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1 **Rice husk biochar and crop residue amendment in subtropical cropping soils: effect on**  
2 **biomass production, nitrogen use efficiency and greenhouse gas emissions**

3 Dai H. Nguyen, Clemens Scheer<sup>✉</sup>, David W. Rowlings, Peter R. Grace  
4 Queensland University of Technology, 2 George St. Brisbane 4001, Australia.

5 <sup>✉</sup> Corresponding author e-mail: clemens.scheer@qut.edu.au

6 ***Abstract***

7 We investigated the effect of maize residues and rice husk biochar on biomass production,  
8 fertiliser nitrogen recovery (FNR) and N<sub>2</sub>O emissions for three different subtropical cropping  
9 soils. Maize residues at two rates (0 and 10 t ha<sup>-1</sup>) combined with three rates (0, 15 and 30 t  
10 ha<sup>-1</sup>) of rice husk biochar were added to three soil types in a pot trial with maize plants. Soil  
11 N<sub>2</sub>O emissions were monitored with static chambers for 91 days. Isotopic <sup>15</sup>N labelled urea  
12 was applied to the treatments without added crop residues to measure the FNR. Crop residues  
13 incorporation significantly reduced N uptake in all treatments but did not affect overall FNR.  
14 Rice husk biochar amendment had no effect on plant growth and N uptake but significantly  
15 reduced N<sub>2</sub>O and CO<sub>2</sub> emissions in two of the three soils. The incorporation of crop residues  
16 had a contrasting effect on soil N<sub>2</sub>O emissions depending on the mineral N status of the soil.  
17 The study shows that effects of crop residues depend on soil properties at the time of  
18 application. Adding crop residues with a high C/N ratio to soil can immobilise N in the soil  
19 profile and hence reduce N uptake and/or total biomass production. Crop residues  
20 incorporation can either stimulate or reduce N<sub>2</sub>O emissions depending on the mineral N  
21 content of the soil. Crop residues pyrolysed to biochar can potentially stabilise native soil C  
22 (negative priming) and reduce N<sub>2</sub>O emissions from cropping soils thus providing climate  
23 change mitigation potential beyond the biochar C storage in soils. Incorporation of crop  
24 residues as an approach to recycle organic materials and reduce synthetic N fertiliser use in  
25 agricultural production requires a thorough evaluation, both in terms of biomass production  
26 and greenhouse gas emissions.

27

28 **Keywords:** Crop residues; greenhouse gas; N<sub>2</sub>O; nitrogen fertiliser recovery; rice husk  
29 biochar

## 30 INTRODUCTION

31 Agriculture is a major emitter of greenhouse gases (GHGs) to the atmosphere. Globally, it is  
32 estimated that there are about 5.1 to 6.1 billion tonnes CO<sub>2</sub>-eq yr<sup>-1</sup> GHG emissions from  
33 agriculture, which contributed 10 – 12% of the total anthropogenic GHG emissions in 2005  
34 (Smith et al. 2007). Croplands are responsible for 60% of total anthropogenic nitrous oxide  
35 (N<sub>2</sub>O) emissions which is a potent GHG with a global warming potential of nearly 300 times  
36 that of CO<sub>2</sub> and also the primary contributor to stratospheric ozone depletion (Ravishankara et  
37 al. 2009; Smith et al. 2007). Nitrous oxide emissions are affected by a number of factors such  
38 as soil organic C (SOC), N fertilisation, and fertiliser N rates. When SOC decomposes, it is  
39 emitted as carbon dioxide (CO<sub>2</sub>) into the atmosphere. On the other hand, agriculture has a  
40 significant climate change mitigation potential which could change the position of agriculture  
41 from the second largest emitter to a much smaller emitter or even a net sink of GHGs with the  
42 greatest mitigation contribution originating from soil C sequestration, but also methane and  
43 nitrous oxide emissions can be considerably reduced (Bellarby et al. 2008).

44 Globally, 3.8 billion tonnes of crop residues are produced annually from cereal, sugar,  
45 legumes, tuber and oil crops (Lal 2005). The management of crop residues in cropping  
46 systems has a significant impact on soil quality and resilience, agronomic productivity, and  
47 GHG emissions from soil to the atmosphere (Lal 1997). Maintaining crop residues on the soil  
48 surface after harvesting reduces water loss, limits weed growth and can improve the physico-  
49 chemical properties of soil. Retention of crop residues on cropping soils offers the potential of  
50 soil C sequestration and may lead to C sequestration at the rate of 0.2 billion tonnes yr<sup>-1</sup> or 5.0  
51 billion tonnes of cumulative C sequestration in the world by the year 2020 (Lal 1997).  
52 However, this potential C sequestration can be offset if crop residues amendment substantially  
53 increases the emissions of other GHGs like N<sub>2</sub>O or CH<sub>4</sub>. To date, the effect of crop residues  
54 incorporation on soil GHG fluxes is not clear and both positive and negative effects have been  
55 observed depending on the N content of the crop residues (Chen et al. 2013). The  
56 incorporation of crop residues with a high C/N ratio may result in net N immobilisation during  
57 crop residue decomposition leading to reduced N<sub>2</sub>O emissions. Conversely, it has been shown  
58 that crop residues can stimulate N<sub>2</sub>O emissions in soils; (i) mineralisable N transformed into  
59 mineral N can provide additional substrate for nitrification and denitrification; (ii)

60 mineralisable C from crop residues can stimulate heterotrophic denitrification activity  
61 generating N<sub>2</sub>O emissions from soil mineral N and crop residue N; and (iii) increased oxygen  
62 consumption from C mineralisation can create anaerobic soil condition and stimulate N<sub>2</sub>O loss  
63 from denitrification (Velthof et al. 2002).

