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Priming of soil organic carbon induced by sugarcane residues and its biochar control the source of nitrogen for plant uptake: a dual ^{13}C and ^{15}N isotope three-source-partitioning study

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Abstract

Sugarcane (*Saccharum* spp.) farming systems globally have largely transitioned away from burning the crop prior to harvest. Harvesting the sugarcane crop 'green' results in large volumes of biomass residues being left on the soil. Despite this, there is little evidence for increased soil organic carbon stocks. We investigated the role of surface application or incorporation (0- 200 mm soil layer) of harvest residues (15 t dry weight residues ha^{-1}) and its biochar (5.4 t ha^{-1} based on the quantity of resource recovered after pyrolysis) on the priming of native soil organic carbon (SOC), the mineralisation of the organic amendments and the source of crop N

uptake (SOC, organic amendment or urea). All treatments received urea at 180 kg N ha^{-1}. To achieve the separation of C and N sources, dual ^{13}C and ^{15}N-enriched sugarcane residues and corresponding biochar (350°C) were used in an 84-d controlled environment study. A three-pool isotope mixing model, utilising two levels of ^{13}C enrichment in residue (16.6‰ and 23.8‰) and biochar (16.8‰ and 24.1‰), was also applied to partition the C from three sources: 1)root respiration, 2) organic amendment mineralisation, and 3) SOC priming. The SOC mineralisation was increased following both surface-applied and incorporated residues, over the nil organic amendment (control) by 72.3 and 78.3 CO_2-C m^{-2} respectively over 84 days. In contrast, biochar lowered the mineralisation of SOC by 62.9 g CO_2-C m^{-2} compared to the control. The cumulative mineralisation of sugarcane residue biochar (18.9 g CO_2-C m^{-2}) was lower (P=0.03) than surface applied residue (50.1 g CO_2-C m^{-2}) and incorporated residue (71.9 g CO_2-C m^{-2}) over the study period. While there were no differences in total crop N uptake between the organic-amended soils and the control, the source of N was significantly different. The sugarcane plants utilised 31.0% and 29.4% of the supplied urea N in the nil organic-amended control and biochar treatment, respectively, but only 24.8% and 20.6% in the surface residue and incorporated residue treatments, respectively. In comparison, the plant uptake of N derived from the organic amendments was 27.8%, 15.4% and 6.4% from incorporated residues, surface-applied residues and biochar, respectively (P<0.001). Results suggest that the increased mineralisation of SOC, partly driven by the C:N ratio (73:1) and the

unbalanced nutrient stoichiometry may lead to low SOC accumulation from residues blanketing and that its biochar results in SOC stabilisation and increase the use efficiency of fertiliser N in sugarcane systems.

Keywords: rhizodeposit, priming effect, N use efficiency, urea, three-pool C partitioning model.

1. Introduction

It has recently been highlighted that there is a need to understand the effects of cropping systems and practices on SOC storage and sequestration, and that there is potential for innovation (Chenu et al., 2019). While sugarcane crops have been traditionally burnt to facilitate easier harvesting, there has been a global transition from burnt to green harvesting in recent years. Green harvesting and residue (sugarcane leaf matter and tops) retention have been reported to improve ratoon crop yields, increase soil moisture and organic matter and to decrease greenhouse gas emissions (Wood, 1991; Panosso et al., 2011). Several reports also suggest that residue retention in sugarcane systems has no effect on soil organic C stocks (Blair et al., 1998; Page et al., 2013). Sugarcane residue retention has the potential to supply N to the following crops (Robertson and Thorburn, 2007a; Ferreira et al., 2016) and may serve as a slow release N fertiliser with potential for N supply over the medium to long term (Meier et al., 2006). Indeed, Ferreira et al. (2016) showed that the average residue-N recovery across the two

46 sites after three crop cycles was 7.6 kg ha^{-1} (or 16.2% of the initial N content in residues). The
47 decomposition rate of residues is the key regulator of residue-N dynamics in soil, which in turn
48 affects the mineralisation of native soil organic carbon (SOC). The change in the mineralisation
49 rates of SOC by soil treatments is defined as the priming effect (Blagodatskaya and Kuzyakov,
50 2008). The challenges in investigating the mechanisms of priming of SOC are the slow turnover
51 of the stable C pool, absence of plants, lack of field mechanisms and short experimental
52 timeframe (Wang et al., 2016; Ding et al., 2018; DeCiucies et al., 2018). However, there is a
53 paucity of information on sugarcane residue-induced priming of SOC. Despite large quantities
54 of residues remaining on soil at harvest (13-20 t ha^{-1}, Thorburn et al., 2012), there was no
55 evidence that total soil C stocks nor fractions as methodologically defined by the authors (Page
56 et al., 2013) differed between green harvest and residue retention and traditional burning at
57 four contrasting sites in Australia. Similarly, Pinheiro et al. (2010) did not find statistically
58 significant differences in soil C stocks between residue retention and burnt crop in Brazil.
59 Balancing stoichiometric ratios of nutrients to C (*i.e.* C: N, P and S) has been shown to be a
60 critical factor in the development of the stable SOC pool (Kirkby et al., 2013). However,
61 sugarcane residues typically has a C: N ratio above 70: 1 (Robertson and Thorburn, 2007), far
62 greater than the ratio of stabilised SOC of 12: 1 (Himes 1998; Kirkby et al., 2013). Indeed,
63 incorporated sugarcane residues decreased N uptake by sugarcane from a Humic Acrisol by
64 immobilising available soil N (Kwong et al., 1987), suggesting the potential for increased SOC

65 mineralisation to meet the microbial demand for N in soil (de Sosa et al., 2018).

66 The thermal conversion, via slow pyrolysis, of cane residues including green harvest residues
67 and bagasse (crop residues after sugar extraction) can produce thermal or electrical energy as
68 well as biochar (Quirk et al., 2012). Biochar can have benefits when used as a soil amendment
69 including increasing SOC through the direct input of a stabilised C as well as further
70 stabilisation of rhizodeposits (*i.e.* new C), resulting in lowered SOC mineralisation (Weng et
71 al., 2015; 2017; 2018). However, the direction and magnitude of biochar-induced priming have
72 not yet reached a consensus with both increased and no change in SOC mineralisation also
73 reported (Zimmerman et al., 2011; Keith et al., 2011, Wang et al., 2016; DeCiucies et al., 2018;
74 Ding et al., 2018). Biochar has also been shown to lower N_2O emissions from soils (Cayuela
75 et al., 2014) and improve crop and pasture P and K nutrition (Slavich et al., 2013; Van Zwieten
76 et al., 2015; 2019), while the effects are variable or transient in other studies (Wang et al., 2014;
77 Ogle et al., 2019).

78 Until now, most studies on the impact of biochar on soil C priming have used two approaches:
79 1) addition of unlabelled biochars to unlabelled soil (Wardle et al., 2008; Singh and Cowie,
80 2012), and 2) addition of ^{13}C (Jones et al., 2011; Zimmerman et al., 2011) or ^{14}C labelled
81 biochars to unlabelled soil (Kuzyakov et al., 2009). However, the use of only two stable
82 isotopes (^{13}C and ^{12}C) constrains the identification of C-partitioning to only two C sources
83 (usually the biochar C and soil organic C). More complex methodological approaches

84 involving $^{13}CO_2$ pulse-labelling techniques or mixture of C3 and C4 compartments can discern
85 three C sources (soil, biochar and plants) in biochar-amended systems in the presence of plants
86 (Weng et al., 2015; Whitman et al., 2014). However, boundary conditions assuming an extreme
87 scenario whereby only one C source was mineralised from the combined C sources of biochar
88 plus root exudates are required to estimate the variations in the three C-pool partitioning system
89 (Weng et al., 2017). A three-source-partitioning approach combining ^{14}C labelling with ^{13}C
90 natural abundance has been adopted in some studies to partition three C sources in soil systems
91 (Blagodatskaya et al., 2011; Luo et al., 2017). Recently, a dual-isotope approach was employed
92 to partition emissions of CO_2 derived from soil organic C, added biochar and root respiration
93 using two levels of ^{13}C enrichment (Whitman and Lehmann, 2015). This approach was utilised
94 in the current study, in conjunction with ^{15}N co-labelled residues or ^{15}N labelled urea to
95 investigate the role of sugarcane residues and its biochar on C and N processes in soil.
96 Specifically, we aimed to quantify the mineralisation of C and N from sugarcane residues and
97 its corresponding biochar, as well as SOC priming and fertiliser N-use efficiency by sugarcane
98 plants. We hypothesised that co-metabolism of labile-C fractions in residues might increase
99 SOC mineralisation due in part to an imbalanced nutrient stoichiometry. This may then
100 accelerate the decomposition of residues and improve residue-N uptake by plants in the short
101 term (<100 d). Based on evidence from other systems (Weng et al., 2017), we hypothesised
102 that residue biochar may lower the mineralisation of SOC, thus providing a means by which to

build new soil C beyond the stabilised C applied via biochar.

