Mineralisation of C and N during decomposition of sugarcane and soybean residues

Fiona Robertson

BSES Ltd, 50 Meiers Road, Indooroopilly, Qld 4068, Australia. Present Address: Department of Primary Industries, Hamilton Centre, Private Bag 105, Hamilton, Vic. 3300, Australia. Email: fiona.robertson@dpi.vic.gov.au

Abstract
Green harvesting of sugarcane (*Sacharum* spp.) produces large quantities of crop residues (trash) of high carbon: nitrogen ratio. Legumes such as soybean are used as green manure crops in sugarcane production systems, and produce residues of low carbon: nitrogen ratio. How these contrasting plant materials interact in terms of decomposition and N availability is not known. The aim of this study was to measure the potential for C mineralisation and N mineralisation or immobilisation when sugarcane and soybean residues and N fertiliser are applied to soil, individually and in combinations. An incubation experiment was conducted where ^{15}N-labelled and unlabelled sugarcane trash, soybean tops, and urea fertiliser were applied to soil in factorial combinations, with a single material in any treatment being ^{15}N-labelled. Mineralisation of C and N were measured for 6 months. Cane trash and soybean tops were good sources of readily decomposable C. Addition of urea had little effect on C mineralisation from soil or residues, except a temporary stimulation during early trash decomposition. Up to 91% urea-N was recovered as soil inorganic N. Soybean tops eventually mineralised 39-67% of their N content. There was evidence of some gaseous loss of mineral N. Trash immobilised all the N mineralised from soil, 80-100% of the N mineralised from soybean tops, and 20-50% of N mineralised from urea. Negligible N was mineralised from trash, and the addition of urea or soybean did not change this. Patterns of C and N mineralisation were generally similar for the amendments alone and in combinations, except where N immobilisation was limited by N availability. By retaining rather than burning sugarcane trash at the end of the crop cycle, significant soil inorganic N may be immobilised and protected from loss.

Key Words
Nitrogen, carbon, mineralisation, immobilisation, crop residue, green manure.

Introduction
Much of the Australian sugarcane (*Sacharum* spp.) crop is harvested green, which leaves large quantities of crop residues (trash) of high carbon: nitrogen ratio on the soil surface. Legumes such as soybean are increasingly used as green manure crops in sugarcane production systems (in the presence or absence of trash), and produce residues of low carbon: nitrogen ratio. The N in trash is released very slowly during decomposition and is of negligible value to the succeeding ratoon crop (Robertson 2003). Legumes such as soybeans contain 2-4 times more N than trash, and their decomposition can lead to rapid accumulation of mineral N in the soil which, if not taken up by a crop, is liable to be lost by leaching or denitrification. How these contrasting plant materials interact in terms of decomposition and N availability is not known, but is potentially important for crop nutrition, fertiliser requirements, and off-farm loss of N.

The aim of this study was to measure the potential for C mineralisation and N mineralisation or immobilisation when cane and soybean residues and N fertiliser are applied to soil, individually and in combinations.

Methods
Preparation of soil and amendments
Soil (non-calcic brown, Holz and Shields 1985) was collected from the 0-10 cm depth of a sugarcane field at Mackay, Queensland (21.10 S, 149.07 E). Particle size distribution was 56, 27, 17% (sand, silt, clay), total N was 0.064%, total C was 1.12% and pH (water) was 4.56. The soil was dried, crushed and sieved (<2 mm) before use.

Unlabelled and ^{15}N-labelled sugarcane and soybean residues were obtained from glasshouse-grown plants. The plants were fertilised weekly with a solution of either ^{15}N-labelled ammonium sulfate (10 atom% excess) or unlabelled ammonium sulfate, plus a N-free general nutrient solution. The soybeans were allowed to grow for approximately 2 months, then harvested before the development of seed pods.

The sugarcane was grown to maturity (approximately 1 year), then all green and senesced leaves were removed from the stalk. All plant material was dried at 70°C, to facilitate handling. All sugarcane leaves were combined as the trash fraction, then shredded in a cutter-grinder (fragment size 2-10 cm long and 0.2-1.0 cm wide). Soybean tops and the shredded trash were then cut into ≤ 4 cm-long pieces with scissors. Unlabelled and ^{15}N-labelled urea solutions were prepared containing 10 mg N/mL. Samples of all materials were taken for analysis. The unlabelled and ^{15}N -labelled residues had similar total C and N contents (Table 1), but total N was slightly lower in ^{15}N -labelled than in unlabelled soybean.