64 In recent years, biochar production via pyrolysis of crop residues and its application to  
65 soil has been proposed as novel approach to sequester atmospheric CO<sub>2</sub> in terrestrial  
66 ecosystems, increase crop yields and reduce GHG emissions from soil (Lehmann et al. 2006).  
67 Organic materials which are pyrolyzed to biochar have a reduced capacity to be oxidised to  
68 CO<sub>2</sub> and thus offer a significant, long-term term C sink (Lehmann 2007). It has been shown  
69 that biochar addition can improve plant growth and soil quality (Chan et al. 2007; Chan et al.  
70 2008; Major et al. 2010). These positive yield responses of biochar addition were related to  
71 improved water holding capacity (Iswaran et al. 1980), increased N uptake (Wardle et al.  
72 1998), liming values (van Zwieten et al. 2010a) and soil physical properties (Asai et al. 2009;  
73 Major et al. 2010). Some studies also observed negative responses to crop growth due to pH  
74 induced micro-nutrient deficiency (Kishimoto and Sugiura 1985). Several studies have shown  
75 that biochar can alter microbial communities and biogeochemical processes in soils (Pereira et  
76 al. 2015; Song et al. 2014), however, there is still contradiction over the effect of biochar on  
77 soil GHG fluxes. Several studies demonstrated reduced N<sub>2</sub>O emissions (Liu et al. 2011; Singh  
78 et al. 2010b; van Zwieten et al. 2010b; Wang et al. 2011; Yanai et al. 2007) and increased CH<sub>4</sub>  
79 uptake (Karhu et al. 2011) associated with the effects of biochar on soil aeration, N  
80 availability and pH, while other studies have shown no effects (Pereira et al. 2015; Scheer et  
81 al. 2011; Suddick and Six 2013) or increased emissions in others (Clough et al. 2010; Singla et  
82 al. 2014).

83 Little is known on the combined effect of biochar and crop residue amendment on GHG  
84 fluxes from cropping soils and no study is available to evaluate the effect of biochar  
85 application in soils which are incorporated with crop residues in relation to fertiliser N  
86 recovery (FNR). Therefore, the objective of this study was to evaluate the effect of crop  
87 residue and rice husk biochar amendment on biomass production, N losses and N<sub>2</sub>O emissions  
88 from three different subtropical cropping soils in Australia. We hypothesised that crop residue

89 addition would increase N immobilisation and in combination with rice husk biochar reduce N  
90 loss, improve FNR and reduce N<sub>2</sub>O emissions from the investigated sugarcane soils.

91

## 92 **MATERIALS AND METHODS**

### 93 **Rice husk biochar and crop residues**

94 Rice husk biochar was produced from rice husk by thermal pyrolysis at 350 – 500°C and  
95 sieved to <2 mm before application to soils. It had a pH 9.1, 0.4% total N, 46.4% total C and  
96 6.8 % moisture (Table 1).

97 The crop residues used in this experiment was maize straw (*Zea mays* L.) collected from  
98 a previous field experiment planted in October 2011. The maize received 200 kg N ha<sup>-1</sup> as urea  
99 and was harvested after 114 days. The maize straw was chopped and dried at 60 °C for 72 h  
100 and ground to pass a 2 mm stainless steel screen. Characteristics of the crop residues are 1.1%  
101 total N and 43.0% total C (Table 1).

### 102 **Soil preparation**

103 Three soils were collected from the coastal humid subtropical cropping region in eastern  
104 Australia. Surface soil (0 – 20 cm) from a sandy loam (Soil 1) and a clay (Soil 2) were both  
105 collected from established (6 months) sugarcane fields near Broadwater, New South Wales  
106 (NSW) (29°00'S 153°25'E), and a sandy loam from a recently planted field near Rocky Point,  
107 Queensland (27°46'S 153°18'E). Rice husk biochar and crop residues were thoroughly mixed  
108 with 7.35 kg (oven-dry weight base) soil sieved to 2 mm into a 7.8 L, 250 mm diameter plastic  
109 pot. Chemical fertiliser was applied identical as a basal requirement for maize industry in  
110 NSW (Garcia undated) (0.981 g N and 0.20 g P and 0.8 g K pot<sup>-1</sup>, equivalent 200 kg N, 40 kg  
111 P and 160 kg K as urea, K<sub>2</sub>HPO<sub>4</sub> and K<sub>2</sub>SO<sub>4</sub> ha<sup>-1</sup>, respectively). All P and 50% K and 40% N  
112 were applied just before sowing, 20% N was applied at 35 days after sowing and the  
113 remaining 50% K and 40% N were applied after 50 days. Treatments without crop residues  
114 were fertilised with <sup>15</sup>N labelled (5.23 % atom excess) urea. Characteristics of soils, crop  
115 residues and rice husk biochar are shown in Table 1.

### 116 **Experimental design**

117 Three rates of rice husk biochar (RB0, RB1, RB2) were compared with and without (CR0,  
118 CR10) the addition of crop residues for each soil. RB was applied at the equivalent of 0, 15  
119 and 30 t ha<sup>-1</sup> (0, 31.8 and 63.6 g C pot<sup>-1</sup> respectively) and maize residues were added at the  
120 rate of 10 t dry matter ha<sup>-1</sup> (equivalent to 19.6 g C pot<sup>-1</sup>). The pots were arranged as a  
121 randomised complete block design with four replicates per treatment.

122 The crop cultivar used was maize *cv.* Pioneer 31H50, a silage variety. Three maize plants  
123 were sown in each pot and after 10 days thinned to one. The trial was irrigated with an  
124 automated drip system set to minimise leaching from the pots. In the first 30 days, 100 mL  
125 water were automatically irrigated twice a day. In the following 30 days the amount of water  
126 applied was increased to 200 ml and irrigated twice a day and in the last 31 days, 300 ml water  
127 was applied three times a day. Soil moisture varied between 55% and 80% water-filled pore  
128 space (WFPS) over the experiment. A plate was placed underneath the pot for collecting  
129 leachate which was returned to the pot. The trial was undertaken in a climate controlled  
130 glasshouse with day/night temperatures of 30/18 °C.

### 131 **Gas sampling**

132 A chamber (140 mm headspace and 100 mm diameter) with rubber septa lid was inserted into  
133 each pot next to the plant to collect gas samples (Figure 1). Gas samples were taken 20 times  
134 over the 91 day trial at 5 day intervals, with more intensive sampling following fertilisation.  
135 Headspace samples were collected at 0, 30 and 60 minute intervals after closure and analysed  
136 for N<sub>2</sub>O concentration using a Shimadzu GC-2014 gas chromatograph equipped with an ECD  
137 <sup>63</sup>Ni detector.