2. Material and Methods

2.1 Soil and amendments

In October 2016, a sandy soil (Arenosol, FAO) ($\delta^{13}C$: -23.2‰) was collected from the 0-200 mm layer using a compositing method (Tan, 2005) in an unfertilised paddock (50 m in diameter) in a subtropical sugarcane plantation near Ballina, New South Wales (NSW), Australia (29°00′S 153°23′E). Soil was air-dried, sieved through 2 mm and thoroughly mixed. Any visible pieces of undecomposed plant materials (*e.g.* roots, leaves and stem) were removed. Soil pH ($CaCl_2$ (1:5)) was 4.0 with a total C content of 23 g kg^{-1} and total N of 2.1 g kg^{-1} (Dumas combustion). Extractable ammonium (in 2 M KCl) was 7.7 mg N kg^{-1} and extractable nitrate (in 2 M KCl) was 12.0 mg N kg^{-1}.

Residues were derived from sugarcane grown in a controlled climate glasshouse at Wollongbar Primary Industries Institute, Wollongbar, NSW, Australia. When mature, the sugarcane tops and leaves were separated from the cane and were processed into 10 mm long sections, air-dried and homogenised. Two sets of ^{13}C and ^{15}N dual-labelled sugarcane residues were produced from the combination of $^{13}CO_2$ pulse-labelling (five pulse labelling events) and the addition of ^{15}N-enriched urea and the only external N source. The following materials were obtained; 1) ^{13}C enriched residues with ^{15}N at natural abundance ($\delta^{13}C$: 23.8‰, $\delta^{15}N$: 31.3‰); and 2) ^{13}C and ^{15}N dual-enriched residues ($\delta^{13}C$: 16.6‰, $\delta^{15}N$: 5360.6‰). Both residue

materials contained 430 g kg^{-1} of total C and 5.9 g kg^{-1} of total N (by Dumas combustion) giving a C: N ratio of 73: 1. The detailed labelling procedures are documented in Supplementary Information.

Biochars were produced from the same sets of sugarcane residues (500 g residues) by slow pyrolysis (5-10 °C min^{-1}) in a modified muffle furnace, at a highest treatment temperature (HTT) of 350 °C, with a residence time at HTT of 30 min under a stream of N_2. The residues were cooled under a stream of N_2. The average conversion rate of residue biomass to biochar was 36% on a mass basis. Two biochars were produced: 1) ^{13}C-enriched biochar with ^{15}N at natural abundance ($\delta^{13}C$: 24.1‰, $\delta^{15}N$: 23.8‰); and 2) ^{13}C and ^{15}N dual-enriched biochar ($\delta^{13}C$: 16.8‰, $\delta^{15}N$: 5163.7‰). Basic chemical properties of the biochars are shown in Table S1, with analytical methods according to Van Zwieten et al. (2019).

2.2 C and N cycling study

An 84-d experiment was established on 2 November 2016 in a temperature-controlled glasshouse at Wollongbar Primary Industries Institute, Wollongbar NSW, Australia. The growth conditions consisted of 16-hour light periods with temperatures of 30°C during the light period and 25°C during the dark period. The experiment comprised four sugarcane residue amendment treatments – 1) sugarcane residues applied to the surface (‘surface residue’), 2) sugarcane residues incorporated (‘incorp residue’), 3) sugarcane biochar incorporated (‘incorp biochar’), and 4) control (nil organic amendment) – with four replicates per treatment. The

141 residues were applied to pots at 15 dry t ha^{-1} on a soil surface area basis (based on Thorburn et
142 al., 2012), either mixed into the top 200-mm soil profile or surface applied. The rate of
143 application in this study is slightly above the realistic application. Based on a 140 kg dry residue
144 per t cane (Waldheim et al., 2001; Malmgren et al., 2005; Caldeira-Pires et al., 2018), the
145 sugarcane yield would be equivalent to 107 t ha^{-1} which is above the common range of 80-100
146 t ha^{-1}. Further, it is recommended not to return all residue back to field to avoid yield penalty
147 (Malmgren et al., 2005; Viator et al., 2006; Cardoso et al., 2015; White et al., 2017; Ottoni et
148 al., 2018; Popin et al., 2019; Silva and Lisboa, 2019). The biochar was applied on a 'resource
149 recovery basis' being incorporated into the 0-200 mm soil profile at 5.4 t biochar ha^{-1}
150 (equivalent to 15 dry t residues ha^{-1} at a conversion rate of 36%).
151 Each pot received N fertiliser in the form of urea at 180 kg N ha^{-1} on a soil surface area basis.
152 To investigate interactions between plant uptake of N from the sugarcane residues and the urea,
153 urea was applied to pots with 5.16 atom% of enrichment or at natural abundance (Sigma-
154 Aldrich, USA). Thus, the treatment design was:
155 1. Surface ^{13}C ^{15}N residue ($\delta^{13}C$: 16.6‰, $\delta^{15}N$: 5360.6‰) + ^{14}N urea (n=4)
156 2. Surface ^{13}C ^{14}N residue ($\delta^{13}C$: 23.8‰, $\delta^{15}N$: 31.3‰) + ^{15}N urea (n=4)
157 3. Incorp ^{13}C ^{15}N residue ($\delta^{13}C$: 16.6‰, $\delta^{15}N$: 5360.6‰) + ^{14}N urea (n=4)
158 4. Incorp ^{13}C ^{14}N residue ($\delta^{13}C$: 23.8‰, $\delta^{15}N$: 31.3‰) + ^{15}N urea (n=4)
159 5. Incorp ^{13}C ^{15}N biochar ($\delta^{13}C$: 16.8‰, $\delta^{15}N$: 5163.7‰) + ^{14}N urea (n=4)

6. Incorp ^{13}C ^{14}N biochar ($\delta^{13}C$: 24.1‰, $\delta^{15}N$: 23.8‰) + ^{15}N urea (n=4)

7. Nil organic amendment: nil residue(δ13C: -23.2‰) + ^{15}N urea (n=4)

8. Nil-urea, nil organic amendment (δ13C: -23.2‰, n=4) was also established as references to determine the ^{15}N enrichment of soil N pools accessed by sugarcane plants for later calculations of ^{15}N recovery by plants (see Equation 8 below).

Phosphorus (P) and potassium (K) were applied as superphosphate and sulfate of potash at 30 kg P ha^{-1} and 100 kg K ha^{-1}, respectively. The basal nutrients were mixed uniformly through the soil for all treatments. The following types and amounts (mg kg^{-1} dry soil) of basal nutrients were added in solution and mixed thoroughly in the soils as per Rose et al. (2007): $CaCl_2.2H_2O$, 180; $MgSO_4.7H_2O$, 50; $ZnSO_4.7H_2O$, 9; $CuSO_4.5H_2O$, 6; $Na_2MoO_4.2H_2O$, 0.4. The mixtures of soil (13.74 kg) and organic amendments were repacked into PVC pots with a diameter of 250 mm and a height of 275 mm (Decor, Australia) to a bulk density of 1.4 g cm^{-3} as assessed on a dry weight per volume basis. The soil was watered to 90% field capacity and allowed to stand in a glasshouse at 30°C overnight before sugarcane was planted.