Table 1. C and N contents of amendments.

Amendment	Total C (%DM)	Total N (%DM)	C:N Ratio	^{15}N Atom % excess
Trash-^{15}N	36.1	0.55	66	6.04
Trash-unlabelled	35.4	0.52	68	0
Soybean-^{15}N	34.0	1.18	29	1.39
Soybean-unlabelled	33.7	1.35	25	0
Urea-^{15}N	-	-		9.80
Urea-unlabelled	-	-		0

The experiment

Amendments were mixed with 250 g of air-dry soil and placed in plastic pots (8.5 cm diameter) with holes on the base but lined with nylon cloth (1 mm mesh) to prevent soil loss. The unlabelled and ^{15}N-labelled trash, soybean and urea were combined as shown in Table 2 to create 15 experimental treatments containing one ^{15}N-labelled amendment, and one treatment containing unamended soil.

Table 2. Experimental treatments, and total C and N applied.

Treatment	Amendments	Total N applied (mg/pot)	Total C applied (mg/pot)	C:N ratio applied
1	CaneTrash-^{15}N	30	1986	66
2	SoybeanTops-^{15}N	32	918	29
3	Urea-^{15}N	66	0	-
4	CaneTrash-unlabelled	29	1947	68
5	SoybeanTops-unlabelled	36	910	25
6	Urea-unlabelled	66	0	-
7	CaneTrash-^{15}N + SoybeanTops-unlabelled	67	2895	43
8	CaneTrash-^{15}N + Urea-unlabelled	96	1986	21
9	CaneTrash-unlabelled + SoybeanTops-^{15}N	60	2865	48
10	CaneTrash-unlabelled + Urea-^{15}N	95	1947	21
11	SoybeanTops-^{15}N + Urea-unlabelled	98	918	9
12	SoybeanTops-unlabelled + Urea-^{15}N	102	910	9
13	CaneTrash-^{15}N + SoybeanTops-unlabelled + Urea-unlabelled	133	2895	22
14	CaneTrash-unlabelled + SoybeanTops-^{15}N + Urea-unlabelled	126	2865	23
15	CaneTrash-unlabelled + SoybeanTops-unlabelled + Urea-^{15}N	131	2857	22
16	Soil only	0	0	18

Amendments were applied at rates commonly encountered under field conditions: trash at 10 t dry matter (DM)/ha (5.5 g/pot), soybean at 5 t DM/ha (2.7 g/pot), and urea at 120 kg N/ha (66 mg/pot). Enough pots were prepared for 4 replicates, and 4 destructive sampling times. Deionised water was added to bring the soil to approximately 80% of field capacity (30% gravimetric water content). The pots were placed inside heavy-duty zip-seal plastic bags (left open) and moved to a constant-temperature room at 25°C, in the dark. Soil water was replenished at approximately weekly intervals. On 15 occasions (starting on days 6, 10, 15, 21, 24, 31, 36, 42, 48, 52, 58, 69, 91, 95, and 120 after set-up), CO_2 evolved from the pots was collected using alkali traps (vials containing 18 mL of 1 M sodium hydroxide suspended 1-2 cm above the pot surface, and the plastic bags sealed for 2-3 days). On 4 occasions (days 17, 43, 91, and 121), a complete set of pots was destructively sampled - the pot contents were thoroughly mixed and analysed for water and inorganic N content. The soils were then dried at 40°C, crushed to <2 mm and retained for further analyses. On day 96, the remaining pots were leached, in an attempt to simulate the depletion of inorganic N under field conditions through plant uptake and leaching (pots mounted in the neck of a 1-L glass jar and leached with 200 ml of deionised water). The leachates were discarded.