138 Fluxes of GHG (N<sub>2</sub>O, CO<sub>2</sub> and CH<sub>4</sub>) emissions from the static chambers were calculated  
139 from the slope of the linear increase or decrease of the three concentrations measured over the  
140 closure time as described by Scheer et al. (2014). Flux rates (F) of N<sub>2</sub>O were calculated using  
141 Eq. 1 and 2.

$$142 \quad F = \frac{b * V_{CH} * MW * 60 * 10^6}{A_{CH} * MV_{corr} * 10^9} \quad (\text{Eq. 1})$$

143 where:  $b$  is the increase in headspace concentration ( $\text{ppb min}^{-1}$ );  $A_{\text{CH}}$  is the basal area of  
 144 the measuring chamber ( $\text{m}^2$ );  $MW_{\text{N}_2\text{O-N}}$  the molecular weight of  $\text{N}_2\text{O-N}$  ( $28 \text{ g mol}^{-1}$ );  $MW_{\text{CO}_2}$ :  
 145 Molecular weight of  $\text{CO}_2\text{-C}$  ( $12 \text{ g mol}^{-1}$ );  $MW_{\text{CH}_4}$ : Molecular weight of  $\text{CH}_4\text{-C}$  ( $12 \text{ g mol}^{-1}$ );  
 146  $MV_{\text{corr}}$ : Temperature corrected molecular volume ( $\text{m}^3 \text{ mol}^{-1}$ );  $V_{\text{CH}}$ : Volume of the measuring  
 147 headspace chamber ( $\text{m}^3$ ); 60 converts from minutes to hours;  $10^6$  converts from g to  $\mu\text{g}$ , and  
 148  $10^9$  converts from ppb to  $\mu\text{L m}^{-3}$ .

$$149 \quad MV_{\text{corr}} = 0.02241 * \frac{273.15 + T}{273.15} \quad (\text{Eq. 2})$$

150 where:  $MV_{\text{corr}}$ : is defined as above; 0.02241: 22.41 L mol volume ( $\text{m}^3 \text{ mol}^{-1}$ ); T: Air  
 151 temperature during the measurement ( $^{\circ}\text{C}$ ).

152

### 153 **Soil and plant sampling**

154 Soil sub-samples (100 g) were collected from 3 of the replicates every four weeks using a two  
 155 cm diameter, stainless steel auger for mineral N analysis. Soil sampling did not disturb in the  
 156 area of gas sampling. Soil bulk density after the experiment was measured by using a soil ring  
 157 to take  $100 \text{ cm}^3$  soil that was dried at  $105 \text{ }^{\circ}\text{C}$  for 24 h. Water-filled pore space (WFPS) was  
 158 determined every fortnight by sub-sampling 50 gram soil, dried at  $105 \text{ }^{\circ}\text{C}$  for 24 h and  
 159 calculated by following formula Eq. 3.

$$160 \quad WFPS(\%) = \frac{GWC * BD}{1 - \frac{BD}{2.65}} \quad (\text{Eq. 3})$$

161 where:

162 - GWC: gravimetric water content (%)

163 - BD: soil bulk density ( $\text{g cm}^{-3}$ )

164 - 2.65 assumes the soil particle density ( $\text{g cm}^{-3}$ )

165 Maize plants were harvested after 91 days and the roots separated and washed in  
 166 deionised water until clean. Both roots and above the ground biomass were dried in an oven at

167 70 °C for 72 hours. Samples were fine ground and analysed using an isotope ratio mass  
168 spectrometer (20-22 IRMS, Sercon Ltd, Crewe, UK).

169 Fresh soil samples (20 g soil) were extracted with 100 mL 2.0 M KCl in 120 mL plastic  
170 vials and analysed for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  colourmetrically (AQ2<sup>+</sup>, SEAL Analytical WI, USA).  
171 Total N in plant and soil was determined by dry combustion (Leco Trumac Series Macro  
172 Determinator, St. Joseph, MI, USA).

173 Fertiliser  $^{15}\text{N}$  recovery (FNR) was calculated as described by Bronson et al. (1991). All  
174  $^{15}\text{N}$  samples were analysed with a SerCon Isotope Ratio Mass Spectrometer.

### 175 **Statistical analysis**

176 All statistical analyses were completed using R Commander Package statistical software  
177 2.13.2 (R Development Core Team 2008). Three-way analysis of variance (ANOVA) was  
178 used to determine the difference between treatments. Multiple comparisons were run in IBM  
179 SPSS 21. Differences were considered significant when P values were lower than 0.05.

## 180 **RESULTS**

### 181 **Biomass production and soil mineral N**

182 Total biomass production from three soils is presented in Figure 2. Above and belowground  
183 biomass production (root and straw) in treatments with crop residue incorporation ranged from  
184 105 to 118 g pot<sup>-1</sup> with a mean of 112 g pot<sup>-1</sup>. This was generally lower than in treatments  
185 without crop residues where above and belowground biomass ranged from 101 to 135 g pot<sup>-1</sup>  
186 with a mean of 121 g pot<sup>-1</sup>. Results of dry biomass production indicated both positive and  
187 negative responses to rice husk biochar additions. In Soil 1, there was no significant difference  
188 in biomass production for the different rice husk biochar rates. The nil crop residue treatments  
189 yielded ca. 112 g straw pot<sup>-1</sup>, 10% higher than the crop residues incorporated treatments. In  
190 Soil 2, there was no significant difference after crop residue or rice husk biochar addition in  
191 straw and total biomass production. Mean straw yield in the treatments with incorporated crop  
192 residues decreased by 7% compared to the nil crop residue treatments. In Soil 3, a significant  
193 difference in biomass production was found at different rates of rice husk biochar and  
194 interaction of rice husk biochar and crop residues, but no significant difference in the absence

195 of crop residues was found. Overall, there was no significant effect of rice husk biochar on  
196 biomass production except for Soil 3 while crop residues significantly reduced biomass  
197 production in all soils.

198 Mineral N (e.g.  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) was not significantly affected by the application of rice  
199 husk biochar and crop residues. Nitrate in Soil 3 was significantly higher than in Soil 1 and  
200 Soil 2. The high rice husk biochar added soils showed a higher N availability, but the  
201 difference was not significant (Figure 3).