Previously, sugarcane stalks were sourced from a sugarcane plantation at Broadwater, NSW, Australia, on the basis of size and vigour to minimise variability associated with plant propagules. Twenty stalks were cut into 1-eye sets and 150 sets were selected for planting in a mixture of fine and coarse sand in the glasshouse at 30°C. Two cane plants (*ca.* 150 mm high) were transferred into each treatment pot. Pots were weighed every 3 d during the experiment

and water was added to maintain 90% of field capacity. Pots were re-randomised weekly within each replicate/block to minimise edge effect and shading from adjacent plants.

2.3 Measurements

2.3.1 Collection and analysis of leachates

The soil pots were leached with Milli-Q water approximating the pore volume of the 0-100 mm amended layer at 14 days after planting (DAP) (1700 ml) and 56 DAP (1800 ml). The mass, pH and EC of the leachate were determined immediately before leachates were frozen for subsequent C and N analyses. Total N and dissolved organic carbon (DOC) of leachates were measured using Sievers InnovOx ES TOC/N analyser. The total amount of N or DOC leached was calculated as a product of concentrations × leachate volume at each leaching time.

2.3.2 CO_2 sampling and analysis

The total CO_2 was sampled using a static chamber (diameter 100 mm, height 150 mm) at 2 to 3 d intervals in the first 3 weeks following organic amendment and sampled at 7 to 14 d intervals thereafter. The static chamber was open-ended heavy-duty polyvinyl chloride (PVC) tube installed 150 mm into soil. A PVC cap (*i.e.* static chamber) with a rubber o-ring was attached during the CO_2 measurement period (described in van Zwieten et al., 2014). No above ground biomass was present within the respiration collars. To capture total CO_2 emissions from soil, a 100-mL jar containing 40 mL of 2 M NaOH was placed in each static chamber for 24 h (Weng et al., 2015). Distilled water was used to make NaOH and the stock solution was checked

198 for background at each measurement. Two empty static chambers with closed bases (*i.e.*
199 blanks) were set up in the same manner to account for headspace CO_2. To determine the total
200 CO_2 respired from each treatment, a 1-mL aliquot of the CO_2 trap solution was titrated against
201 0.1 M HCl on a TitraLab® auto-titrator (TIM840, Radiometer analytical, Lyon, France), with
202 a terminal pH of 8.2. The blank CO_2 trap solution was titrated in the same fashion and the
203 amount of atmospheric CO_2-C captured within the static chamber headspace was subtracted
204 from each sample. A 10-mL aliquot of the CO_2 trap solution (*i.e.* NaOH) was precipitated with
205 10 mL of 1.25 M $SrCl_2$ to form $SrCO_3$ (Weng et al., 2015). 1.5 mg of $SrCO_3$ with 3 mg of WO_3
206 (as an oxidant) was prepared in tin capsules and sent to the University of California (Davis
207 Stable Isotope Facility, CA, USA) for $\delta^{13}C$ analysis. The $\delta^{13}C$ signatures of these $SrCO_3$
208 mixtures were measured by a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ
209 Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The $\delta^{13}C$ signatures
210 of the trapped total CO_2 fluxes were corrected:

211 $$\delta Total = \frac{\delta Measured \times (CTotal + CBlank) - \delta Blank \times CBlank}{CTotal} \quad (1)$$

212 where δ_{Total} is the $\delta^{13}C$ signature of the total CO_2 fluxes from the planted residues/biochar and
213 soil mixture, $\delta_{Measured}$ is the measured $\delta^{13}C$ signature of each sample, and δ_{Blank} is the $\delta^{13}C$
214 signature of headspace CO_2 at the time of measurement. C_{Total} is the total CO_2-C evolved from
215 the planted residue/biochar-amended soils and C_{Blank} is the amount of atmospheric CO_2-C
216 captured within the blank static chamber headspace.

217 *2.3.3 Soil and plant sampling and analysis*

218 The soil was destructively sampled at the end of the experiment. The roots were carefully
219 separated from the soil and were cleaned under running water. Shoots were divided into leaf/
220 tops and cane tissue, which was washed once in RO water, once in 0.1 M HCl and rinsed twice
221 in deionised water. Plant tissue was oven-dried at 70°C for 2 days for dry matter determination
222 and soil was oven-dried at 40°C for 5 days.

223 Subsamples of homogenised plant (*i.e.* cane + tops and leaf) (50 g) and soil (200 g) materials
224 were ground to a fine powder for analyses using a laboratory grinding mill (Polymix MFC-
225 90D, Switzerland). For the assessment of pH, a subsample of ground soil (5 g) was extracted
226 with 0.01 M $CaCl_2$ (1: 5) using an end-over-end shaker for 1 h, then centrifuged at relative
227 centrifugal force of 839 *g* for 5 min. The pH of each soil extract was determined before aliquots
228 were filtered for subsequent analyses. Soil pH was measured using a Thermo Orion 720 pH
229 meter. Total C and total N in soil and plant samples were assessed by Dumas combustion (Chan
230 et al., 2008). The $\delta^{13}C$ and ^{15}N signatures of biochar, soil and plant material were determined
231 at the Davis Stable Isotope Facility, CA, USA, as described above.

232 *2.4 Calculations*

233 *2.4.1 Three-pool C source partitioning*

234 In a three-pool system with two levels of enrichments:

235 $$f_{Soil} + f_{Amendment} + f_{Root} = 1 \quad (2)$$

$$\delta_{Total\ 1} = f_{Soil} \times \delta_{Soil} + f_{Amendment} \times \delta_{Amendment\ 1} + f_{Root} \times \delta_{Root} \quad (3)$$

$$\delta_{Total\ 2} = f_{Soil} \times \delta_{Soil} + f_{Amendment} \times \delta_{Amendment\ 2} + f_{Root} \times \delta_{Root} \quad (4)$$

where f_{Soil}, $f_{Amendment}$ and f_{Root} are the proportion of soil CO_2-C, amendment-derived CO_2-C and root CO_2-C in the total CO_2-C fluxes from the planted residue/biochar and soil mixture, respectively. δ_{Total} is the $\delta^{13}C$ signal of the total CO_2-C evolved from the planted residue/biochar-amended soils after corrected for blanks. δ_{Soil} is the averaged $\delta^{13}C$ signal of CO_2-C evolved from the unplanted controls at the time of sampling and $\delta_{Amendment}$ is the $\delta^{13}C$ signature of the initial amendment (residue/biochar). δ_{Root} is the $\delta^{13}C$ signature of root respiration from individual pot at each sampling (details below). Note that three organic amendments (surface residue, incorp residue and incorp biochar) would potentially generate different proportions of amendment-derived CO_2.

To determine the $\delta^{13}C$ signatures of root respiration (δ_{Root}), washed root material (0.6 g) from each pot was incubated in a 100-mL glass flask in the dark for 6 h (Weng et al., 2015; Biasi et al., 2012). This approach has limitations in determining the true isotopic signals of all components contributing to autotrophic respiration (Section 4.1). In this study, roots were subsampled weekly to 80 mm depth outside the static chambers using a stainless steel soil corer (30mm in diameter) to capture the changes in $\delta^{13}C$ signatures of root respiration. The respired CO_2 was trapped in 2.5 mL of 2 M NaOH and ^{13}C was analysed as described above.