Calculations and statistical analysis
Cumulative C mineralisation during the experiment (ΔC) was estimated from the area under the curve of CO_2 accumulation against time. Mineralisation of C due to the amendment (ΔC_A) was calculated as ΔC from amended soil *minus* ΔC from unamended soil. Apparent net mineralisation or immobilisation of N (ΔN) was calculated as soil inorganic N at the end of the incubation period *minus* soil inorganic N at the start. Apparent net mineralisation or immobilisation of N due to the amendment (ΔN_A) was calculated as ΔN from amended soil *minus* ΔN from unamended soil. Percentage ^{15}N recovery at the end of the incubation period was calculated from:
% ^{15}N recovery = [(Soil ^{15}N atom% excess x mg total N in soil) / mg ^{15}N originally applied] x 100

Percentage ^{15}N loss (including leached ^{15}N) was calculated as 100 *minus* % ^{15}N recovery. Total mineralisation of N from all the amendments in any treatment (N_{min}) was thus estimated from:
N_{min} = ΔN + ^{15}N loss + (Unlabelled N loss)

where Unlabelled N loss is N loss from all the unlabelled amendments in that treatment, calculated by assuming that the percentage loss of each unlabelled amendment was the same as the percentage loss from the corresponding ^{15}N-labelled amendment in the treatment containing the same combination of amendment types.

Treatment effects were tested by 1-way Analysis of Variance (ANOVA) for repeated measures. Least significant differences (LSD) were calculated when ANOVA indicated significant effects ($P<0.05$).

Analytical Methods
Soil inorganic N (ammonium + nitrate) was determined by extracting fresh soil in 2 M potassium chloride, followed by automated colorimetric analysis of the extracts (Rayment and Higginson, 1992, method 7C2). Total N and C were determined in dried residues (< 0.5 mm) using a Leco combustion analyser. Carbon dioxide in the sodium hydroxide traps was measured by titration against dilute hydrochloric acid (Zibilske 1994). The ^{15}N content of soil and residues was determined in pulverised samples on a Europa mass spectrometer.

Results
There was good agreement between unlabelled and ^{15}N-labelled amendments in measurements of C mineralisation, soil total N and inorganic N (Fig. 1), thus the aim of producing ^{15}N-labelled and unlabelled residues of similar chemical composition and decomposition characteristics was achieved.

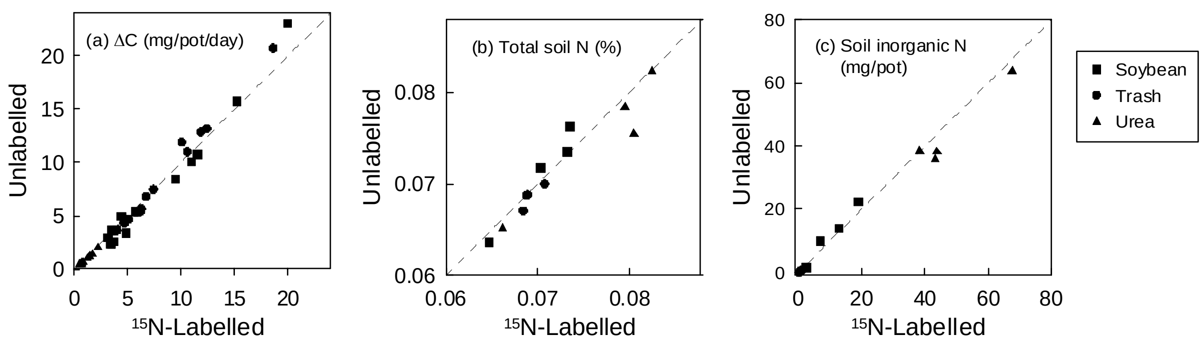

Figure 1. C mineralisation (a), total soil N (b), and inorganic soil N (c) from ^{15}N-labelled and unlabelled amendments. The broken lines shows the 1:1 relationship.

The C mineralisation (Fig. 2) from unamended soil was much less than from all the other treatments except the urea-amended soils. Soil ΔC_A (Fig. 3 b,d) was not affected by application of urea on its own.

Trash and soybean on their own produced similarly large increases in ΔC_A for the first few weeks, then the rate of C mineralisation continued at a lower rate. By day 121, trash had mineralised slightly more C than soybean (780 and 620 mg C/pot, respectively), representing 39% of trash C and 67% of soybean C. Application of urea temporarily increased ΔC_A from soybean (to day 10) and trash (to day 58) (Fig. 2). At day 121, the cumulative effect of urea on soybean had disappeared, but cumulative ΔC_A from trash was still increased by urea addition (to 1100 mg C/pot, or 57% of trash C) (Fig. 3 b,d). Application of trash and soybean together caused a very large ΔC_A, which showed the same pattern of decline with time as that shown by the residues alone. Cumulative ΔC_A for trash and soybean together was 1500 mg C/pot, or 52% of the applied C. Urea addition to the trash and soybean mixture increased ΔC_A at most of the samplings, and significantly increased cumulative ΔC_A (to 1700 mg C/pot, or 61% of residue C).