### 202 **Total N uptake and fertiliser N recovery**

203 Crop residue addition significantly decreased total plant N uptake in Soil 1 and Soil 3 (Table  
204 2). Increased biochar rates had no significant effect on N uptake except for Soil 3 where N  
205 uptake was increased. Nitrogen uptake ranged from 0.77 to 0.99 g N pot<sup>-1</sup> in Soil 1 and from  
206 0.90 to 1.19 g N pot<sup>-1</sup> in Soil 3. No influence of the amendments on N uptake in maize straw  
207 was observed in Soil 2, which ranged from 0.74 to 1.13 g pot<sup>-1</sup>.

208 Rice husk biochar addition and soil types had no significant influence on the fertiliser <sup>15</sup>N  
209 recovery (% FNR) in the soil or plant (Table 3). Fertiliser N recovery in soils varied between  
210 16.7% and 29.2% (average 25.1%). Plant FNR ranged from 68.7% to 81.0% (average 71.4%)  
211 with the highest mean FNR in Soil 2 (75.0%) while Soil 1 and Soil 3 were almost identical at  
212 70%. An average of 3.5% of added fertiliser over three soils could not be recovered in the soil  
213 or in plant. Highest N losses were from Soil 3, with almost twice as much N lost compared to  
214 Soil 1 and Soil 2.

### 215 **Greenhouse gas emissions**

216 The incorporation of crop residues significantly increased CO<sub>2</sub> emissions in all three soils  
217 (Table 4). Adding rice husk biochar generally reduced CO<sub>2</sub> emissions, except for the nil crop  
218 residues in Soil 1 and with crop residues in Soil 3. The percentages of CO<sub>2</sub> reduction varied  
219 from 15.4% in the nil crop residues plus RB1 in Soil 3 to 42% in the nil crop residues plus  
220 RB2 in Soil 2.

221 Maximum emissions of N<sub>2</sub>O occurred in the first three days after maize sowing (Figure  
222 4). The addition of the rice husk biochar significantly decreased N<sub>2</sub>O emissions from Soil 1

223 and Soil 2 under both the nil residue and crop residue treatments. Daily N<sub>2</sub>O emissions were  
224 the lowest in the Soil 2 and the highest in Soil 3 where crop residue incorporation increased  
225 emissions over the first 60 days. Total N<sub>2</sub>O emissions during growing time (91 days) were  
226 significantly affected by the addition of rice husk biochar (P<0.001), except in Soil 3, but there  
227 was no significant interaction between the two sources of amendments (Table 4). Total N<sub>2</sub>O  
228 emissions decreased with increasing rice husk biochar rates in the three soils. Crop residue  
229 incorporation decreased N<sub>2</sub>O emissions in Soil 1, but significantly increased N<sub>2</sub>O emissions in  
230 Soil 3 (Table 4). Average N<sub>2</sub>O emissions over the experimental period varied from 0.012 to  
231 0.075 mg N<sub>2</sub>O pot<sup>-1</sup> d<sup>-1</sup> in treatments without crop residues and from 0.017 to 0.108 mg N<sub>2</sub>O  
232 pot<sup>-1</sup> d<sup>-1</sup> in treatments incorporated with crop residues.

233 Methane fluxes were measured and low fluxes were observed, which were not  
234 significantly different from the fluxes in the control for each soil. Cumulative CH<sub>4</sub> emissions  
235 varied from 0.007 mg C pot<sup>-1</sup> to 0.20 mg C pot<sup>-1</sup>. Since, there were no significant difference  
236 between rice husk biochar rates and crop residue incorporation, the data is not shown here.

## 237 **DISCUSSION**

### 238 **Effect of RB and crop residues on maize growth and N uptake**

239 There was no significant effect of rice husk biochar rates on biomass production which is  
240 contrast to the literature where it is frequently suggested that biochar applications to soil can  
241 increase agricultural productivity (Blackwell et al. 2009). However, plant response to  
242 biochar addition only has been shown to vary according to biochar characteristic, soil type  
243 and plant species. Improved crop growth after biochar addition has been explained by  
244 mechanisms such as changed microbial activity, soil physical properties, pH, and CEC (Chan  
245 et al. 2007; Rondon et al. 2007; Yamato et al. 2006). We assume that in our study biochar did  
246 not affect maize growth probably due to the adequate supply of mineral N fertiliser and  
247 irrigation water to the experimental pots in all treatments. Another reason for this result  
248 could be that the short period of 91 days in this study was not long enough for the biochar to  
249 establish change in the microbial activity of the soils. Other studies reported an effect of  
250 biochar on biomass production only one year after application suggesting that biochar

251 application to soil can provide increasing benefits over time (Kimetu et al. 2008; Major et  
252 al. 2010; Steiner et al. 2007).

253 Biochar had no effect on soil mineral N content but biochar application significantly  
254 increased N uptake in maize in Soil 3 which is most likely attributed to the higher mineral N  
255 content at the start of the experiment. The increased N uptake in the rice husk biochar  
256 treatments is consistent with earlier finding of Zhang et al. (2012) who found that increased  
257 biochar rates significantly increased N uptake in maize. Results of this study suggest that the  
258 increased N uptake in Soil 3 applied with biochar can partly be explained by reduced gaseous  
259 N losses and increased N retention.

260 The effects of crop residues on biomass production depended on soil properties. It  
261 reduced biomass production in all three soils after its incorporation into the soil. This was  
262 most likely caused by N immobilisation over the cropping season, although this effect was no  
263 longer apparent in the mineral N content after harvest (Figure 3). Organic matter with a C/N  
264 ratio higher than 30 generally results in N immobilisation (Alexander 1977). We assume that  
265 the high C/N ratio of the maize residues (C/N =38.4) used in this study immobilised soil  
266 mineral N and resulted in low N uptake.

### 267 **Effect of RB on fertiliser N recovery in soil and in plant**

268 The amount of fertiliser <sup>15</sup>N recovery in the soils varied between 16.7% and 29.2% with a  
269 higher amount of residual <sup>15</sup>N found in Soil 1 and Soil 3 where high SOC content promoted N  
270 immobilisation. Total <sup>15</sup>N recoveries were not significantly different, despite differences in  
271 total soil N in all three soils when <sup>15</sup>N was added (Table 1 and 3). These results suggest that  
272 initial SOC content had little effect on total N retention in the cropping system.