The proportion of amendment-derived CO_2-C in the total CO_2-C fluxes from the planted

255 residue or biochar and soil mixture ($f_{Amendment}$) was calculated using the following three-pool

256 ^{13}C isotopic mixing model:

257 $$f_{Amendment} = \frac{\delta Total\ 1 - \delta Total\ 2}{\delta Amendment\ 1 - \delta Amendment\ 2} \quad (5)$$

258 The proportion of soil CO_2-C in the total CO_2-C fluxes from the planted residue/biochar and

259 soil mixture (f_{Soil}) was determined using the following three-pool ^{13}C isotopic mixing model:

260 $$f_{Soil} = \frac{\delta Total\ 1 - \delta Root + famendment \times (\delta Root - \delta Amendment\ 1)}{\delta Soil - \delta Root} \quad (6)$$

261 The proportion of root respiration in the planted system was calculated by subtracting the

262 proportion of amendment-C and soil-C from 100.

263 We adapted the calculations of the uncertainty in source partitioning using first order Tyler

264 series approximations of the variances of respiration from each end-member (Whitman and

265 Lehmann, 2015).

266 $$\sigma^2 C_{End\text{-}member} = (f_{End\text{-}member})^2 \times \sigma^2 C_{Total} + (C_{Total})^2 \times \sigma^2 f_{End\text{-}member} \quad (7)$$

267 where $C_{End\text{-}member}$ and $f_{End\text{-}member}$ is the amount of respiration of each end-member (*i.e.* soil, root

268 respiration and organic amendments) and its proportion in the total CO_2-C evolved from the

269 planted residue/biochar-amended soils (C_{Total}), respectively.

270 *2.4.2 Total N uptake by cane plants and recovery of ^{15}N*

271 The N content of roots, cane and leaves were determined by multiplying the biomass by the

272 respective N concentration, and total plant N uptake was calculated by summing the N content

273 of the three tissues. The % of N in each tissue derived from fertiliser (NDF: urea) or organic

amendment (OA: residue/biochar) (%NDF/OA) was calculated using the equation:

$$\%\text{NDF/OA} = \frac{100 \times (a-b)}{c-b} \quad (8)$$

where '*a*' is the atom% ^{15}N in the tissue, '*b*' is the atom% ^{15}N in respective tissue of plants from the nil-N control pots, and '*c*' is the atom% ^{15}N in the organic amendments or urea.

The percentage of applied fertiliser or organic amendment ^{15}N recovered in root, leaf and cane tissue was calculated using the equation:

$$\%\text{N recovered} = \frac{\%\text{NDF/OA} \times \text{d}}{e} \quad (9)$$

where 'd' is the N content of the plant tissue (mg N) and '*e*' is the amount of N applied in organic amendments or urea (mg N). Total plant ^{15}N recovery from urea or from residue/biochar was calculated by summing the ^{15}N recovery of the three plant tissues.

The content of N in each of the three tissues derived from urea or organic amendment sources was calculated by multiplying the tissue N content by the respective %NDF/OA, and the values for each tissue were summed to calculate total cane N uptake from urea or organic amendments. Cane uptake of N from the soil was calculated by subtracting the urea or organic amendment-derived cane N from the total cane N uptake.

2.5 Statistical analysis

A one-way analysis of variance (ANOVA) was conducted to test the effects of organic amendment treatment (surface residue, incorp residue, incorp biochar or urea only) on CO_2 flux, C mineralisation of organic amendments, root respiration, total N and DOC of leachates

293 and plant characteristics including leaf, cane and root biomass, N content and ^{15}N recovery
294 from fertiliser and organic amendments using GenStat 19th edition (VSN International, Hemel
295 Hempstead, England). The normality of residuals and homogeneity of variances were checked.
296 A least significant difference (LSD) test at P=0.05 was performed to test differences between
297 means. For plant biomass and N content (leaf, cane, root and total), preliminary analysis
298 indicated no difference in biomass or N content between plants grown with ^{15}N urea and with
299 ^{14}N urea (4 replicate pots), and these data were therefore combined for the analysis (8 replicate
300 pots per treatment).

301 **3. Results**

302 *3.1 Biomass production*

303 Total sugarcane biomass was significantly increased with the surface residue treatment
304 (P<0.001) but did not differ between the incorporated residue, incorporated biochar and the nil
305 organic amendment (Table 1). This difference was predominantly driven by the increase in
306 cane biomass by 54% from 49 g pot^{-1} in the nil organic amendment control to 75 g pot^{-1} in the
307 surface residue treatment (P=0.02, Table 1).

308 *3.2 N uptake*

309 While there were no differences in the N concentration in leaf tissue between the treatments,
310 the N content of cane was significantly lower in the surface residue treatment than the other
311 treatments (P<0.001, Table 1). However, there were no significant differences in the total N

uptake between treatments.

3.3 Plant uptake of ^{15}N from urea and organic amendments

The plant uptake of ^{15}N from urea was significantly lower in the incorporated residue treatment (183 mg pot^{-1}) than in the incorporated biochar and nil-amended control which were not significantly different from each other (261 and 276 mg pot^{-1}, respectively). Both surface application and incorporation of residues significantly (P=0.02) lowered urea-N recoveries from 31% in the control to 25% and 21%, respectively (Table S2).

The plant uptake of N derived from the organic amendments was in the order of incorporated residues (225 mg pot^{-1} or 27.8% of supplied ^{15}N from residues) > surface residues (125 mg pot^{-1} or 15.4%) > incorporated biochar (15.4 mg pot^{-1} or 6.37%) (Tables 2 and S1; P=0.01).

There was no significant effect of organic amendments on the total uptake of native soil N. The most noticeable feature of the percentage of total plant N derived from each N source was that cane plants had only 2% of their total N derived from the incorporated biochar, compared to 18% and 30% from the surface residues and incorporated residues treatments, respectively.

3.4 Priming of native SOC, cumulative amendment-C mineralisation and root respiration

The incorporation of biochar lowered the SOC mineralisation by 62.9 g CO_2-C m^{-2} *c.f.* the control, whereas both surface residues and incorporated residues increased SOC mineralisation over the control by 72.3 and 78.3 CO_2-C m^{-2}, respectively, towards the end of the 84-d study (Fig. 1). Similarly, the cumulative total soil respiration was greater in the surface and

331 incorporated residue treatments and lower in the incorporated biochar treatment compared with
332 the control over 84 days (Fig. S1). The mineralisation of incorporated residues (71 g CO_2-C m^{-2})
333 and surface applied residues (50 g CO_2-C m^{-2}) over 84 days was significantly greater
334 (P=0.03) than the incorporated biochar (18 g CO_2-C m^{-2}) (Fig. 2). Both surface application
335 and incorporation of the residues had three times higher cumulative root respiration (P=0.03)
336 over 84 d compared with the incorporation of biochar (Fig. 3). The normalised root respiration
337 (*i.e*. root respiration/ total root biomass) in surface and incorporated residue were 0.17 ± 0.04
338 and 0.14 ± 0.03 g CO_2-C g^{-1} root respectively compared with incorporated biochar of 0.07 ±
339 0.05 g CO_2-C g^{-1} root.

340 *3.5 Total N and dissolved organic carbon in leachates*

341 At 14 DAP, total N in the leachate in the biochar treatment was similar to that in the nil-
342 amended control but was significantly lower than that in the surface and incorporated residue
343 treatments (Table 3). At 56 DAP, total N in the leachate from the biochar-treated pots (53 mg
344 N pot^{-1}) was significantly greater than that from the incorporated residues and nil organic
345 amendment pots (32 and 27 mg N pot^{-1}, respectively), while the lowest N was from the surface
346 residue treatment (0.54 mg N pot^{-1}) (P=*0*.04). The sum of N leachate however was not
347 significantly different amongst the treatments. The cumulative DOC in the leachate was
348 significantly higher with the incorporated residues compared to other treatments (Table 3).

