Fate of $^{15}$N-labeled Inorganic Fertilizer in an Upland Soil Applied with Sweet Sorghum Bagasse and N Uptake Efficiency by Komatsuna Plants

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Abstract: Sweet sorghum bagasse (SSB) is a soil amendment with potential for biofuel production. This study was conducted to determine the appropriate techniques for application of SSB and the effect of incorporation of inorganic fertilizer (IF) on the production of komatsuna (Brassica rapa) plants. SSB was applied to the surface of the plant or incorporated into soil. The N fate of IF was evaluated by using $^{15}$N-labeled IF. The combination of surface application of SSB and incorporation of IF to soil decreased the N uptake by komatsuna plants but increased dry weight, whereas the incorporation of IF and SSB gave lower komatsuna dry weight than IF treatment alone. Moreover, the application of SSB tended to increase the N distribution from IF to komatsuna with decreased N loss from the plant-soil system. These results showed that surface application of SSB is effective for increasing crop production due to reduction of N loss and improved N use efficiency.

Key words: Biofuel production, $^{15}$N, N uptake, N loss, Sweet sorghum bagasse.

Sweet sorghum (Sorghum bicolor (L.) Moench) has attracted great interest as an alternative feedstock for biofuel production, because it has high yielding potential and a high content of fermentable sugars in the stem. The extracted sugar can be fermented directly into ethanol (Srinivasa Rao et al., 2013). Establishment of an effective and sustainable biofuel production system by cultivation of the biofuel crop sweet sorghum will help minimize CO$_2$ discharge derived from fossil fuels. Moreover, maintenance of a high soil fertility is important to obtain a consistently high yield of sweet sorghum. Soil fertility can be effectively improved by the application of organic matter such as crop residue, legumes, and animal manure compost, because these materials offer beneficial effects for soil physiochemical and biological properties, thereby increasing plant growth and yields (Othieno, 1973; Anderegg and Naylor, 1988; Barajas-Guzmán et al., 2006; Fang et al., 2007). After squeezing sugar juice out of a sweet sorghum stem, a large amount of residue (bagasse), a type of organic matter, is generated. Therefore, application of sweet sorghum bagasse (SSB) to sweet sorghum-cultivated soil seems to be a useful method for maintaining soil fertility to improve the growth of sweet sorghum and, consequently, sugar productivity.

Soil N is often the most constraining element for plant growth and yield. N dynamics in soil applied with organic matter may affect plant growth through processes such as N mineralization and immobilization in the soil. The rates of N mineralization and immobilization of N applied to soil with organic matter depend on the nature of the organic materials applied. N is mineralized if the C/N ratio in organic materials applied to the soil is below 20 or if the percentage of N exceeds 2.5 (Singer and Munns, 2006). N is likely to be immobilized and decomposed when the C/N ratio exceeds 20. Therefore, regarding N fertilization, organic matter with a low C/N ratio (i.e., below 20) may represent an important alternative source of N to inorganic fertilizer (IF) for crop production. However, organic materials with a high C/N ratio (i.e., above 20), like SSB, also possess a low N supply potential to plants.
because of the high N immobilization rate during decomposition in soil. However, N immobilization in the soil to which these materials have been applied might offer several advantages, including retention of inorganic N in the soil and decreased nitrate leaching and denitrification. Several reports have shown that the combination of crop residues with high C/N ratios and IF inputs reduces environmental N losses and creates a positive interactive effect on crop N uptake (Gentile et al., 2009, 2011). Moreover, mulching with high C/N organic matter has positive effects to control weeds, increase infiltration, reduce evaporation, supply available nutrients, and increase crop yields (Othieno, 1973; Gaur and Mukherjee, 1980; Ssali et al., 2003). Therefore, by using SSB as mulch (i.e., surface application of SSB) in sweet sorghum cultivation, an effective and sustainable biofuel production system with sweet sorghum could be developed in a more environmentally and economically suitable manner. However, the combined effects of SSB and IF input on N fate in soil-plant systems and crop productivity have not yet been evaluated.

In this study, we conducted a preliminary study on the effects of SSB application on plant (komatsuna, *Brassica rapa* L. *perviridis* Group) growth and yield, and the N fate of the *15*N-labeled IF in potted upland soil in order to assess the potential of SSB.

