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RESEARCH ARTICLE

Interactive effects of rice-residue biochar and N-fertilizer on soil functions and crop biomass in contrasting soils

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Abstract

There is limited understanding of the effects of rice residue biochar, particularly when applied in combination with nitrogen (N) fertilizer on soil fertility, soil C sequestration and crop productivity. A one-year pot experiment was established to examine effects of rice residue biochar (0, 10, 20 and 40 t ha^{-1}) and N (0, 60, 90, 120 and 150 kg N ha^{-1}) in soils with contrasting texture (loamy sand and sandy clay loam) in a wheat-maize cropping sequence. Biochar was only applied once before sowing wheat. Biochar alone or in combination with N did not significantly increase wheat biomass in both soils, whereas biomass of maize (next crop) was significantly increased from the residual effect of biochar, alone or in combination with N fertilizer. In both soils, electrical conductivity (EC) and pH, oxidisable organic carbon (OC), microbial biomass carbon (MBC), dissolved organic carbon (DOC) and available nutrients (NPK) increased with increasing rates of biochar addition. However, addition of N with biochar (cf. biochar alone) did not change pH and oxidisable OC values but increased EC significantly. After one year, the soil organic carbon (SOC) stocks increased beyond the input of biochar-C, that is, by 0.1–2.1 t ha^{-1} and 1.8–4.8 t ha^{-1} in loamy sand and sandy clay loam, respectively, across all treatments. It may be concluded that the potential benefits of rice residue biochar to soil functions and crop production may encourage growers to minimise open field burning of straw, which is a common practice in the region.

Keywords: Biochar rates, wheat-maize cropping system, nitrogen levels, C stocks, available nutrients, soil texture.

1. Introduction

Increased grain productivity during green revolution in the developing economies of the world has also resulted into a proportionally surplus crop residue generation. In Asian countries, rice and wheat are the major crops, contributing around 30% of the total biomass production (Haefele *et al*. 2011). Generation of such large amount of crop residues however causes considerable crop management problems in fields. Farmers commonly burn excess crop straw/stubble to clear their fields for sowing the next crop. This open air burning results in the loss of nearly the entire amount of carbon (C), 80% of nitrogen (N) and sulphur (S) and 10–20% of phosphorus (P) and potassium (K) present in the straw. In addition, dangerous gases such as carbon monoxide, methane, nitrogen oxide, sulphur dioxide and very small aerosols are released into the air, thus adversely affecting atmospheric composition (Singh *et al*. 2014).

In recent years, a high temperature (e.g. 300-600 ^{o}C) conversion of crop residues into biochar and biofuel under controlled conditions, with no or low oxygen (known as pyrolysis), has been proposed as an option to manage and thereby minimise the adverse impacts of open air burning of crop residues (Lehmann and Joseph 2009). Particularly, if biochar, a stable C-rich solid residue, is returned to the same field where residues would have been removed for pyrolysis, this process may return many nutrients, and improve soil properties such as soil bulk density, water holding and cation exchange capacity, favouring the retention of water and nutrients (Zheng *et al*. 2013). Further, biochar application together with chemical fertilizers has been shown to increase agronomic benefits in terms of crop production especially in soils with low fertility, either by acting as a direct source of nutrients or by enhancing nutrient availability due to improvements in soil physical, chemical and biological properties (Lashari *et al*. 2015). For example, combined application of biochar and N fertilizer has been found to have positive impacts on soil functions, and crop N uptake and productivity (Laird *et al*. 2010; Jeffery *et al*. 2011; Lori *et al*., 2013). On the other hand, immobilization of N due to addition of C-rich biochar and reduced bio-availability of essential nutrients through their sorption on biochar has also been reported (de Sousa *et al*. 2014). These processes have been observed particularly at higher application rates of biochar (Haefele *et al*. 2011), or as biochar ages in soil (Singh *et al*., 2010). Thus, there is a further need to assess responses of plant growth and key soil functionality attributes to the combined application of biochar and N fertiliser at different rates. Earlier literature suggests that biochar can improve plant growth and yield by acting as a direct nutrient source or altering nutrient availability through cation exchange, surface interactions and water availability processes (Laungani *et al*. 2016). However, all of these processes are dependent on biochar type and application rate, biochar ageing in soil, soil type and environmental conditions (Lori *et al*., 2013; Olmo *et al*. 2016). Similarly, Haefele *et al*. (2011) suggested that the effects of rice residue biochar addition on soil fertility and crop yield will depend on site-specific conditions. Thus, ambiguity about the benefits of biochar made from different feedstocks in diverse ecological regions has limited its use in agriculture.