349 *3.6 Final soil analyses*

There was no difference in the total soil C content in the 0-100 mm soil layer across all treatments (Table S3) at the completion of the study. Surface residues and incorporated biochar also resulted in small but significantly higher total soil N content compared to the control and incorporated residues. While there were no significant differences in soil ammonium concentrations amongst the treatments, soil nitrate concentration in the surface residue treatment (7.70 mg kg^{-1}) was significantly lower (P<0.001) than the incorporated residues (34.6 mg kg^{-1}), incorporated biochar (29.5 mg kg^{-1}) or the control (28.5 mg kg^{-1}).

4. Discussion

4.1 Quantification of SOC priming and N dynamics using a dual-label three-pool partitioning model

We used a dual ($^{13}C/^{15}N$) isotopically labelled sugarcane residues and its biochar (350°C) with a three C source partitioning model (Whitman and Lehmann, 2015) to quantify priming of native SOC and to establish relations with N dynamics in a controlled environment study. The ^{13}C signature of CO_2 from the unplanted controls varied around ±1.5 ‰ over 84 days. Because of differential discrimination of isotopes, the $\delta\,^{13}C$ signatures of respiration from soil, root and organic amendments can vary from those of their solid form (Ehleringer et al., 2000; Bowling et al., 2008) and can gradually change over time (Whitman et al., 2014). In the field, a root signature collar packed with acid-washed sand and planted with ryegrass was used to determine the ^{13}C signatures of root respiration (Weng et al., 2017). In this study, the uncertainty in source

369 partitioning was propagated using first order Tyler series approximations of the variances of
370 respiration from soil, root and organic amendments (Whitman and Lehmann, 2015). For future
371 research, a respiration collar for each end-member will be useful to minimize the potential
372 impact of isotopic discrimination on C source partitioning.
373 Sugarcane biochar (350°C) lowered SOC mineralisation in the presence of actively-growing
374 plants at 40 d after incorporation, whereas surface applied or incorporated sugarcane residues
375 increased SOC mineralisation over the entire 84-d study (Fig. 1). This confirms recent studies
376 showing that biochar incorporation into soil can lower mineralisation of native SOC in the
377 presence of plants by 16-48% compared to the unamended controls (Ventura et al., 2014;
378 Whitman et al., 2014; Keith et al., 2015; Hernandez-Soriano et al., 2016; Weng et al., 2015;
379 2017). In our study, there was no change in SOC mineralisation in the first 40 d, possibly
380 because of positive rhizosphere priming counteracting the biochar-induced stabilisation of
381 SOC. Similar findings were observed in previous plant/soil-based studies (Weng et al., 2015;
382 Wang et al., 2017; 2018). For example, in a 5-year laboratory study, manure-based biochars
383 (400°C) increased SOC mineralisation in a low-C clayey soil over the first 2.3 years (Singh
384 and Cowie, 2014), and increased SOC mineralisation was also observed over 28 d after the
385 addition of a maize-derived biochar (400°C) in two Luvisols (Luo et al., 2017). These studies
386 suggested that biochars had a labile C component giving short-term increase in SOC
387 mineralisation in the absence of plants. In another unplanted study, a grass-based biochar

388 increased SOC mineralisation over the first 18 d, and then decreased from Days 18 to 158
389 (Maestrini et al., 2014). This was consistent with earlier research on a variety of biochar-soil
390 combinations, which showed that increased SOC mineralisation frequently occurred within the
391 first 90 d after incorporation, especially with biochars produced at low temperatures (250-
392 450°C), whereas lowered SOC mineralisation was more common from Days 250 to 500,
393 particularly with biochars produced at high temperatures (525-650°C) (Zimmerman et al.,
394 2011).
395 In our study, the mineralisation of surface-applied or incorporated sugarcane residues was
396 consistently greater than its "resource equivalent" biochar addition after incorporation over the
397 84-d experimental period (Fig. 2). The reason for this increased SOC mineralisation from
398 surface-applied and incorporated sugarcane residues is likely due to stimulation of microbial
399 co-metabolism induced from labile C fractions of the residues, root-C and SOC (Liang et al.,
400 2012). The normalised root respiration over total root mass was also lowered in biochar
401 treatment compared with residue which may suggest suppressed mineralisation of root-derived
402 C. This is mirrored by the quantity of DOC in leachates where incorporated residues had greater
403 leached DOC compared with surface-applied residues and incorporated biochar at 14 and 56
404 DAP (Table 3). Fiorentino et al. (2019) recently showed that biochar lowered labile C (glucose)
405 in soil and suggested that the lowering of labile C sources, from root exudates for example,
406 buffered the immobilisation of inorganic N. The increased SOC mineralisation has been

407 described as a distinct N-mining response of the microbial biomass (Murphy et al., 2015), thus
408 as the C: N ratio of sugarcane residues in our study was 73: 1, it is conceivable the soil microbes
409 were seeking N from soil organic matter (C: N ratio of 11: 1).
410 The increased SOC mineralisation observed in the residue treatments is consistent with field
411 studies showing that retention of residues did not necessarily increase soil C stocks compared
412 to other management practise such as burning cane residues (Page et al., 2013; Miles et al.,
413 2016). Where residue retention did not lead to modest increases in soil C in surface layers
414 compared to burning residues, modelling studies suggested that an equilibrium might be
415 reached within decades, limiting the potential for further increases in soil C (Meier and
416 Thorburn 2016). Like most sugarcane farms in Australia, the site where the soil was collected
417 had been continually producing sugarcane for over 60 years. Typically, sugarcane is grown for
418 between 5-8 years, with a summer soybean crop and winter fallow before being replanted to
419 sugarcane. Certainly, the high C:N ratio of residues in our study (73:1) is well above the optimal
420 ratio for stabilised soil organic matter (Kirkby 2013, 2016), thus increased mineralisation of
421 SOC is likely to have resulted from the need to satisfy microbial demand for N (de Sosa et al.,
422 2018). Indeed, Blair et al. (1998) showed that residue retention had lowered the total soil C
423 stocks of a Typic Tropaquept soil following the crop cycle of plant cane followed by four ratoon
424 crops over 7 years compared with the uncropped soil. They also showed an increase in labile
425 soil C, demonstrating potential demand for N during the mineralisation of the residues. Thus,

426 the application of stable C from biochar and subsequent lowered SOC mineralisation may
427 represent a management practice that could provide long-term increases in soil C storage in
428 warm, wet tropical environments where turnover of soil C is typically high. Nevertheless, our
429 short-term study only captured the transient breakdown of residue over 84 days. To determine
430 the full breakdown of the residue, a longer-term field study is recommended. Further, there are
431 numerous publications already showing no longer term effects of residue retention on SOC
432 stocks, but, no stable isotope methods were to differentiate the effect of residue on SOC pools
433 (Blair et al., 1998; Page et al., 2013; Miles et al., 2016). However, these potential improvements
434 in soil C need to be evaluated in light of other factors such as N cycling that could influence
435 crop growth, as discussed below.