273 There was no significant response of biochar amendment on plant FNR. Our hypothesis  
274 that rice husk biochar will increase FNR was not supported by this experiment where FNR in  
275 Soil 1 and Soil 3 was identical (70%), but lower than that in Soil 2 (75%). Our results showed  
276 a relatively high FNR because leaching was minimised as leachate water was collected and  
277 returned to the pot, which reduced N leaching losses from the root zone. The current results  
278 are, therefore, at the upper level of FNR reported for agricultural crops varying between 30%  
279 and 78% (Broadbent and Carlton 1978; Kirda et al. 2001).

280 After balancing the fertiliser N proportions between plant and soils, on average 3.5%  
281 fertiliser N was lost. Soil 3 had the highest N loss accounting for 5.25% of N lost. Although  
282 this soil had a sandy texture combined with high N levels and low CEC, these losses couldn't  
283 have resulted through leachate which was returned to the pots with a negligible amount. The N  
284 was, therefore, most likely lost via gaseous N emissions such as N<sub>2</sub> and N<sub>2</sub>O. The observed  
285 emissions of N<sub>2</sub>O only accounted for 0.4% of fertiliser N added (Table 4). Whilst gaseous N  
286 emissions of N<sub>2</sub> were not specifically monitored in this study, it has been shown that gaseous  
287 N losses can constitute the largest part of N losses in sugarcane systems with up to 100 times  
288 as much N loss as N<sub>2</sub> than as N<sub>2</sub>O (Weier 1999). Since the soil pH during the experiment was  
289 below 5.5 (data not shown) also NH<sub>3</sub> volatilisation can be considered negligible.  
290 Consequently, we hypothesise that the majority of N loss in this study was emitted as N<sub>2</sub> from  
291 denitrification.

#### 292 **Effect of RB and crop residues on GHG emissions**

293 All treatments amended with crop residues increased CO<sub>2</sub> emissions but the magnitude varied  
294 according to soil types and interaction with rice husk biochar rates. The increased CO<sub>2</sub>  
295 emissions in the crop residue treatments confirmed that the added C substrate is immediately  
296 broken down by soil microbes after incorporation (Muhammad et al. 2011); (Lehtinen et al.  
297 2014). Furthermore, soil moisture in this study was maintained between 55% – 80% WFPS by  
298 irrigation which stimulated microbial activity and mineralisation because microbial activity is  
299 optimised at, or near field capacity, equivalent to 60% WFPS (Hofman and Van Cleemput  
300 2004).

301 There was a significant reduction of soil CO<sub>2</sub> emissions in treatments where rice husk  
302 biochar had been added. Increasing rates of rice husk biochar addition also had an influence on  
303 the magnitude of the CO<sub>2</sub> reduction. Such a “negative priming effect” of biochar addition on  
304 SOM degradation has been reported by other (Spokas et al. 2009; Zimmerman et al. 2011).  
305 The exact cause of this reduction is not known. One potential explanation is that application of  
306 biochar affects soil physical properties such as soil structure, soil porosity, thereby changing  
307 oxygen content, water holding capacity and microbial activities (Downie et al. 2009). It can  
308 also reduce bioavailability of soluble organic substrate by OM sorption to biochar and  
309 physical protection which slows down mineralisation and decomposition of SOM. Moreover,

310 a change in microbial abundance and community structure due to biochar presence may affect  
311 not only biochar mineralisation itself but also mineralisation of existing soil C (Lehmann et al.  
312 2011). The results of this short term study suggest that rice husk biochar has the potential to  
313 enhance SOC preservation by retarding its mineralisation (negative priming) and thus could  
314 stabilise existing SOC in cropping soils.

315 The effect of crop residues on N<sub>2</sub>O emissions varied depending on soil chemical  
316 properties, in particular on the initial mineral N levels of the soil. The increase of N<sub>2</sub>O  
317 emissions after incorporating crop residues in Soil 3 was depending on the addition of mineral  
318 N fertiliser and organic matter substrate, stimulating denitrification capacity (Baggs et al.  
319 2003). A recent meta-analysis of agricultural soils in Europe showed that N<sub>2</sub>O emissions are  
320 12 times higher following crop residue incorporation due to increased denitrification  
321 stimulated by the added substrate and the creation of anaerobic micro sites by increased soil  
322 respiration (Lehtinen et al. 2014). In Soil 3, a high initial NO<sub>3</sub><sup>-</sup>, high decomposable C and  
323 suitable moisture regulated the denitrification process to produce more N<sub>2</sub>O. In contrast to the  
324 findings of Soil 3, we found a significant reduction in N<sub>2</sub>O emissions in Soil 1. This contrast  
325 likely depended on the low NO<sub>3</sub><sup>-</sup> content in the initial soil (Table 1) and after the experiment  
326 (Figure 3) which limited available NO<sub>3</sub><sup>-</sup> for the denitrification process. The results suggest that  
327 under low soil mineral N levels in Soil 1 the addition of crop residues increased the  
328 immobilisation of available NO<sub>3</sub><sup>-</sup> which in turn led to reduced N<sub>2</sub>O emissions.

329 The hypothesis that the application of biochar would reduce N<sub>2</sub>O emissions was  
330 confirmed in this pot trial and confirmed what was already reported (Nguyen et al. 2014;  
331 Singh et al. 2010a; Yanai et al. 2007). Nitrous oxide emissions from treatments applied with  
332 RB decreased by increasing rice husk biochar rates in all three soils with or without  
333 incorporation of crop residues confirming results of previous studies (Rondon et al. 2005;  
334 Wang et al. 2012; Zhang et al. 2010). Suppression of N<sub>2</sub>O emissions in treatments amended  
335 with biochar may result from increased soil aeration which inhibited denitrification rates.  
336 Biochar can also alter the soil microbial communities (Song et al. 2014) and promote  
337 microbial immobilisation of the available N in soil (Rondon et al. 2005; Singh et al. 2010a),  
338 although this effect was not apparent in the plant N uptake. The fact that both CO<sub>2</sub> and N<sub>2</sub>O

339 production were reduced also suggests that biochar amendment might suppress microbial  
340 activities.