Therefore, the present study addresses the proposition that biochar produced from surplus rice straw may provide benefits to soil properties and enhance crop biomass/yield of both, first and second, rotation crops in a wheat–maize cropping sequence when added in varied textured soils at different rates either alone or in combination with N fertilizers.

The findings may have important implications for subsistence of agriculture and for the sustainable use of surplus rice straw residue in the region.

2. Materials and Methods

2.1. Soil collection and characterization

Two soils varying in texture i.e. loamy sand (LS; 82% sand, 1.8% silt and 16.2% clay) and sandy clay loam (SCL; 59% sand, 8.6% silt and 32.4% clay) were collected from the A horizon (0-30 cm) of a research farm situated at Punjab Agricultural University, Ludhiana, (30°56′ N, 75° 52′ E) India, after harvest of the maize crop from a non-experimental area. The study area is characterized as a sub-tropical and semi-arid type of climate with hot and dry summers from April to June followed by a hot and humid period during July to September and cold winters from November to January.

Average minimum and maximum air temperature range between 18 °C and 40 °C during summer, and 6.7 °C and 22.6 °C during winter, with a mean annual rainfall of 700-800 mm. From a given soil, 10 sub-samples were collected using a shovel and thoroughly mixed to get a composite sample.

The soils were air dried, passed through a 2 mm sieve and put into pots. The pot experiment was conducted during the cropping year 2013-14 (on wheat 2013-2014 and maize 2014) in a glasshouse.

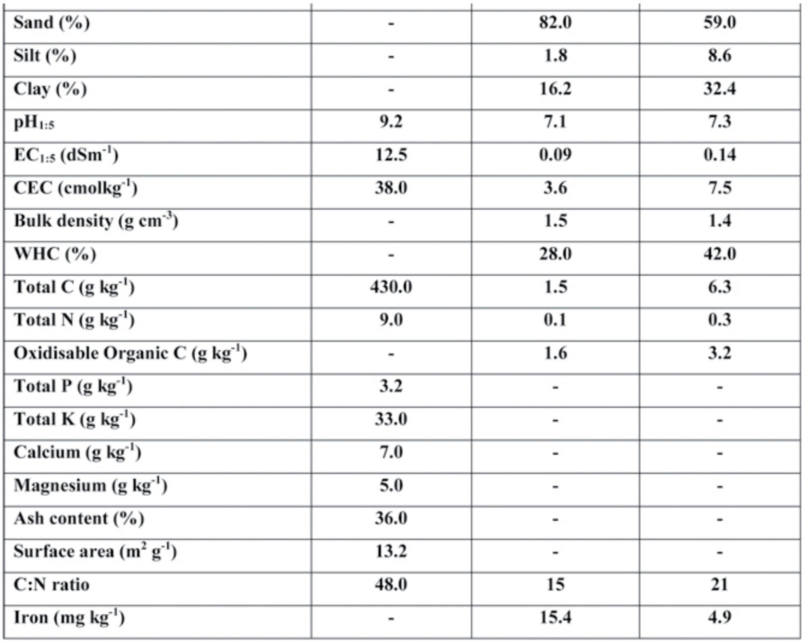
2.2. Biochar production

This study used rice straw derived biochar and the feedstock was generated after harvesting of a rice crop in the Punjab region in India. An intermediate pyrolysis technique was used to produce biochar. Pyrolysis, a thermo-chemical conversion process occuring inside a reactor, separates the ash rich biochar from the vapour fraction. Prior to pyrolysis the straw was pelletised into pellets with a size of 8 mm in diameter and 10 to 12 mm in length. The pyrolysis temperature was fixed at 380 °C with the residence time of 5 min and the resultant biochar yields were around 35% (dry weight). The biochar pellets were homogenized, powdered and passed through a 2 mm sieve before application.

2.3. Soil and biochar analysis

Initial samples of both soils (LS, SCL) and the biochar were analysed for various properties (see Table 1). Standard methods of AOAC (2000) were followed for the determination of proximate and chemical composition of the biochar. The surface area (13.2 m^2g^{-1}) was determined by a Brunauer, Emmet and Teller (BET) method, via the measurements obtained by N_2 adsorption an ASAP-2400 Micrometrics apparatus (Subedi *et al*. 2016). The rice residue biochar was rich in ash content (36%), with higher pH (9.2) and EC (12.5 dSm^{-1}) values. The total C, N and P concentrations in the biochar were found to be 430 g kg^{-1}, 9 g kg^{-1} and 3.2 g kg^{-1}, respectively.