**Materials and Methods**

1. **Site, treatments, and plant cultivation**

An experiment was conducted using pots in a greenhouse at the College of Agriculture, Ibaraki University, Ami, Japan (36°04’N, 140°21’E).

SSB was obtained after squeezing the juice from the stems of sweet sorghum (*Sorghum bicolor* L. *Moench ‘FS905’*) plants cultivated from May to October 2009 and used as the organic matter. This residue was air-dried and fragmented into small pieces <3-cm long, with a total C content of 46.5%; total N content of 0.215% and C/N ratio of 216. Total C and total N of the SSB were measured by a CN Corder (JM3000CN; J-SCIENCE LAB Co. Ltd., Kyoto, Japan).

The treatments were (1) no N fertilizer application (NF), (2) incorporation of IF (INC/IF), (3) surface application of SSB with IF (SUR/SSB), and (4) incorporation of SSB with IF (INC/SSB). Table 1 shows the application rates of SSB and IF. *15*N-labeled IF [(NH$_4$)$_2$SO$_4$, 1.00 atom%] was applied to the soil to estimate the fate of N derived from IF in the soil-plant system. Samples (1.66 kg, <2-mm fraction) of dry clay loam Andisol texture with a total C content of 1.86%; total N content of 0.21% were placed into Wagner pots (diameter: 16 cm, height: 19 cm). These pots did not have holes in the bottom for draining irrigated water.

In the INC/SSB treatment, SSB was incorporated into the soil and mixed well, and in the INC/IF, SUR/SSB, and INC/SSB treatments, P$_2$O$_5$ (as superphosphate) and K$_2$O (as potassium chloride) were applied and mixed thoroughly with the soil. The soil was placed into Wagner pots and its moisture content was maintained at around 60% of water holding capacity (WHC) with a solution of *15*N-labeled IF [(NH$_4$)$_2$SO$_4$, 1.00 atom%] in the INC/IF, SUR/SSB, and INC/SSB treatments, and SSB was applied to the soil surface of the pots in the SUR/SSB treatment. Then, 9 komatsuna seeds were sown in each pot and thinned to 3 seedlings after emergence. This plant is a typical Japanese leafy vegetable that is used as an experimental crop in order to evaluate the effects of organic matter application on plant growth and yield. Komatsuna plants were cultivated in the greenhouse with 4 replicates per treatment. Plants were irrigated manually in order to maintain soil moisture content close to 60% of WHC during cultivation. The cultivation period was from 14 September to 22 October of 2010 (38 d).

2. **Sampling and chemical analysis**

Growth parameters of the komatsuna plants (plant length and leaf chlorophyll content index) were measured during the cultivation period. The chlorophyll content index was measured using a chlorophyll meter (SPAD-502; KONICA MINOLTA Co. Ltd., Tokyo, Japan).

SSB residues (>5-mm fractions) in the SUR/SSB treatment were collected from the soil surface manually. After harvesting the plant tops, the roots (>5-mm fractions) were collected by lifting the soil from each pot, sieving the soil, and washing off the soil under running tap water. Plant tops and roots, soil, and SSB residues were collected carefully at harvest, oven-dried at 70°C, and the dried samples were weighed and ground into a fine powder with

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**Table 1. Application rates of sweet sorghum bagasse (SSB) and inorganic fertilizer (IF).**

| Treatment                  | SSB (g m$^{-2}$) | IF (g m$^{-2}$) |
|----------------------------|------------------|-----------------|
|                            | Dry matter | N P$_2$O$_5$ | K$_2$O | N P$_2$O$_5$ | K$_2$O |
| No N fertilizer (NF)       | 0         | 0    | 0    | 0    | 0    | 0    | 12.7  | 14.0  |
| Incorporated IF (INC/IF)   | 0         | 0    | 0    | 0    | 14.0  | 12.7  | 14.0  |
| Surface-applied SSB (SUR/SSB) | 920      | 1.98  | 2.28 | 3.36 | 14.0  | 12.7  | 14.0  |
| Incorporated SSB (INC/SSB) | 920      | 1.98  | 2.28 | 3.36 | 14.0  | 12.7  | 14.0  |
an electric mill (SSB residues in the INC/SSB treatment group were mixed with soil and pulverized). Total N concentration and the abundance of $^{15}$N in each subsample (the plants, soil, and SSB residues) were measured using a mass spectrometer (Integra CN; SerCon Ltd., Cheshire, UK).

