foliar greenness during grain filling, known as stay-green (Borrell & Hammer 2000). It was reported that stay-green traits affected the increase in sugar content in the stem (McBee *et al.* 1983, Duncan 1984) particularly under water-limited conditions, because the stay-green type plants retain chlorophyll in their leaves and maintain their ability to carry out photosynthesis longer than senescent types. Serrao *et al.* (2012) also reported that leaf N concentration was the best indicator for predicting sugar yields in sweet sorghum. The present study revealed a significant correlation of leaf N concentration at the maturity stage with juice volume and sugar yield (Fig. 2). These results suggest that topdressed N at 30 DAS increased N accumulation in leaves, which could boost sugar productivity by extending foliar greenness. Due to the importance of stay-green for sorghum productivity, QTLs associated with stay-green traits were identified (San-

chez *et al.* 2002, Harris *et al.* 2007).

### 3. Fate of N unabsorbed by sweet sorghum

In the un-labeled experiment, $NRE_{difference}$ did not differ significantly among the six N treatments (Table 2). This matches the results reported by Moraghan *et al.* (1984) for grain sorghum. They studied the effects of urea input rates ranging from 40 to 160 kg N $ha^{-1}$ on N uptake by grain sorghum hybrid and revealed that $NRE_{difference}$ did not differ significantly among N input rates. These results indicate that the amount of N not absorbed by the sorghum rose with increasing N input rate.

In the present labeled experiment, 67% of all N applied could not be absorbed by sorghum plants and about half the unabsorbed N remained in the soil on physiological maturity (Table 3, Fig. 3). In particular, 68% of basal applied N remained in the soil and this ratio exceeded that for topdressing of N. This result is well supported by Jia *et al.* (2011), who reported that 37-51% of the basal applied N remained in the soil on harvest and the ratio exceeded that from topdressed N. Stevens *et al.* (2005) reported that 20-55% of inorganic $^{15}N$ was converted into an immovable form, including organic and clay-fixed forms, during the maize growing season. Sugihara *et al.* (2010) studied the effect of rainfall after the dry period on microbial biomass and N dynamics and revealed increasing microbial biomass and a drastic decline in inorganic N after rewetting by rainfall, which occurred within 50 hours of rainfall. In the present study, although the microbial biomass was not measured, the inorganic N derived from the applied urea was probably converted to organic N by stimulated microbial activity (in a process known as immobilization) following rewetting by irrigation and/or rainfall after a long dry spell of post-rainy season.

In general, the N not absorbed by plants and soil is lost from agricultural fields through various processes, including $NH_3$ volatilization, $N_2O$ emissions (Follett & Delgado 2002) and nitrate N leaching. Ramu *et al.* (2012) monitored $N_2O$ emissions from the field site where this study was conducted; and the results showed that only 0.90% of the applied urea-N was lost as $N_2O$. In contrast, it is reported that 47.7% of the applied urea was lost due to $NH_3$ volatilization from Alfisol fields in the semi-arid tropical regions (Reddy & Sharma 2000). Burford & Sahrawat (1988) also reported that $NH_3$ volatilization was one of the major N loss pathways from Alfisols in the semi-arid tropical regions. In the present study, therefore, it is hypothesized that most of the unaccounted for N was lost due to $NH_3$ volatilization.

Nitrate N leaching is also one of the possibilities causing N loss from agricultural fields. Although nitrate N leaching was not monitored in the present study, it is known that Alfisols were well-drained soils due to their low water-holding capacity and high saturated hydraulic conductivity (Pathak *et al.* 2013b). In the present study, therefore, part of the unabsorbed N might leach out from the rooting zone in association with water percolation to a deeper soil layer. In particular, it was reported that high rainfall markedly increased deep water drainage on Alfisols (Pathak *et al*. 2013b). Since precipitation around second topdressing was very high compared to other application timing in 2010 (Fig. 1), this facilitated nitrate N leaching and resulted in a relatively higher ratio of unaccounted for N loss in $TP_2$-$^{15}N$ than in B-$^{15}N$ and $TP_1$-$^{15}N$ (Fig. 3).

However, there is a possibility that various processes other than those listed above (such as NO and $N_2$ emissions due to denitrification) also contributed to N losses from agricultural fields. Further investigations to monitor gaseous emissions and N leaching are needed to clarify understanding of N dynamics in sweet sorghum production systems on Alfisols.

## Conclusions

In our present and previous studies (Uchino *et al.* 2013a), although N uptake rose with increasing N rate, grain and sugar yields and net incomes for farmers increased at rates of up to 90 kg N $ha^{-1}$ and higher N rates did not significantly affect productivity or income. In addition, the un-labeled urea study revealed that the amount of N not absorbed by the sorghum rose with increasing N rate. These results suggest that an input of 90 kg N $ha^{-1}$ is optimum for sweet sorghum production on Alfisols in the semi-arid tropical regions. However, since the NRE was much lower for basal applications than topdressings, the NRE might be increased by improving basal N application methods, such as the deep application of fertilizer, using slow-release fertilizer (Raun & Johnson 1999) and so on.

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