Table 1. Properties of soils and biochar



Sand (%)	**-**	**82.0**	**59.0**
Silt (%)	**-**	**1.8**	**8.6**
Clay (%)	**-**	**16.2**	**32.4**
$pH_{1:5}$	**9.2**	**7.1**	**7.3**
$EC_{1:5}$ (dSm^{-1})	**12.5**	**0.09**	**0.14**
CEC ($cmolkg^{-1}$)	**38.0**	**3.6**	**7.5**
Bulk density ($g\ cm^{-3}$)	**-**	**1.5**	**1.4**
WHC (%)	**-**	**28.0**	**42.0**
Total C ($g\ kg^{-1}$)	**430.0**	**1.5**	**6.3**
Total N ($g\ kg^{-1}$)	**9.0**	**0.1**	**0.3**
Oxidisable Organic C ($g\ kg^{-1}$)	**-**	**1.6**	**3.2**
Total P ($g\ kg^{-1}$)	**3.2**	**-**	**-**
Total K ($g\ kg^{-1}$)	**33.0**	**-**	**-**
Calcium ($g\ kg^{-1}$)	**7.0**	**-**	**-**
Magnesium ($g\ kg^{-1}$)	**5.0**	**-**	**-**
Ash content (%)	**36.0**	**-**	**-**
Surface area ($m^2\ g^{-1}$)	**13.2**	**-**	**-**
C:N ratio	**48.0**	**15**	**21**
Iron ($mg\ kg^{-1}$)	**-**	**15.4**	**4.9**

The symbol '-' means the property was not applicable or measured

At the end of first year of the wheat-maize cropping sequence, collected soil samples from the pot experiment were air-dried, ground and passed through a 2 mm sieve to remove fine roots and analysed for various soil properties (reported in Table 1). Both EC and pH were measured in a 1:5 soil/biochar:water suspension after 1 h end-over-end shaking at 25 °C. Microbial biomass C (MBC) was determined on the freshly-collected, sieved (2 mm) soil samples by the fumigation–extraction method. Total elemental concentrations in the soils and biochar were determined by digesting with di-acid and thereafter samples were analysed using inductively coupled plasma emission spectrometry. Oxidizable organic C (OC) in the soil or soil-biochar mixture was estimated using dichromate digestion method (Walkley and Black 1934) and total C (TC) in the biochar and soil samples was determined by dry combustion using a CHNS Elementar analyzer (model Vario EL III). Available N, P and K in the samples were analysed using standard methods (Page *et al.* 1982). Total C stock (t/ha) in the soil or biochar-soil mixture collected from the pots (15 cm soil depth) was calculated using the following equation:

$$\text{C stock (t/ha)} = \text{C content (\%)} \times \text{soil bulk density (t m}^{-3}\text{)} \times \text{depth (m)} \times 10^4 \quad (1)$$

Increase in total C (ΔTC) and soil organic C (ΔSOC) stock (t ha^{-1}) in soils amended with different rates of biochar and N was calculated using equations 2 and 3, respectively.

$$\Delta TC = \text{Total C in biochar amended soil} - \text{Total C in unamended soil} \quad (2)$$

$$\Delta SOC = \Delta TC - \text{Biochar C input in the amended soils} \quad (3)$$

2.4. Pot experiment

Treatments of the experiment were set up in PVC plastic pots measuring 0.018 m^3 (0.284 m height and 0.284 m diameter). The 2 mm sieved soils (with or without biochar) were packed in the pots to achieve field bulk density of about 1.52 g cm^{-3} for LS and 1.37 g cm^{-3} for SCL. The soils in the pot were adjusted to 50-60% of water holding capacity and were regularly watered to maintain this level of moisture as required, depending on the prevailing weather conditions. All pots were placed on a raised platform allowing drainage and a suspended glass was used to reduce the sunlight exposure and rain damage to the plants. There were 40 treatments, which were replicated three times in 120 pots in a factorial completely randomized design. The treatments included: four biochar (B) levels, i.e. 0 (B0), 10 (B10), 20 (B20) and 40 (B40) t ha-1; five N levels i.e. 0 (N0), 60 (N60), 90 (N90), 120 (N120) and 150 (N150) kg N ha^{-1}; and two soil types (LS, SCL).