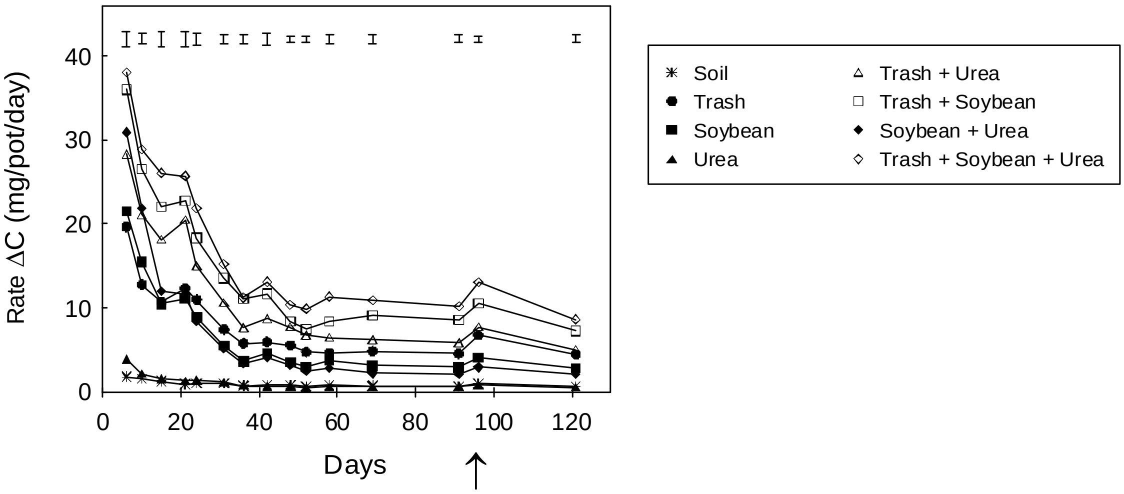

Figure 2. C mineralisation rate during incubation (ΔC). Bars are LSD (P=0.05) for comparing treatments. Arrow indicates time of leaching.

Apparent net N mineralisation in unamended soil (ΔN) reached a maximum of 4 mg N/pot (Fig. 3c). Trash by itself resulted in net immobilisation, with ΔN_A of up to -6 mg N/pot, or 21% of the original trash N content (Fig. 3a,c). Soybean immobilised N to day 17 (ΔN_A -1 mg N/pot), then gradually mineralised N to a maximum ΔN of 15 mg N/pot (43% of soybean N content) at day 91. Urea produced a ΔN_A of ≤36 mg N/pot during the first 43 days, and this rose to a maximum of 60 mg N/pot (91% of urea applied) at day 91. Addition of trash completely negated the apparent N mineralisation from soybean (maximum ΔN_A -2 mg N/pot), and approximately halved apparent mineralisation from urea (maximum ΔN_A 36 mg N/pot). Soybean and urea together apparently mineralised only slightly more N than urea on its own (maximum ΔN_A 68 mg N/pot, or 67% of applied N). Soybean, trash and urea together (maximum ΔN_A 49 mg N/pot, 37% of applied N) mineralised slightly more N than trash and urea together. Large amounts of inorganic N remained in the soil after the leaching on day 96.