436 *4.2 Amendment impacts on plant growth and N uptake from different N sources*

437 As pressure mounts globally to increase agricultural production for a growing population, while
438 sustaining the natural resource base, there is an urgent need to improve N-use efficiency in
439 farming systems. While the increased mineralisation of SOC may have resulted in greater soil
440 N uptake by plants in an N-limited system, N was not limiting in the current study as urea was
441 supplied (at the agronomically recommended dose) in all treatments. Indeed, there was no
442 significant difference in total N uptake among the treatments; only the source of N differed.
443 The most notable result was where plants compensated for lack of bioavailable N supplied by
444 the biochar treatment (only 15.4 mg pot^{-1} of N from the biochar was taken up, representing 2%

445 of total N uptake) through significantly higher uptake of urea-N. Biochar amendment did not
446 result in a significantly different uptake of soil N compared to the nil organic amendment
447 control. Thus, the increased N uptake from applied urea is particularly relevant where increased
448 fertiliser N-use efficiency is required.
449 Current losses of fertiliser-N to the environment in sugarcane farming systems can be
450 substantial, with losses of 45-59% of fertiliser-N reported (Prasertsak et al., 2002). These losses
451 are predominantly attributed to ammonia (NH_3) volatilisation and nitrate (NO_3^-) leaching
452 (Nachimuthu et al., 2017), but nitrous oxide emissions of > 18 kg N ha^{-1} $year^{-1}$ have also been
453 reported under some conditions (Wang et al., 2016). The study indicates that the incorporation
454 of residue biochar to sugarcane systems could improve N-use efficiency and lower
455 environmental losses of N.
456 The low bioavailability of N from the residue biochar was expected since biochar-N is
457 generally slow release and often associated with aromatic and heterocyclic structures (Clough
458 et al., 2013). The amount of N taken up by the plants from organic amendments was in the
459 order of incorporated residues (225 mg pot^{-1}) > surface residues (125 mg pot^{-1}) > incorporated
460 biochar (15.4 mg pot^{-1}) (Table 2), and is consistent with the corresponding rates of C
461 mineralisation observed (Fig. 2). However, given the increased mineralisation of SOC observed
462 following the amendment of soil with residues, a greater uptake of soil N into plants would be
463 expected (Dijkstra et al., 2013). However, this was not the case in our study. Rather the

proportion of N uptake from the applied urea was lower than the control or biochar amendment (P<0.01), at only 20.6% for the incorporated residues and 24.8% for the surface applied residues, compared to the control (31.0%).

5. Conclusions

While green sugarcane harvest leaves substantial volumes of leaf residue in the field, with many practitioners anticipating increases in SOC from this practice, in reality little if any evidence of changes to SOC stocks from this practice have been reported. Using dual ^{13}C labelled leaf residues ($\delta^{13}C$: 23.8‰ and $\delta^{13}C$: 16.6‰) co-enriched with ^{15}N, we were able to demonstrate that both residue retention and residue incorporation increased mineralisation of SOC, thus limiting potential for SOC accumulation. Contrary to this, biochars produced by pyrolysing the residues at 350°C lowered SOC mineralisation with actively-growing sugarcane plants, thus stabilising SOC. Importantly, the biochar resulted in increased uptake of fertiliser (urea) ^{15}N, while the residue retention and incorporation resulted in greater plant uptake of soil N. This improved mechanistic understanding of the role of sugarcane residues and biochar on priming effects and N-use efficiency allows new management strategies to be implemented to mitigate environmental N losses while improving SOC stocks. Future research is needed to validate the findings from this controlled-environment study under field conditions.

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Figures

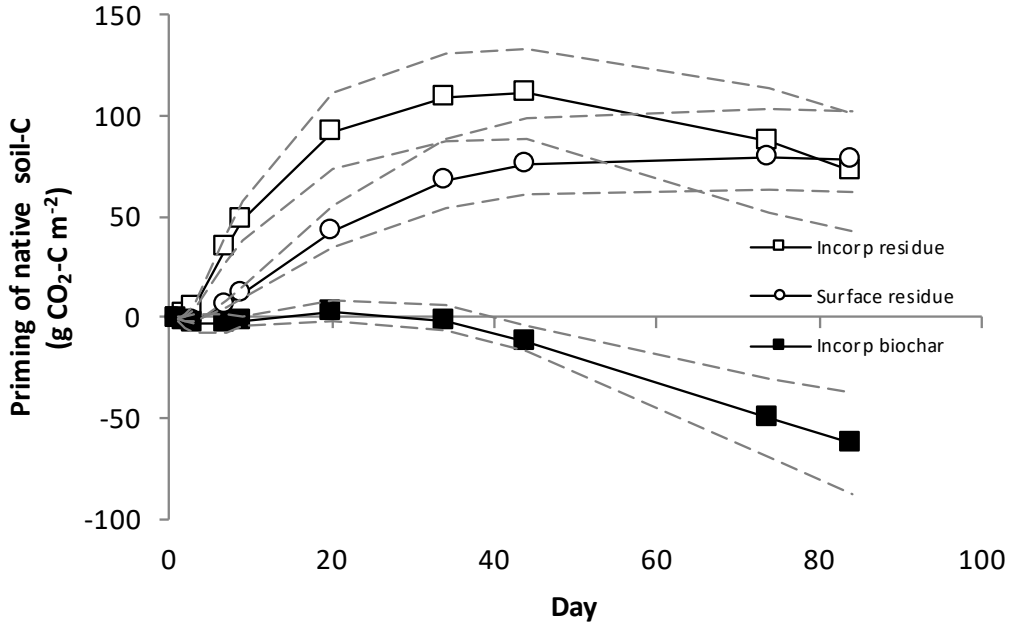

Fig. 1. Priming as the difference between cumulative mineralisation (CO_2-C) of native soil organic C in the organic-amended soils (vs the control soil). Confidence intervals (95%) of incorporated residues and biochars were plotted in dashed lines and normalised against the mean squares across all treatments at each sampling event (n= 4).

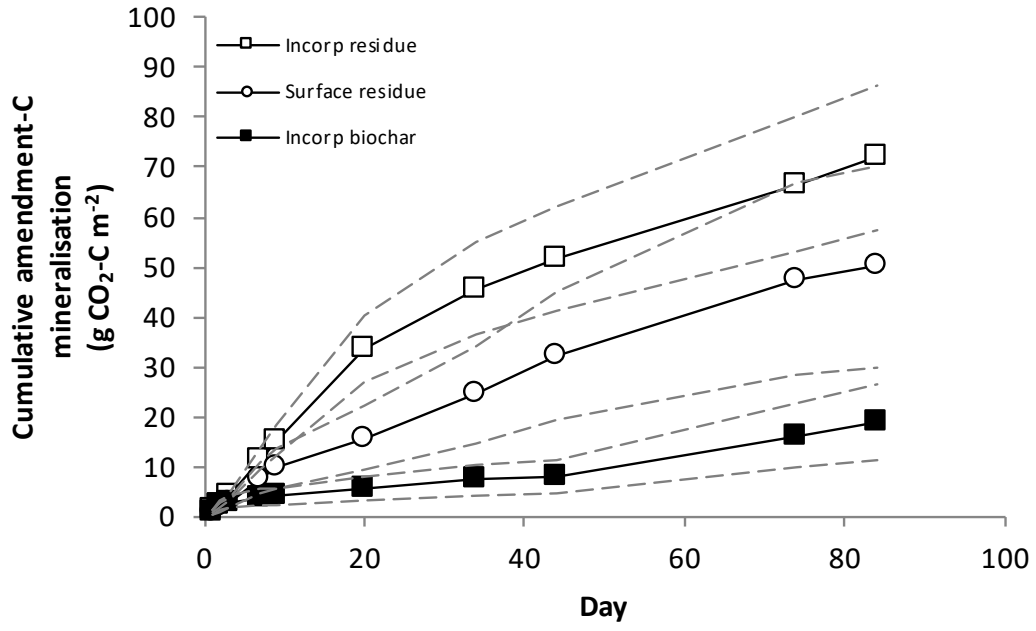

Fig. 2. Cumulative C mineralization from organic amendments over 84 d. Confidence intervals (95%) (n= 4) are plotted using dashed lines for the incorporated residues and its biochar and normalised against the mean squares across all treatments.