Rice straw biochar was mixed well with the soil before filling the pots at the start (i.e. before sowing wheat). Crop variety (var. PBW 373) for wheat (*Triticum aestivum*) was sown during winter season in the 1st week of November and maize (*Zea mays* -var. PMH-1) in the following year during the summer season in June with eight seeds in each pot. Thinning was done 15 days after sowing and five seedlings were retained for wheat and three for maize per pot. All other recommended package of practices by Punjab Agricultural University (PAU), Ludhiana were followed for raising both crops. For both wheat and maize, similar doses of N fertiliser at different rates (0, 60, 90, 120 and 150 kg N ha^{-1}) using urea were applied in two splits; the first half at the time of sowing and the second half four weeks after sowing. In addition, PAU recommended basal doses of phosphorus at 60 kg P_2O_5 ha^{-1} in the form of single super phosphate (SSP), potassium as muriate of potash (MOP) at 30 kg K_2O ha^{-1} and Zn at 12 kg Zn ha^{-1} as ZnSO4 were applied at the time of sowing. Three sprays of 0.5% $FeSO_4$ were applied at weekly intervals at 2.5 kg $FeSO_4$ ha^{-1} to both crops. All fertilizers were applied after dissolving in water. Wheat was harvested at maturity (after 4 months), and maize at the tillering stage (after 2 months). Dry biomass for both wheat (grain + straw together) and maize crop (straw only) was recorded after drying the samples to a constant weight in an oven at 60 °C.

2.5. Statistical analyses

Data were analysed using the two-way (nitrogen x biochar level) analysis of variance (ANOVA) with SPSSv16.0 (SPSS for Windows, Linux/UNIX and MAC, 2013). The Tukey post-hoc test was used to determine significant differences ($p<0.05$). Least significant difference test (LSD) was performed for treatment means comparison. Regression was performed to interpret the relationship between maize biomass, biochar addition rates and measured soil properties using SPSSv16.0.

3. Results

3.1. Crop biomass

Irrespective of the soil types, the addition of biochar at different rates, with or without N fertiliser, did not significantly influence wheat biomass (grain + straw), whereas N addition alone increased wheat biomass in both soils. In contrast, a favourable residual effect of the added biochar on the biomass of the subsequent crop (maize) was observed. Compared to the unamended control (B0N0), biochar at 10 and 20 t ha^{-1} (B10N0; B20N0) increased maize biomass by 26% and 40% in LS, and 18% and 34% in SCL, respectively (Figure 1). Additionally, there were significant positive relationships between maize biomass and measured soil properties (Figure 2) in the biochar amended soils. But, at the highest rate of biochar addition (40 t ha^{-1}), maize biomass slightly decreased in both soils compared to lower rates (10 and 20 t ha^{-1}), even though it was still higher than the control. Further, application of N along with biochar increased maize biomass compared to treatments without added N. The greatest positive effect on biomass was observed in treatments where biochar at 20 t ha^{-1} was applied with 60 kg N ha^{-1} in LS and 90 kg N ha^{-1} in SCL, respectively. But, at the highest N application rate (150 kg N ha^{-1}), maize biomass decreased or had no additional benefit irrespective of biochar rate and soil type (Figure 1). Consequently, no significant relationships were observed between biomass yield (wheat or maize) and measured soil properties when N was added along with biochar at different rates.

3.2. Soil properties

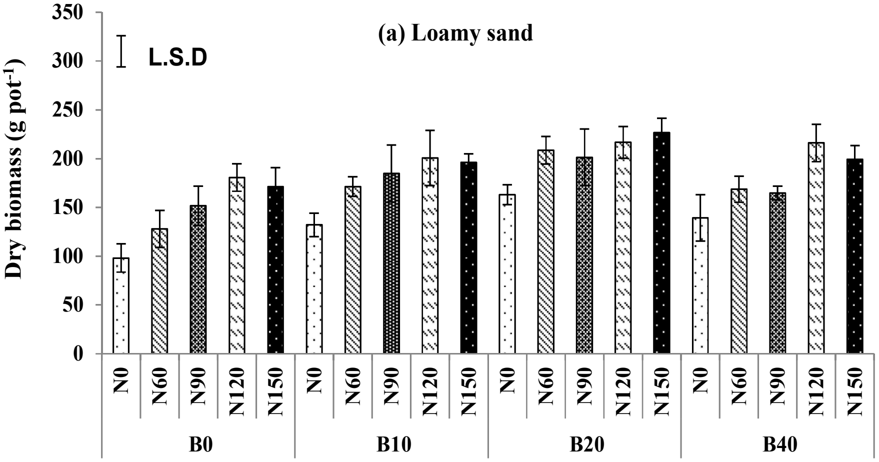
Soil EC and pH.