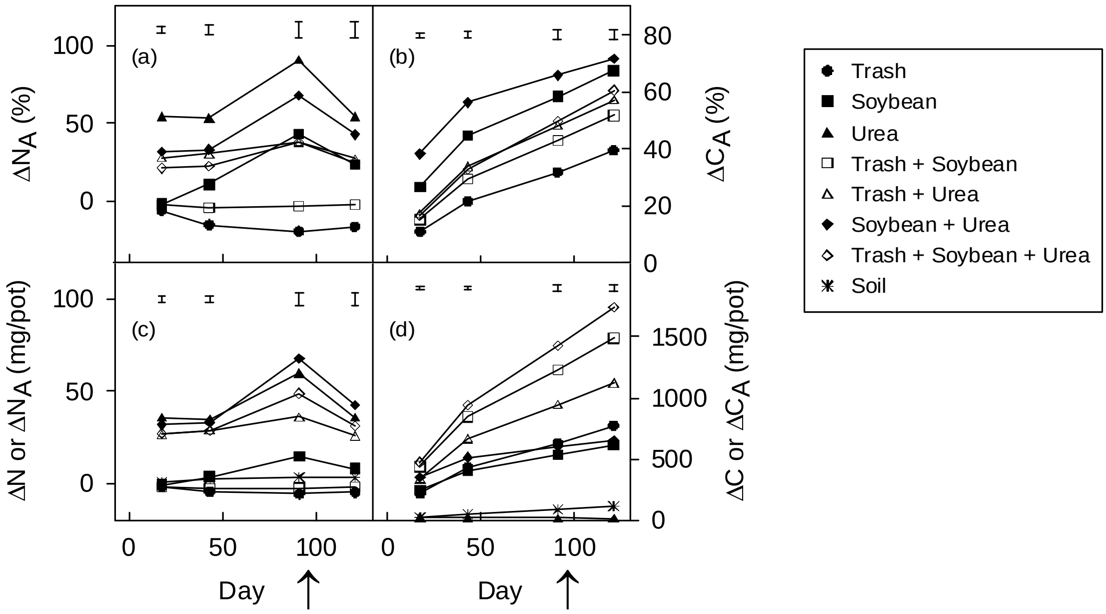

Figure 3. C mineralisation and apparent N mineralisation from soil (ΔC, ΔN) and amendments (ΔC_A, ΔN_A), expressed as mg and as percentage of applied. Bars are LSD (P=0.05) for comparing treatments. Arrow indicates time of leaching.

Loss of ^{15}N from trash, soybean, and urea in the various mixtures is shown in Fig. 4. Trash by itself lost ≤11% of its original ^{15}N content. Addition of soybean and/or urea did not significantly change this (Fig. 4a). The ^{15}N loss from soybean alone was ≤18% until day 91, and increased to 40% at day 121 (Fig. 4b). The ^{15}N loss from soybean was not significantly affected by addition of urea, but was reduced (to ≤16%) at day 121 by addition of trash or trash plus soybean. The ^{15}N loss from urea alone gradually increased to 15% by day 91, and had increased to 43% by day 121 (Fig. 4c). Soybean addition slightly increased ^{15}N loss from urea (to 20%) until day 43, but reduced ^{15}N loss at day 121. Trash or trash plus soybean addition had no effect on ^{15}N loss from urea until day 121, when ^{15}N loss was reduced to ≤12%.