### 3. Calculations of the fate of $^{15}$N-labeled IF

The fraction of N derived from applied IF N (%Ndff) in the potted plant (soil) and the fraction of N from native soil (%Ndfs) in the potted plant were calculated using Eqs. (1) and (2), as described by Li et al. (2001):

\[
%Ndff \text{ in the potted plant (soil)} = \frac{(^{15}\text{N atom}\% \text{ excess of potted plant (soil))}}{(^{15}\text{N atom}\% \text{ excess of applied IF N})} \times 100
\]  

\[
%N dfs \text{ in the potted plant} = 100 - %Ndff \text{ in the potted plant}
\]

where $^{15}$N atom% excess = $^{15}$N atom% in the INC/IF, SUR/SSB, or INC/SSB group - $^{15}$N atom% (natural abundance) in the NF ($^{15}$N non-amended) group, as described by Tagoe et al., 2008.

The amount of N derived from applied IF N (Ndff) in the potted plant (soil) was calculated using Eqs. (3):

\[
Ndff (\text{g pot}^{-1}) \text{ in potted plant (soil)} = \text{total N (g pot}^{-1}) \text{ in the potted plant (soil)} \times (%Ndff) / 100
\]

The N uptake efficiency by the plant (NUE, the recovery rate of N by the plant from applied IF N) and the rate of N remaining in the soil (NRS) were calculated from Eq. (4):

\[
\text{NUE (NRS) } (%) = \frac{[\text{Ndff in the potted plant (soil) at harvest}] \times 100}{\text{amount of applied IF N}}
\]

The unaccounted portion of N from IF applied to the plant-soil system (U), including denitrification loss, was calculated by using Eq. (5):

\[
U(\%) = 100 - (\text{NUE} + \text{NRS})
\]

### 4. Statistical analysis

Differences among means were analyzed using the Tukey-Kramer test with KyPlot software (KyensLab Inc., Tokyo, Japan).

### Results and Discussion

1. **Effects of SSB on komatsuna growth, yield, and N uptake**

   In all treatments, plant length and the leaf chlorophyll content index of komatsuna plants increased over time (Fig. 1). The plant length at harvest tended to be longer in the SUR/SSB group than in the INC/IF and INC/SSB groups, but no significant difference was found among the 3 treatments (Fig. 1A). In addition, the leaf chlorophyll content index was higher in the INC/IF and INC/SSB groups than in the SUR/SSB group during cultivation (Fig. 1B).

   The dry weight of komatsuna tops at harvest was heaviest in the SUR/SSB treatment, followed by the INC/IF, INC/SSB, and NF treatments (Table 2). The top dry weights in the SUR/SSB and INC/IF treatments were significantly heavier than that in the NF treatment. The heaviest dry weight of roots at harvest was obtained in the SUR/SSB group, followed by the INC/IF, NF, and INC/SSB groups; however, no significant difference was found among treatments. We sampled SSB residues from the soil surface in the SUR/SSB treatment at komatsuna harvest (the residues in the INC/SSB group were not separated). The proportion of SSB residues (> 5-mm fractions) remaining...
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on the soil surface was 96.3%. Therefore, the decomposition rate was about 4% of applied SSB in the SUR/SSB treatment.

Table 3 shows the total N concentrations in the komatsuna plants, soil, and SSB residues. The total N (%) in komatsuna top was significantly higher in the INC/IF group compared to the other groups. The highest $^{15}$N abundance in plants was obtained in the INC/SSB group, followed by the SUR/SSB, INC/IF, and NF groups (Table 4). The soil $^{15}$N abundance was also highest in the INC/SSB group compared to the other groups. We evaluated the fate of IF in plant-soil systems by using values of the dry weight, total N concentration, and $^{15}$N abundance in the komatsuna plants, soil, and SSB residues (Tables 2, 3 and 4).