On average, compared to unamended control, the value of pH increased by 0.1-0.3 units with the highest increase observed for the highest biochar addition rate in both soils (Table 2). Addition of N with biochar did not significantly change pH values in the various treatments. Furthermore, compared to unamended control, EC values increased significantly ($p<0.05$) with increasing rate of biochar and N addition (Table 2). In both soils, the highest increase in EC was found in the treatments with the highest rates of biochar (40 t ha^{-1}) and N addition (150 kg N ha^{-1}).

Carbon dynamics.

In general, oxidisable OC and MBC values increased with increasing application rates of biochar in both soils (Figure 3 and 4). On average, compared to unamended control (B0), addition of biochar alone at different rates (10, 20 and 40 t ha^{-1}) increased oxidisable OC by 20-59% and MBC by 6-61% in LS; and increases were 21-31% and 25-53% in SCL, respectively. In addition, linear and polynomial relationships were observed between oxidisable OC and rate of biochar addition in LS ($R^2= 0.88$; $p<0.05$) and SCL ($R^2= 0.93$; $p<0.05$), respectively.

Addition of N along with biochar did not significantly influence the oxidisable OC values in both soils, except at the higher rates of N addition (120 and 150 kg N ha^{-1}) (Figure 3). On the other hand, application of N with different rates of biochar significantly increased MBC only in the treatments with 60 and 90 kg N ha^{-1} in both soils. At the higher levels of N addition (i.e. 120 and 150 kg N ha^{-1}), soil MBC was lower compared to the other N levels (i.e. 60 and 90 kg N ha^{-1}) irrespective of biochar rates, but was still higher than in the unamended (no biochar) treatment (Figure 4).



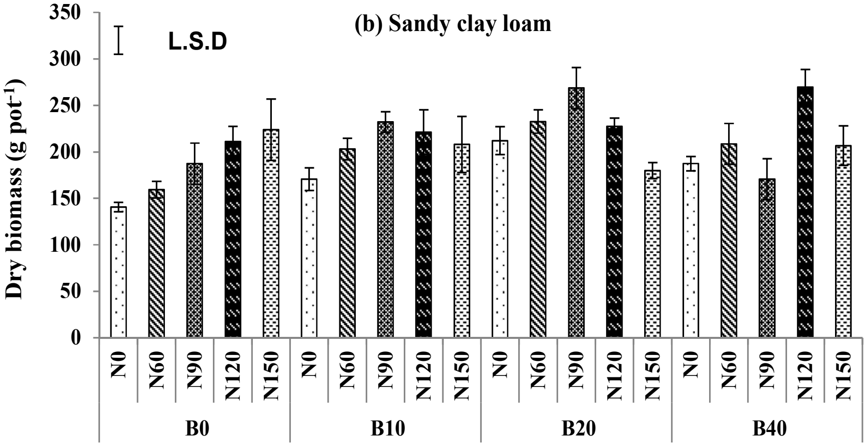


Figure 1. Maize dry biomass (g pot^{-1}) in (a) loamy sand and (b) sandy clay loam soil amended with different rates of biochar (0, 10, 20, 40 t/ha) and N (0, 60, 90, 120, 150 kg ha^{-1}). Vertical lines on the column indicate standard deviation of the mean (n=3). Least significant difference (LSD; P<0.05) is provided in the left hand panel of the graph.