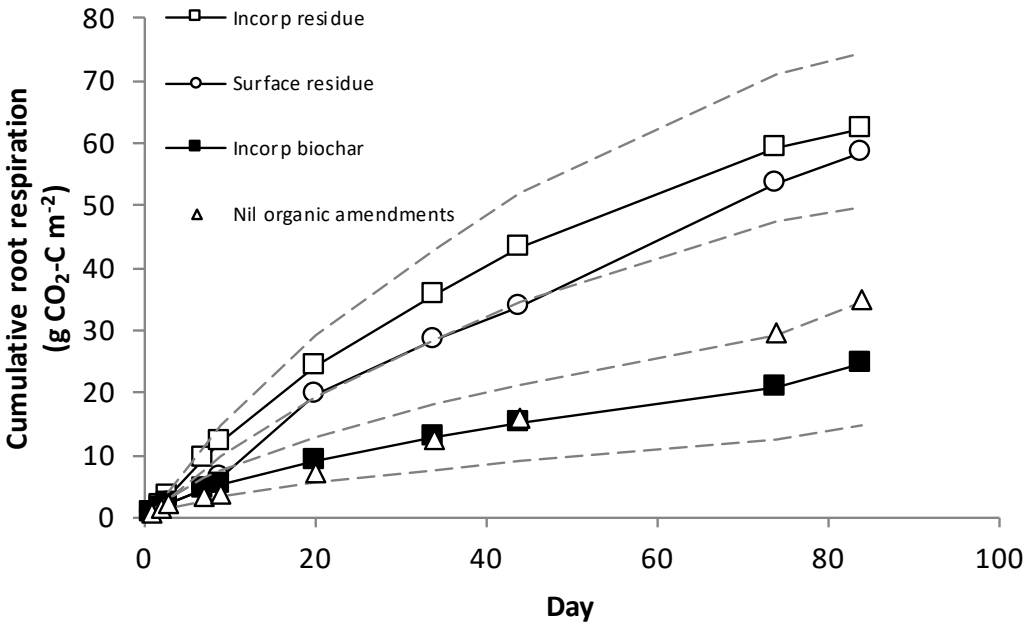

Fig. 3. Cumulative root respiration (CO_2-C) from the organic-amended and control soils over 84 d. Confidence intervals (95%) (n= 4) are plotted using dashed lines for the incorporated residues and its biochar and normalised against the mean squares across all treatments. The nil organic amended control was plotted as triangles.

Table 1. Biomass as leaf, cane and shoot, their total N concentrations and total N uptake of sugarcane grown for 84 days. One-way analysis of variance (ANOVA) of the effects of the amendments was performed (n=4). All treatments received urea at 883 mg N pot^{-1}.

	Biomass (g pot^{-1})				Concentrations of N (g N kg^{-1} biomass)				Total N uptake into biomass (mg pot^{-1})			
	Leaf	Cane	Root	Sum	Leaf	Cane	Root	Sum	Leaf	Cane	Root	Sum
Surface residue	46.0	75.0	20.8	142	9.36	2.46	8.36	20.2	366	180	190	737
Incorp residue	39.6	45.0	17.7	102	9.13	4.82	11.24	25.2	331	222	194	747
Incorp biochar	42.0	51.2	16.2	109	8.63	4.29	8.11	21.0	346	218	135	699
Nil organic amendment	41.6	48.6	14.2	104	8.35	4.68	9.74	22.8	348	228	136	712
Significance level	***	***	***	***	ns	***	***	***	*	***	***	ns
LSD (*P*= 0.05)	3.0	8.4	3.7	10		1.08	2.12	3.0	23	26	25	

Not significant (ns), *, ** and *** indicate $P>0.05$, $P<0.05$, $P<0.01$ and $P<0.001$, respectively. LSD, Least significant difference at $P=0.05$.

Table 2. The N content in plant (leaf, cane, root and total biomass) derived from N fertilizer, amendments (residues or biochar) and soil N. One-way analysis of variance (ANOVA) of the effects of the amendments was performed. Soil N uptake was derived as the total plant N content subtracted from N uptake from urea and/or amendments (n=4). All treatments received urea at 883 mg N pot^{-1}.

	^{15}N uptake from urea (mg ^{15}N pot^{-1})				^{15}N uptake from organic amendment (mg ^{15}N pot^{-1})				Soil N uptake (mg N pot^{-1})			
	Leaf	Cane	Root	Total	Leaf	Cane	Root	Total	Leaf	Cane	Root	Total
Surface residue	101.5	59.4	59.5	220.4	58.9	23.2	42.6	124.7	140	102	120	362
Incorp residue	83.7	59.4	40.1	183.2	70.7	41.9	112.7	225.2	134	102	106	341
Incorp biochar	138.9	81.4	40.6	260.9	3.7	7.0	4.8	15.5	231	130	94	455
Nil organic amendment	138.1	89.0	48.4	275.6					210	139	88	436
Significance level	*	*	ns	*	***	***	***	***	*	ns	ns	ns
LSD (P= 0.05)	38.6	21.8		54.0	7.4	13.8	53.3	55.4	65			

Not significant (ns), *, ** and *** indicate P>0.05, P<0.05, P<0.01 and P<0.001, respectively. LSD, Least significant difference at P=0.05.

Table 3. Total N and dissolved organic carbon (DOC) in leachate collected 14 and 56 days after planting (DAP) and sum of two events, calculated as a product of concentrations × volume of leachate. One-way analysis of variance (ANOVA) of the effects of the amendments was performed (n=4). All treatments received urea at 883 mg N pot^{-1}.

	Total N (mg N pot^{-1})			DOC (mg C pot^{-1})		
Leachate	14 DAP	56 DAP	Sum	14 DAP	56 DAP	Sum
Surface residue	80.9	0.5	81	0.67	1.46	2.13
Incorp residue	80.3	32.0	112	2.53	1.16	3.69
Incorp biochar	66.1	52.7	119	0.73	0.80	1.53
Nil organic amendment	74.7	26.8	102	1.00	0.55	1.55
Significance level	***	***	ns	***	***	**
LSD (P= 0.05)	10.9	8.1		0.56	0.52	0.95

Not significant (ns), *, ** and *** indicate, $P>0.05$, $P<0.05$, $P<0.01$ and $P<0.001$, respectively. Least significant difference (LSD) at $P=0.05$.

Supplementary information

Dual ^{13}C labelling of sugarcane biomass with single ^{15}N enrichment

Two sets of labelling pots were: $^{13}C^{15}N$ (Enrichment 1) and $^{13}C^{14}N$ (Enrichment 2), n=10. Two cane plants (*ca.* 150 mm high) were transferred into each labelling pot. Sugarcane stalks were sourced from a sugarcane plantation at Broadwater, NSW, Australia, on the basis of size and vigour to minimise variability associated with plant propagules. Twenty stalks were cut into 1-eye sets and 150 sets were selected for planting in a mixture of fine and coarse sand in the glasshouse at 30°C. Each pot received N fertiliser in the form of urea at 180 kg N ha^{-1} on a soil surface area basis. Urea was applied to pots four times over the growth period with 5.16 atom% of enrichment (Sigma-Aldrich, USA) or at natural abundance. Phosphorus (P) and potassium (K) were applied as superphosphate and sulfate of potash at 30 kg P ha^{-1} and 100 kg K ha^{-1}, respectively. The basal nutrients were mixed uniformly through the soil for all treatments. The following types and amounts (mg kg^{-1} dry soil) of basal nutrients were added in solution and mixed thoroughly in the soils as per Rose et al. (2007): $CaCl_2.2H_2O$, 180; $MgSO_4.7H_2O$, 50; $ZnSO_4.7H_2O$, 9; $CuSO_4.5H_2O$, 6; $Na_2MoO_4.2H_2O$, 0.4. Pots were weighed every 3 d during the experiment and water was added to maintain 90% of field capacity. Plants were maintained under identical growing conditions in the constant-temperature glasshouse with 30°C during the day and 25°C night.

Five $^{13}CO_2$ pulse labelling campaigns were carried out weekly after three weeks of growth. All plants were enclosed in two insulated enclosures (2.5 m long, 2 m wide and 1.5 m high) during each campaign, one for Enrichment 1 and the other for Enrichment 2 The sides of the enclosures were transparent polyethylene, and the top was acrylic, allowing 90% transmittance of photosynthetically active radiation. A 40 mm 12V DC ball bearing fan (Sirocco, Taiwan) was installed inside each enclosure to ensure air mixing during the labelling period.