Table 5 shows the N uptake by komatsuna plants (top + root). The Ndff (g pot$^{-1}$) was highest in the SUR/SSB.
Table 5. Uptake of N derived from inorganic fertilizer, and N uptake efficiency by komatsuna (top + root).

| Treatment | N uptake (g pot⁻¹) | NUEa (%) | Dry weight/N uptake in plant top |
|-----------|--------------------|----------|---------------------------------|
|           | Ndff*              | Ndfs**   | Total                           |
| NF        | 0.076 ± 0.009 b    | 0.076 ± 0.009 b | 20.2 ± 2.0 a                |
| INC/IF    | 0.075 ± 0.006 a    | 0.128 ± 0.008 a | 25.0 ± 2.1 a                |
| SUR/SSB   | 0.131 ± 0.014 a    | 0.044 ± 0.005 bc | 43.8 ± 4.5 a                |
| INC/SSB   | 0.105 ± 0.022 a    | 0.027 ± 0.005 c | 34.9 ± 7.3 a                |

Values are means ± SE (n = 4).

Values followed by the same letter in each column are not significantly different at P < 0.05, as determined by the Tukey-Kramer test (n = 4).

*Ndfs: N derived from inorganic fertilizer.

**Ndff: N derived from native soil.

NUE: N uptake efficiency, the recovery rate of N by komatsuna (top + root) at harvest from applied inorganic fertilizer N.

NF, no N fertilizer; INC/IF, incorporated inorganic fertilizer; SUR/SSB, surface-applied sweet sorghum bagasse; INC/SSB, incorporated sweet sorghum bagasse.

Group, followed by the INC/SSB and INC/IF groups, but no significant difference was found among the 3 treatments. By contrast, the concentration of Ndff (g pot⁻¹) was significantly higher in the INC/IF group, and significantly lower in the INC/SSB group compared to that in the NF group. The N uptake by komatsuna plants from the applied IF was higher and N from native soil was lower in the SSB-applied treatment groups compared to those in the INC/IF group. Total N uptake (Ndff + Ndfs) was significantly higher in the INC/IF and SUR/SSB groups than in the NF group. The highest NUE (recovery rate of N by komatsuna from applied IF) was obtained in the SUR/SSB treatment, followed by INC/SSB, and the lowest NUE was in the INC/IF treatment; however, no significant difference was found among the 3 treatments. Previous studies estimated the NUE by komatsuna to be 19 – 43% of the input 15N-labeled IF (Asagi and Ueno, 2008; Ebidi et al., 2008). In the present study, the NUE was 25 – 44% of applied inorganic N; therefore, these efficiencies were within the reported range. Total N uptake (Ndff + Ndfs) by komatsuna was lower in the SSB treatments, but the NUE tended to increase when using a combination of IF and SSB input treatments compared to the IF treatment alone. The dry weight/N uptake ratio tended to be higher in the treatments where SSB was applied compared to that of the INC/IF group.

Experiments with 15N-labeled fertilizers often show that plants given N fertilizer take up more N from the soil than plants not given N fertilizer (Hart et al., 1986; Azam et al., 1992; Diekmann et al., 1993; Ashraf et al., 2004; Asagi and Ueno, 2009). This interaction is known as a positive added N interaction (ANI) (Jenkinson et al., 1985). Positive ANIs can represent real effects if, for example, N fertilizer increases the volume of soil explored by roots. By contrast, positive ANIs could represent an apparent effect if they are caused by pool substitution or by isotope displacement reactions (Jenkinson et al., 1985). Azam et al. (1992) reported that the application of NH₄-N resulted in a significant increase in the plant uptake of unlabeled N (native soil N and N derived from rhizospheric N₂ fixation), suggesting the occurrence of a positive ANI, and both apparent and real ANIs were likely to have contributed to the total reactions. In the present study, compared to the NF group, the Ndfs was significantly higher in the INC/IF group, suggesting a positive interaction, and was significantly lower in the INC/SSB group, suggesting a negative interaction (Table 5). No significant difference in the root dry weight was found among the treatments (Table 2). Therefore, the apparent positive interaction in the INC/IF group might have been caused by pool substitution or isotope displacement reactions. Although the reasons for the occurrence of a negative interaction in the INC/SSB group are not clear, they might be related to N mineralization and immobilization in the soil with SSB. These results suggest that the combination of SSB and IF input changes the outcomes related to N uptake derived from the soil.