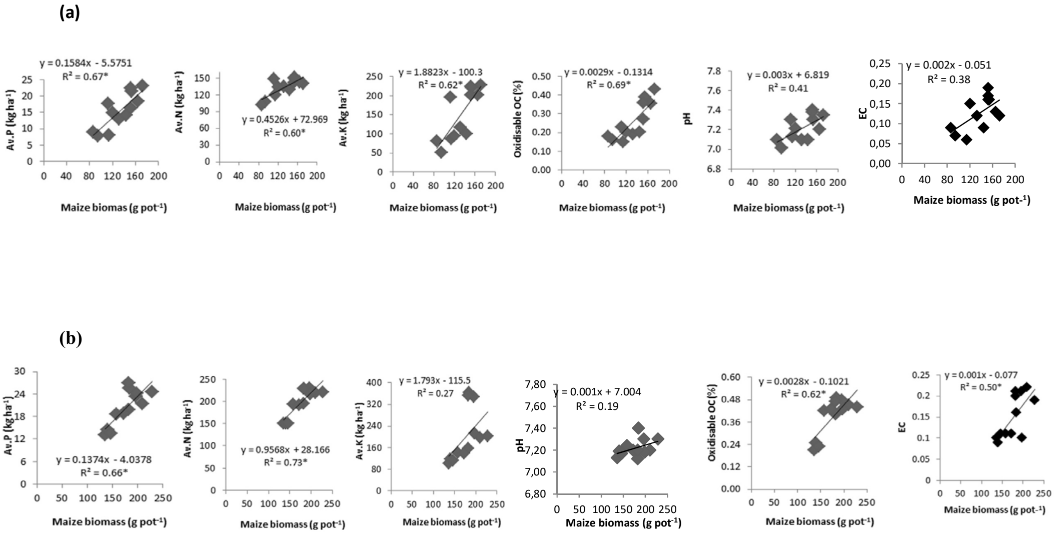


Figure 2. Relationships between maize crop biomass and measured soil properties (Available (Av.) N, P, K, Oxidisable OC, pH and EC) in the biochar amended (without N) loamy sand (a) and sandy clay loam (b) soil after one year of wheat-maize cropping sequence. *Represents significance at P<0.05 level.

Even though addition of biochar increased oxidisable OC stock across various biochar and N treatments relative to the unamended control (Figure 3), these increases in oxidisable OC were only 20-60% in LS and 17-30% in SCL, respectively. Interestingly, the total SOC in the biochar-soil mixture (Table 3) was found to be higher than the total initial input of biochar C at the end of the experiment (i.e. higher by 0.1-2.1 t C ha^{-1} and 1.8-4.8 t C ha^{-1} in LS and SCL, respectively)

3.3. Available nutrients (N, P, K).

Application of biochar at increasing rates (10, 20 and 40 t ha^{-1}) increased available N compared to the unamended control (B0N0) by 17.4%, 23.2% and 27% in LS and 22%, 31% and 34% in SCL, respectively (Table 4). Consistent with these results, a significantly positive relationship was found between available N and rates of biochar addition in both LS (R^2= 0.77; $p<0.05$) and SCL (R^2= 0.81; $p<0.05$) respectively (Figure 2). Higher available N values were found when N application was combined with biochar in SCL, which increased with increasing biochar and N application rates, but the results of changes in available N were not consistent in LS. Similar to available N, available P and K increased with increasing application rate of biochar in both soils (Table 4). However, addition of N with biochar did not influence P and K availability consistently in all the treatments.

Table 2. Soil pH and EC (dS m^{-1}) in loamy sand and sandy clay loam soil amended with different rates of biochar (0, 10, 20, 40 t ha^{-1}) and N (0, 60, 90, 120, 150 kg ha^{-1}) after one year of wheat-maize cropping sequence.

Treatments		**pH$_{1:5}$**		**EC$_{1:5}$**	
		Loamy sand	**Sandy clay loam**	**Loamy sand**	**Sandy clay loam**
N0	**B0**	**7.08**	**7.16**	**0.07**	**0.10**
	B10	**7.14**	**7.19**	**0.12**	**0.14**
	B20	**7.30**	**7.23**	**0.14**	**0.17**
	B40	**7.34**	**7.30**	**0.17**	**0.19**
N60	**B0**	**7.12**	**7.12**	**0.10**	**0.13**
	B10	**7.19**	**7.23**	**0.12**	**0.15**
	B20	**7.37**	**7.26**	**0.18**	**0.17**
	B40	**7.42**	**7.48**	**0.21**	**0.18**
N90	**B0**	**7.03**	**7.07**	**0.08**	**0.15**
	B10	**7.12**	**7.13**	**0.10**	**0.21**
	B20	**7.20**	**7.21**	**0.15**	**0.27**
	B40	**7.33**	**7.30**	**0.25**	**0.36**
N120	**B0**	**7.10**	**7.07**	**0.09**	**0.13**
	B10	**7.19**	**7.12**	**0.18**	**0.22**
	B20	**7.27**	**7.26**	**0.22**	**0.30**
	B40	**7.34**	**7.35**	**0.26**	**0.39**
N150	**B0**	**7.10**	**7.04**	**0.13**	**0.14**
	B10	**7.12**	**7.22**	**0.17**	**0.22**
	B20	**7.28**	**7.32**	**0.24**	**0.33**
	B40	**7.31**	**7.42**	**0.33**	**0.42**
LSD (P<0.05)		**B=0.05** **N=0.06** **B x N = 0.12**	**B=0.09** **N=0.10** **B x N = 0.20**	**B=0.05** **N=0.06** **B x N = 0.12**	**B=0.03** **N=0.04** **B x N = 0.07**