## 341 **CONCLUSIONS**

342 To our knowledge this is the first study that investigates the combined effect of biochar and  
343 crop residue amendment on soil GHG emissions and plant FNR. Applications of rice husk  
344 biochar and crop residues significantly influenced overall N<sub>2</sub>O emissions but did not affect  
345 biomass production and FNR. This study highlights that incorporation of crop residues as an  
346 approach to recycle organic materials and reduce synthetic N fertiliser use in agricultural  
347 production requires a thorough evaluation, both in term of biomass production and GHG  
348 emissions. In order to optimise the benefits of crop residue incorporation soil properties need  
349 to be taken into account. The study also showed that application of rice husk biochar could  
350 potentially stabilise native and added soil C and reduce N<sub>2</sub>O emissions from these subtropical  
351 cropping soils at the same time. Thus crop residues pyrolysed to biochar as a soil amendment  
352 has a climate change mitigation potential beyond the biochar C storage in soils. However, this  
353 study only investigated a short time frame right after the addition of rice husk biochar and did  
354 not show a positive effect on crop growth and FNR. Future work is needed to assess if biochar  
355 can improve agricultural productivity and provide a long lasting mitigation as it has been  
356 indicated in current results.

357

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515 **Table 1** Properties of organic amendments and soils

	Crop residues	Rice husk biochar	Sandy loam Broadwater (Soil 1)	Clay Broadwater (Soil 2)	Sandy loam Rocky Point (Soil 3)
pH (1:5 H <sub>2</sub> O)	-	9.00	4.7	5.18	4.42
CEC (cmol <sup>(+)</sup> kg <sup>-1</sup> )	-	5.65	8.9	11.0	7.6
Moisture (%)	7.1	6.80	-	-	-
Total C (g C kg <sup>-1</sup> )	430	464	36.4	22.8	32.2
Total N (g N kg <sup>-1</sup> )	11.2	4.0	2.9	1.8	2.5
C/N ratio (%)	38.4	116.1	12.7	12.7	13.1
NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> soil)	-	-	12.8	5.5	17.3
NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> soil)	-	-	19.9	19.8	31.0
Soil bulk density (g cm <sup>-3</sup> )	-	-	1.12	1.05	1.08
Texture (USDA)	-	-	Sandy loam	Clay	Sandy loam
Sand (%)	-	-	75.4	17.2	64.9
Silt (%)	-	-	12.6	33.1	21.3
Clay (%)	-	-	12.0	49.7	13.8

517 **Table 2** Nitrogen uptake (g N pot<sup>-1</sup>) in maize in the three different soils after maize harvest

	<b>Soil 1</b>		<b>Soil 2</b>		<b>Soil 3</b>	
	Straw	Root	Straw	Root	Straw	Root
Nil crop residues (CR0)						
RB0	0.97±0.03	0.11±0.01	0.99±0.03	0.12±0.01	1.08±0.01	0.12±0.01
RB1	0.99±0.04	0.11±0.01	1.13±0.10	0.08±0.01	1.19±0.06	0.13±0.01
RB2	0.93±0.07	0.14±0.01	0.98±0.10	0.10±0.01	1.14±0.04	0.08±0.01
With crop residues (CR10)						
RB0	0.79±0.02	0.09±0.01	0.74±0.10	0.06±0.01	0.90±0.01	0.09±0.01
RB1	0.83±0.08	0.07±0.01	0.95±0.08	0.09±0.02	1.04±0.03	0.09±0.01
RB2	0.77±0.08	0.07±0.01	1.05±0.06	0.07±0.01	1.07±0.01	0.10±0.01
Significance test (P value)						
For RB	0.600	0.340	0.107	0.720	0.003	0.123
For CR	0.005	0.001	0.089	0.013	0.001	0.116
For RBxCR	0.969	0.048	0.149	0.040	0.215	0.057

518

519

520 **Table 3** Fertiliser N recovery (FNR) in soils and in plant and N loss in different rates of rice  
 521 husk biochar in different soils after maize harvest

Treatments	FNR in soil (%)	FNR in plant (%)	N loss (%)
Soil 1	27.4	70.0	2.63
RB0	28.7±1.0	69.0±2.4	2.28±1.6
RB1	24.3±4.0	73.8±3.8	1.87±0.5
RB2	29.2±8.5	67.0±7.4	3.76±1.1
Soil 2	22.3	75.0	2.71
RB0	27.5±0.9	70.2±1.4	2.32±0.6
RB1	16.7±4.8	81.0±6.9	2.35±2.2
RB2	22.8±3.8	73.8±5.5	3.45±1.9
Soil 3	25.6	69.2	5.25
RB0	24.9±1.1	69.4±0.9	5.62±0.6
RB1	27.1±3.6	68.7±3.2	4.18±2.1
RB2	24.7 ± 4.1	69.3±4.2	5.96±0.6
<b>Mean of 3 soils</b>	<b>25.1</b>	<b>71.4</b>	<b>3.53</b>
Significance test (P value)			
For RB	0.451	0.358	0.396
For Soils	0.341	0.257	0.057
For RBxSoils	0.619	0.743	0.983

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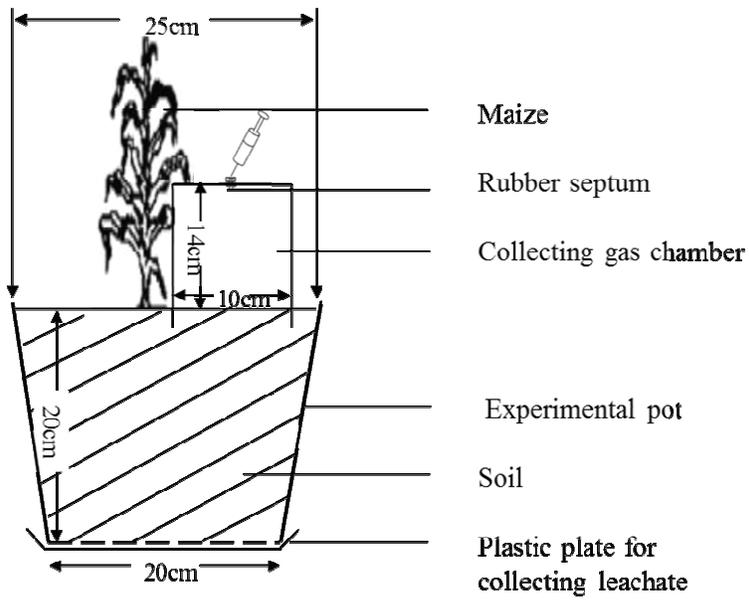
524 **Table 4** Effects of rice husk biochar and crop residues on total N<sub>2</sub>O and CO<sub>2</sub> emissions from  
 525 three different soils.