In all treatments except for NF, the application rate of IF was the same; however, the leaf chlorophyll content index during cultivation in the INC/IF group was higher than that in the SUR/SSB or INC/SSB groups (Fig. 1B). Moreover, the total N concentration and total N uptake by komatsuna (Ndff + Ndfs) at harvest were also higher in the INC/IF treatment compared to the SSB treatments (Tables 3 and 5). These results indicate that N uptake by komatsuna decreased when using a combination of IF and SSB inputs. This may be because of the N immobilization that occurred when applying SSB with a high C/N ratio, regardless of the application method (i.e., surface application or incorporation). The N supply potential to komatsuna was lower in the soil with both IF and SSB compared to that with IF alone, supporting the hypothesis suggested by Gentile et al. (2011). However, the dry weight/N uptake ratio (Table 5) and dry weight of
komatsuna (Table 2) were higher when using a combination of IF and SSB surface application compared to IF application alone. The reason for this higher efficiency when using both IF and SSB surface application is not clear; however, it might result from differences in the balance between N assimilation and C consumption in komatsuna (Osone and Tateno, 2003). Moreover, SSB surface application might affect soil physiochemical and biological attributes in the plant-soil system, such as soil water content, microorganisms, and phosphorus uptake in komatsuna, which consequently have positive effects on komatsuna yield (Othieno, 1973; Barajas-Guzmán et al., 2006). In contrast, the combination of IF and SSB incorporation led to a lower yield than that obtained in the treatment with IF alone. SSB has been reported to show allelopathic potential (Cheema and Khaliq, 2000). In fact, SSB incorporation might cause negative impacts due to N immobilization and because of slow root growth resulting from the toxic substances generated during SSB decomposition, which would result in the regulation of komatsuna growth. However, SSB surface application does not appear to have a negative impact on root growth because of the smaller contact area between the roots and toxic substances from the SSB in surface application treatment compared to the area of contact associated with SSB incorporation. Therefore, plant growth and N uptake were affected by both the application of SSB and the particular application method used (i.e., surface application or incorporation), and SSB surface application seems to have a more positive effect on plant yield.

2. Distributions of N derived from IF applied to the plant-soil system

The N distributions derived from the application of IF to the komatsuna and soil system at harvest are shown in Table 6. N distributions to komatsuna and soil (+ applied SSB) were higher in the SUR/SSB and INC/SSB groups compared to the INC/IF group. Therefore, the ratio of unaccounted N was significantly lower in the SSB treatments compared to the INC/IF treatment.

Nishio and Oka (2003) reported that the unaccounted portions of applied $^{15}$N in upland soil were 40–60% of the total applied N. The unaccounted N was considered to represent N loss from the plant-soil system, presumably by denitrification (Nishio and Oka, 2003). In this study, application of SSB showed a tendency to increase the N distribution from applied IF to the plant and soil, thereby decreasing the rate of unaccounted N (SUR/SSB: 18%, INC/SSB: 19%, INC/IF: 46% in Table 6). This result implies that a combination of organic matter of a high C/N ratio with IF has positive effects on the soil, air environment, and soil fertility, and is in agreement with previous reports on reduction of environmental N losses by crop residue and IF inputs (Gentile et al., 2009, 2011).

3. Conclusions

In the present study, application of SSB combined with IF incorporation affected plant growth, yield, N uptake, and the N fate of IF. N uptake by plants decreased upon surface application of SSB and incorporation of IF; however, the plant dry weight/N uptake ratio increased, producing a higher or equal yield compared to that obtained by IF incorporation alone. These results might be attributed to changes in the interaction between mineralization and immobilization during the decomposition of SSB with high C/N in the soil. The N loss of IF from the plant-soil system when using SSB surface application decreased without any accompanying decrease in plant yield. Therefore, using a combination of surface application of high C/N SSB with IF might help reduce the rate of IF application while simultaneously increasing plant yields, which would improve the sustainability of crop production systems. It will be necessary to evaluate the effects of SSB application on sweet sorghum yield and N dynamics in the field in order to establish an effective and sustained biofuel production system.
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