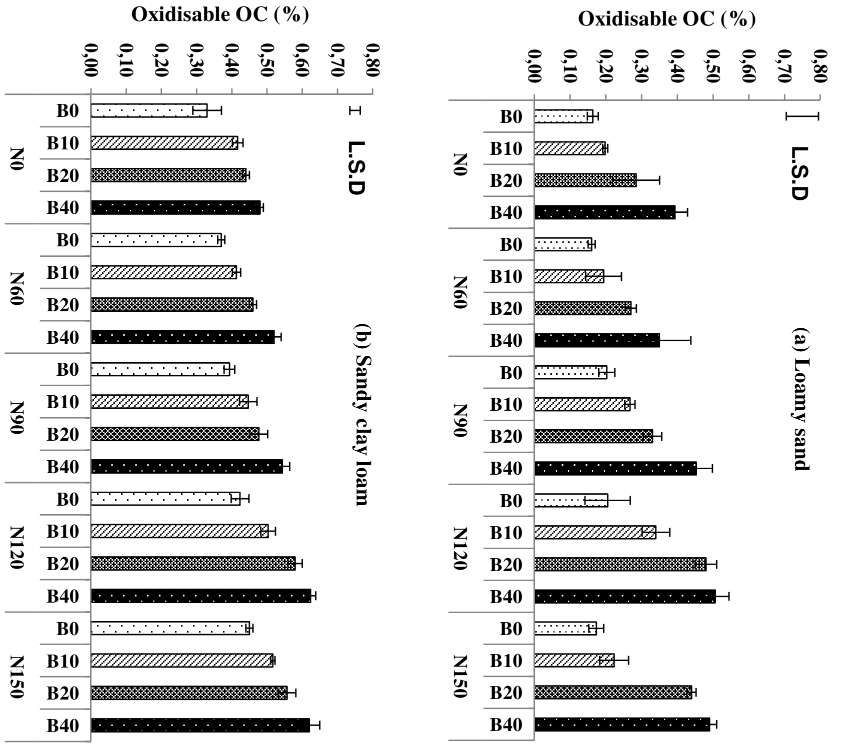


Figure 3. Oxidisable organic carbon (OC) in (a) loamy sand and (b) sandy clay loam soil amended with different rates of biochar (0, 10, 20, 40 t ha^{-1}) and N (0, 60, 90, 120, 150 kg ha^{-1}) after one year of wheat-maize cropping sequence. Vertical lines on the column indicate standard deviation of the mean (n=3). Least significant difference (LSD; P<0.05) is provided in the left hand panel of the graph.