526

Treat- ments	Soil 1		Soil 2		Soil 3	
	N <sub>2</sub> O*	CO <sub>2</sub> <sup>§</sup>	N <sub>2</sub> O	CO <sub>2</sub>	N <sub>2</sub> O	CO <sub>2</sub>
Nil crop residues (CR0)						
RB0	5.0±0.9	8.7±1.6	2.8±0.2	10.5±1.4	6.8±0.8	9.1±0.6
RB1	2.2±0.2	11.3±1.4	1.8±0.3	7.4±1.2	5.7±0.6	7.7±0.5
RB2	1.6±0.2	8.7±0.9	1.0±0.1	6.1±0.3	6.7±1.0	5.2±0.8
Mean	2.9	9.6	1.9	8.0	6.4	7.3
With crop residues (CR10)						
RB0	2.9±0.4	14.2±2.2	3.0±0.1	11.0±2.0	9.9±1.6	11.6±2.1
RB1	1.6±0.1	11.2±0.8	2.3±0.3	13.4±1.7	8.1±1.3	12.7±2.2
RB2	1.6±0.2	9.8±0.2	1.9±0.3	8.4±1.4	6.4±0.5	12.5±1.8
Mean	2.0	12.0	2.4	11.0	8.1	12.2
Significance test (P value)						
For RB	0.001	0.018	0.001	0.005	0.222	0.299
For CR	0.023	0.002	0.009	0.002	0.051	0.001
For RBxCR	0.078	0.017	0.403	0.043	0.245	0.095

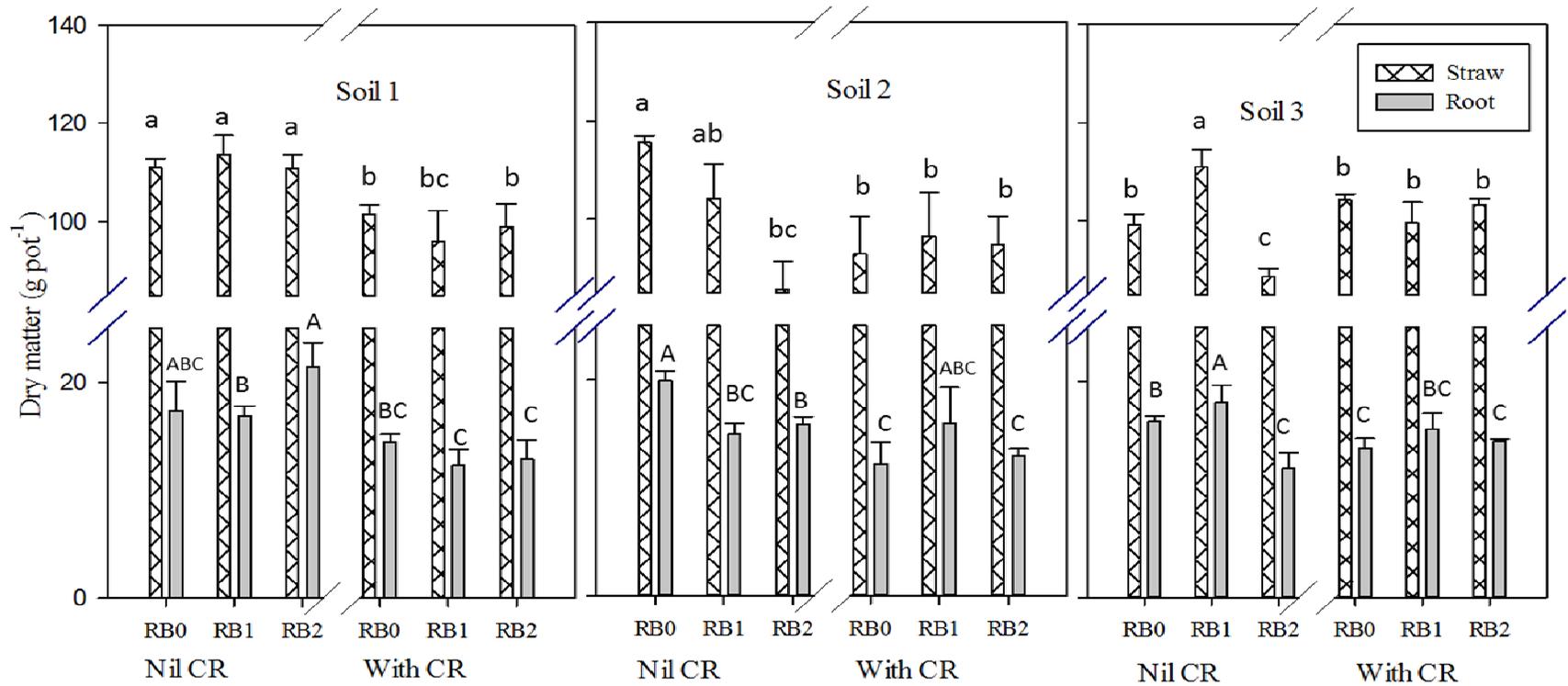
527 \* mg N<sub>2</sub>O-N, §g CO<sub>2</sub>-C pot<sup>-1</sup>, respectively