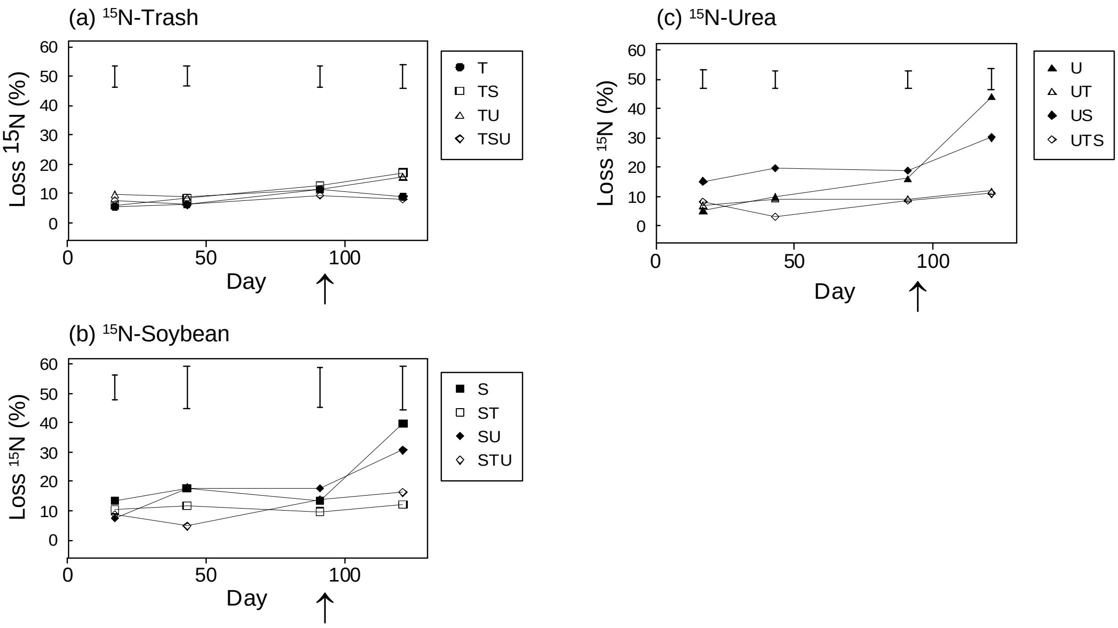

Figure 4 Loss of ^{15}N from labelled trash, soybean and urea, decomposing alone or with other unlabelled amendments. Bars are LSD (P=0.05) for comparing treatments. Arrow indicates time of leaching.

Calculated total N mineralisation (N_{min}) (Fig. 5) was greatest in the soybean + urea and the urea treatments. The absolute quantity of N mineralised was greatest with soybean + urea (45-86 mg/pot, 45-

86%), but the proportion of amendment-N mineralised was greatest with urea alone (39-72 mg/pot, 59-110%). The N_{min} from soybean alone increased gradually from day 17 to 121, to a maximum of 20 mg/pot and 63%. Trash alone immobilised N throughout the incubation (N_{min} –2 to 0 mg/pot). Trash addition markedly reduced N_{min} from the other amendments at most sampling times.

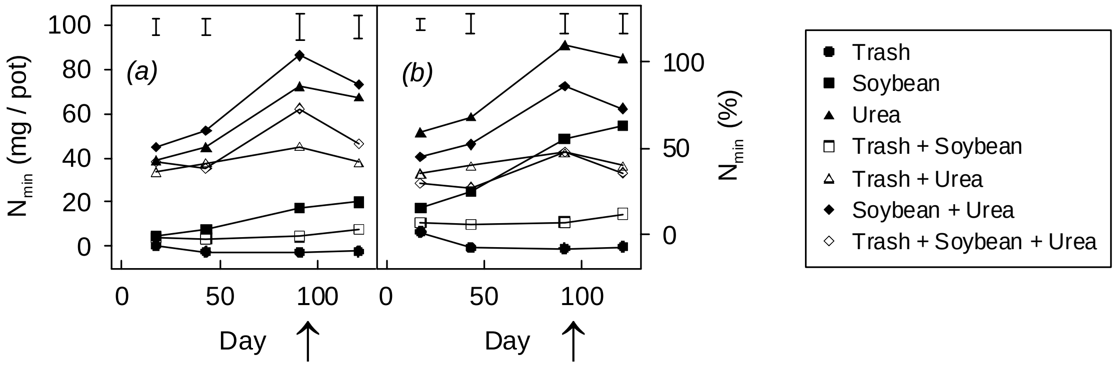

Figure 5. Calculated N mineralisation (N_{min}) expressed as mg (a) and as percentage of applied (b). Bars are LSD (P=0.05) for comparing treatments. Arrow indicates time of leaching.

Discussion

Trash and soybean tops were both significant sources of labile C, with 39-67% of their original C being mineralised after 121 days (Fig. 3). The pattern of C mineralisation in these residues, however, was different. Soybean-C was more labile, with rapid mineralisation during the first few weeks, followed by slower mineralisation as the labile C supply became depleted. Trash-C was mineralised more slowly than soybean at first, but mineralisation was sustained for longer. The peak in C mineralisation, representing decomposition of the most accessible residue components, occurred within 10-15 days (Fig. 2).

Urea had no effect on C mineralisation from soil. Application of urea to plant residues would not be expected to increase C mineralisation unless decomposition was limited by N availability (Alexander 1977). Thus, cumulative C mineralisation from soybean was not increased by urea. However, the initial stimulation of C mineralisation from soybean by urea (first 10 days) suggested that decomposition of the most labile C fraction was limited by N supply. Mineralisation of C from trash was apparently N-limited for the first 58 days (Fig. 2), the effect of which was still evident in cumulative ΔC at day 121 (Fig. 3). This confirmed other findings that N addition to trash may stimulate C mineralisation during the early stages (<2 months) of decomposition, but had no effect on C mineralisation over 8 months (F. Robertson unpublished data). Urea applied to trash and soybean together caused a similar increase in ΔC during this incubation experiment.