The headspace air was flushed with 99.9% N_2 (Coregas, Yennora, NSW, Australia) for 5 min with approximately 2 headspace volumes immediately prior to the pulse labelling. Theoretical $^{13}CO_2$ concentrations of 350 and 700 µL L^{-1} were generated in the labelling enclosures by dispensing a 1.5 mL aliquot of 32% HCl into 10 mL of 0.179 and 0.358 mmol mL^{-1} $NaH^{13}CO_3$ (99 atom % ^{13}C, Icon Isotope, USA). The plants were exposed to $^{13}CO_2$ enrichment for 5 h from 1100 to 1600 h. During the pulse labelling, the CO_2 concentration inside the enclosures was monitored by collecting 25 mL headspace gas every 15 min within the first hour and then at 2 h intervals into pre-evacuated 12 mL blue-cap Exetainer vials with grey silicon septa (Labco, Lampeter, UK). Samples were then analysed using a flame ionisation detector on an Agilent 7890A gas chromatograph (Wilmington, USA). Details of the analytical procedure are found in van Zwieten et al. (2010). To maximize $^{13}CO_2$ uptake by the plants, the labelling enclosures were replaced over the experimental plots at sunset after each labelling event (~1700 h) to capture overnight $^{13}CO_2$ respiration, and then removed the following morning (~0800 h) after CO_2 levels in the enclosure dropped below 250 µL L^{-1} (Kong and Six, 2010). The

remaining $^{13}CO_2$ within each labelling enclosures was then evacuated through a vacuum pump (Büchi, V-500,Flawil, Switzerland) for 5 min, and the exhaust gas was disposed off-site (i.e. 5 m away, down-wind).

Table S1. Characterization of biochars.

	Unit	Detection limit	$^{13}C^{15}N$ biochar	^{13}C biochar
Total C	g kg^{-1}	2	600	610
Total N	g kg^{-1}	0.2	15	17
Total P	g kg^{-1}	0.03	6.5	7.2
Total K	g kg^{-1}	0.004	53	57
Total S	g kg^{-1}	0.006	6.3	7.0
Agronomic analyses				
KCl-extractable NH_4^+-N	mg kg^{-1}	0.3	1.5	1.5
KCl-extractable NO_3^- -N	mg kg^{-1}	0.2	0.54	<0.2
Electrical conductivity	dS m^{-1}	0.01	12	11
pH ($CaCl_2$)		0.04	8.9	9.2
Acid neutralising	% $CaCO_3$	0.5	10.0	8.1
Water-soluble P	%	0.0003	0.13	0.23
Formic acid-soluble P	%	0.0003	0.63	0.56
Available P	%	0.0003	0.79	0.79
Exchangeable cations				
Al	cmol(+) kg^{-1}	0.1	<0.1	<0.1
Ca	cmol(+) kg^{-1}	0.03	4.5	2.6
K	cmol(+) kg^{-1}	0.01	120	120
Mg	cmol(+) kg^{-1}	0.007	10	8.5
Na	cmol(+) kg^{-1}	0.03	0.56	0.52

Table S2. The recovery of applied ^{15}N as N fertilizers or amendments (residue or biochar). One-way analysis of variance (ANOVA) of the

effects of the amendments was performed (n=4). All treatments received urea at 883 mg N pot^{-1}.

	^{15}N plant recovery from ^{15}N-urea (%)				^{15}N plant recovery from ^{15}N labelled organic amendment (%)			
	Leaf	Cane	Root	Total	Leaf	Cane	Root	Total
Surface residue	11.4	6.7	6.7	24.8	7.3	2.9	5.3	15.4
Incorp residue	9.4	6.7	4.5	20.6	8.7	5.2	13.9	27.8
Incorp biochar	15.6	9.2	4.6	29.4	1.5	2.9	2.0	6.4
Nil organic amendment	15.6	10.0	5.5	31.0				
Significance level	*	*	ns	*	***	ns	*	***
LSD (*P*=0.05)	4.3	2.5		6.1	1.0		6.7	7.0

Not significant (ns), *, ** and *** indicate $P>0.05$, $P<0.05$, $P<0.01$ and $P<0.001$, respectively. LSD, Least significant difference at $P=0.05$.

Table S3. The chemical properties of soil (0-100 mm layer) at the completion of the study. One-way analysis of variance (ANOVA) of the

effects of the amendments was performed (n=4). #Total C in soil treated with surface residue is the adjusted amount of C of soil plus remaining residue on the surface at Day 84. The average weight of residue was 13.22 g (n=4) with C content of 44% in 13.74 kg of soil with C content of 2.14%.

	Total C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	KCl-extractable NH_4-N ($mg\ kg^{-1}$)	KCl-extractable NO_3-N ($mg\ kg^{-1}$)	Electrical conductivity ($dS\ m^{-1}$)	pH ($CaCl_2$)
Surface residue	22.4[#]	1.7	1.41	7.7	0.05	3.90
Incorp residue	23.8	2.0	5.83	34.6	0.11	4.00
Incorp biochar	23.1	1.7	5.49	29.5	0.10	3.98
Nil organic amendment	22.3	2.0	3.75	28.5	0.08	3.98
Significance level	ns	***	ns	***	***	***
LSD (*P*= 0.05)		0.1		8.2	0.02	0.08

Not significant (ns), *, ** and *** indicate *P*>0.05, *P*<0.05, *P*<0.01 and *P*<0.001, respectively. LSD, Least significant difference at *P*=0.05.

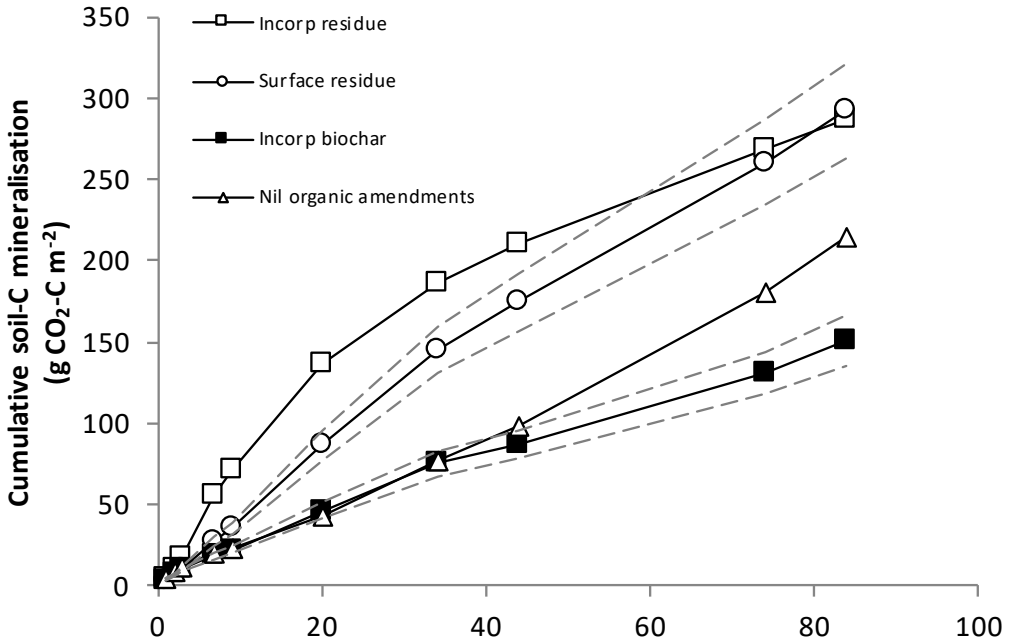

Fig. S1 Cumulative total soil respiration over 84 d. Confidence intervals (95%) (n= 4) are plotted using dashed lines for the surface residues and its biochar and normalised against the mean squares across all treatments.