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529

530 **Figure 1** Simple apparatus for collecting gas samples

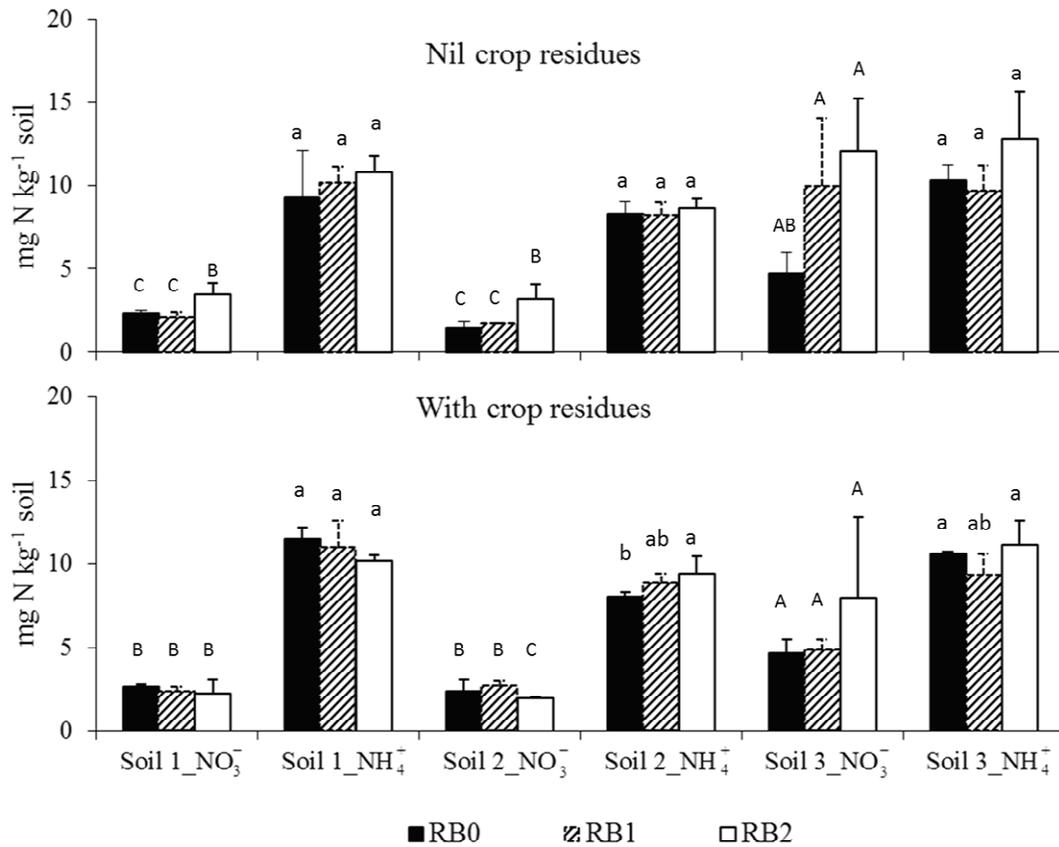


531

532 **Figure 2** Effects of rice husk biochar (RB) and crop residues on biomass production in different soils. Vertical bars represent standard error.

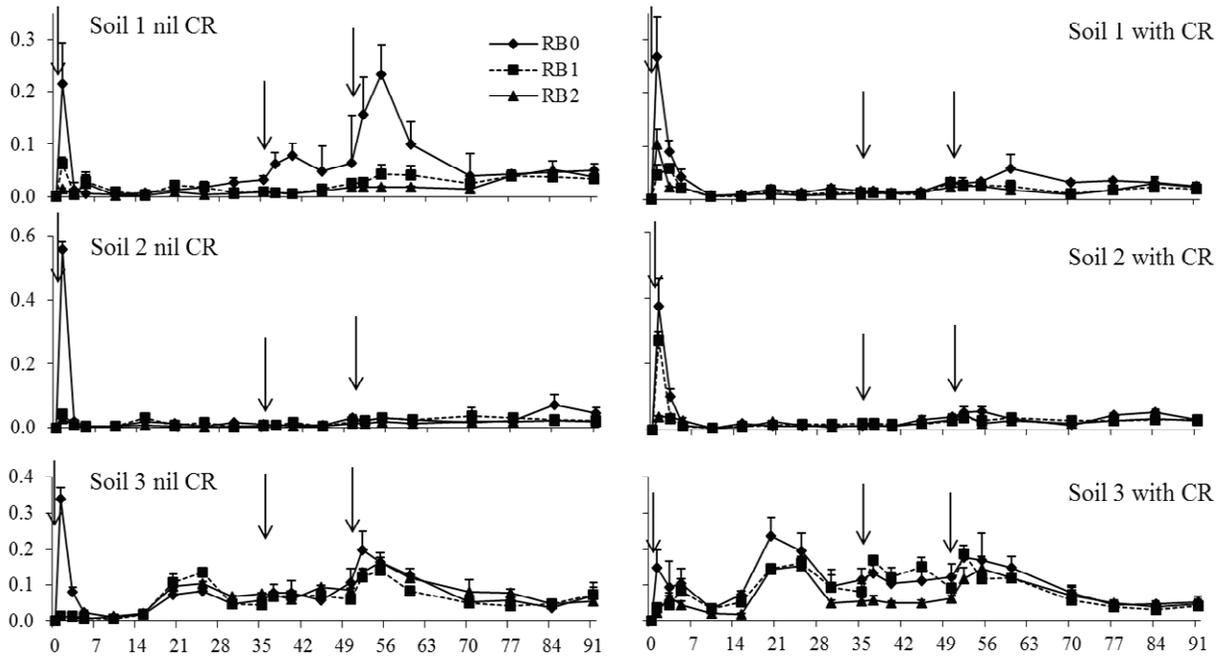
533 Different letters above the bars indicate significant difference between treatments in each soil (P<0.05). Lowercases refer for straw biomass and

534 uppercase refer for root biomass. CR is crop residues.



535  
 536 **Figure 3** Effects of rice husk biochar (RB) and crop residues on NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> contents of  
 537 three soils after maize harvest. Vertical bars represent standard error. Different letters above  
 538 the bars indicate significant difference between treatments in the categories of nil or with CR  
 539 (P<0.05). Lowercases refer for NO<sub>3</sub><sup>-</sup> and uppercases refer for NH<sub>4</sub><sup>+</sup>.

540  
 541



542

543 **Figure 4** Daily fluxes of N<sub>2</sub>O emissions during the experiment growing time under different  
 544 management of rice husk biochar (RB) rates and crop residues. Vertical bars represent  
 545 standard error. Arrows indicate time of N fertilisation. CR is crop residues.

546