Apparent net mineralisation of N estimated from ΔN and ΔN_A suggested that soil N mineralisation was <14 µg N/g soil, and that trash immobilised all the inorganic N in the soil (Fig. 3). The soybean initially caused net immobilisation, but >40% of the soybean-N was finally mineralised, providing additional soil inorganic N of 51 µg N/g soil. The increase in soil inorganic N caused by urea was surprisingly slow, accounting for only about half of the applied urea on day 43 (Fig. 3). This may have been due to slow hydrolysis or to microbial immobilisation of hydrolysed urea. However, most (91%) of the applied urea was later recovered as inorganic N.

The ΔN_A from soybean and from urea were very much reduced by the addition of trash. This net immobilising effect of trash was much greater than for trash alone, reflecting the greater N availability in the mixtures (apparent immobilisation –58 to –81 µg N/g soil, assuming soybean and urea mineralised the same alone and in combination with trash). Trash had a similar effect on ΔN_A in the Trash + Soybean + Urea treatment (apparent immobilisation –61 µg N/g soil). The ΔN_A in mixtures was mostly similar to the total ΔN_A of the same amendments decomposing alone, suggesting that microbial utilisation of the amendments was not limited in the mixtures.

The ^{15}N lost from the pots comprised soluble N that was removed by leaching at day 96, and gaseous N lost through denitrification or volatilisation. As the pots were not leached until day 96, and there was no drainage from the pots after watering, the ^{15}N lost at the first 3 samplings must have been through denitrification or volatilisation. It should be recognised, however, that some of the errors associated with the ^{15}N results were quite large, particularly for soybean. Loss of ^{15}N from trash was small (<11%), and not affected by the presence of any of the other amendments (Fig. 4). Loss of ^{15}N from soybean reached 40%, with up to half of this apparently lost in gaseous forms. Loss of ^{15}N from urea was 43%, with one third of it apparently gaseous loss. Addition of soybean increased loss of urea-^{15}N at the first 3 samplings, perhaps because the greater labile C supply stimulated denitrification. Addition of trash markedly reduced ^{15}N loss from soybean and urea at the end of the experiment, probably because mineralised N was protected from being leached by being immobilised.

The ^{15}N data is useful for understanding the mineralisation-immobilisation behaviour of individual components in the decomposing mixtures. Of greater agronomic importance, though, is the net mineralisation or immobilisation of the complete mixture (N_{min}), as this is what determines N supply to the crop. The N_{min} results (Fig. 5) suggested that 63% of soybean N was mineralised at the end of the experiment. The N_{min} from trash was negative. Urea N_{min} accounted for 93-110% of applied N. Trash addition reduced N_{min} from soybean and urea. Soybean and urea together had the greatest N_{min}.

This study demonstrated the capacity of trash to immobilise large quantities of inorganic N from soil, urea, or soybean tops. This characteristic could be used to advantage by sugarcane growers. e.g. by retaining rather than burning the trash at the end of the crop cycle, significant soil inorganic N may be immobilised and protected from loss.

It should be recognised, however, that this study relates only to the first 6 months after application of the amendments, and to a soil with no history of trash or soybean addition. Patterns of C and N mineralisation from the current amendments would, of course, eventually change with time. Furthermore, mineralisation from old residues accumulated from several annual applications may influence mineralisation from the current amendments.

Conclusions
Cane trash and soybean tops were good sources of readily decomposable C. Addition of urea had little effect on C mineralisation from soil or residues, except a slight stimulation in the first 2 months of trash decomposition. Urea produced the greatest increase in soil inorganic N, 85-110% being recovered. Soybean tops eventually mineralised 40-70% of their N content. There was evidence of some gaseous loss of N mineralised from soybean and urea. Trash immobilised all the N mineralised from soil, 80-100% of the N mineralised from soybean tops, and 20-50% of N mineralised from urea. Negligible N was mineralised from trash, and the addition of urea or soybean did not change this. Patterns of C and N mineralisation were generally similar for the amendments alone and in combinations, except where N immobilisation was limited by N availability.

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