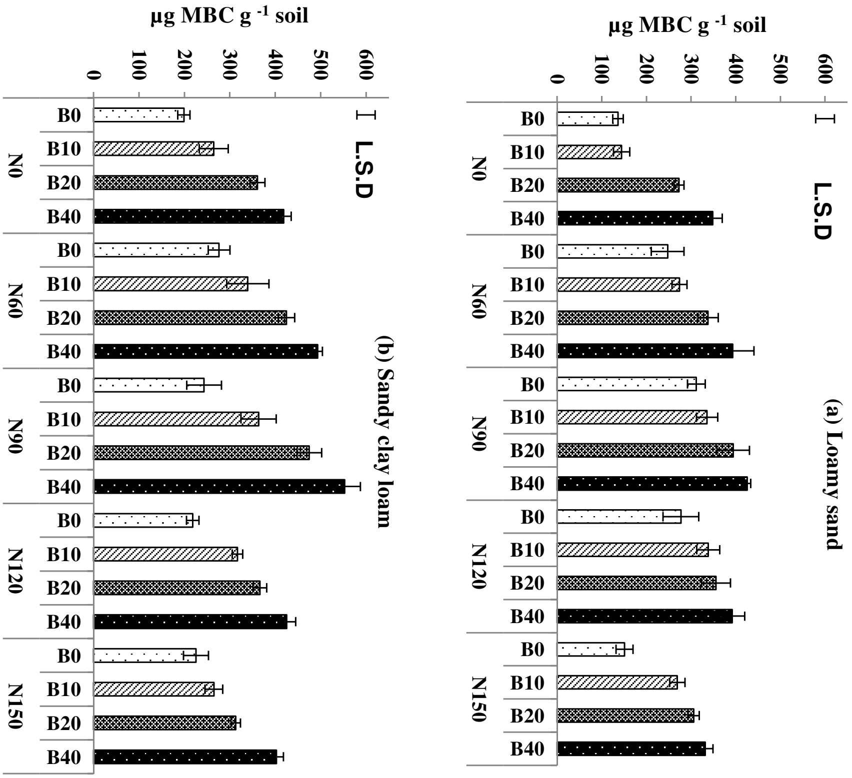


Figure 4. Microbial biomass carbon (μg MBC g^{-1} soil) in (a) loamy sand and (b) sandy clay loam soil amended with different rates of biochar (0, 10, 20, 40 t/ha) and N (0, 60, 90, 120, 150 kg ha^{-1}) after one year of wheat-maize cropping sequence. Vertical lines on the column indicate standard deviation of the mean (n=3). Least significant difference (LSD; P<0.05) is provided in the left hand panel of the graph.

Table 3. Increase in total C (ΔTC) and soil organic C (ΔSOC) stock (t ha^{-1}) in loamy sand and sandy clay loam soil amended with different rates of biochar (10, 20, 40 t ha^{-1}) and N (0, 60, 90, 120, 150 kg ha^{-1}) after one year of wheat-maize cropping sequence.

Treatments		*ΔTC stock (t ha^{-1})		ΔSOC stock (t ha^{-1})	
		Loamy sand	**Sandy clay loam**	**Loamy sand**	**Sandy clay loam**
N0	**B10**	**4.4**	**6.1**	**0.6**	**2.7**
	B20	**8.0**	**11.5**	**0.4**	**4.8**
	B40	**15.7**	**16.1**	**0.4**	**2.7**
N60	**B10**	**4.8**	**6.1**	**1.0**	**2.8**
	B20	**8.2**	**8.5**	**0.5**	**1.8**
	B40	**15.4**	**17.6**	**0.1**	**4.2**
N90	**B10**	**4.0**	**5.9**	**0.2**	**2.5**
	B20	**8.0**	**9.3**	**0.4**	**2.6**
	B40	**15.4**	**17.8**	**0.1**	**4.4**
N120	**B10**	**5.0**	**5.8**	**1.2**	**2.5**
	B20	**9.8**	**9.3**	**2.1**	**2.6**
	B40	**15.8**	**17.7**	**0.5**	**4.3**
N150	**B10**	**4.7**	**7.5**	**0.9**	**4.1**
	B20	**9.5**	**9.2**	**1.8**	**2.5**
	B40	**15.6**	**17.0**	**0.3**	**3.6**

*ΔTC = Total C in biochar amended pots (B10; B20; B40) – Total C in unamended pots (B0)

ΔSOC =ΔTC – Biochar C input in the amended soils

Table 4. Soil available N, P and K (kg ha^{-1}) in loamy sand and sandy clay loam soil amended with different rates of biochar (0, 10, 20, 40 t ha^{-1}) and N (0, 60, 90, 120, 150 kg ha^{-1}) after one year of wheat-maize cropping sequence.

Treatments		**Available N (kg ha^{-1})**		**Available P (kg ha^{-1})**		**Available K (kg ha^{-1})**	
		Loamy sand	**Sandy clay loam**	**Loamy sand**	**Sandy clay loam**	**Loamy sand**	**Sandy clay loam**
N0	**B0**	**109**	**150**	**8**	**14**	**73**	**109**
	B10	**132**	**194**	**14**	**19**	**105**	**145**
	B20	**142**	**219**	**17**	**23**	**200**	**203**
	B40	**149**	**230**	**22**	**25**	**224**	**355**
N60	**B0**	**123**	**198**	**10**	**13**	**168**	**134**
	B10	**133**	**220**	**16**	**19**	**196**	**149**
	B20	**145**	**238**	**21**	**20**	**207**	**153**
	B40	**171**	**255**	**24**	**29**	**219**	**228**
N90	**B0**	**129**	**218**	**13**	**15**	**136**	**146**
	B10	**142**	**250**	**16**	**22**	**140**	**179**
	B20	**151**	**259**	**21**	**25**	**146**	**291**
	B40	**173**	**289**	**24**	**27**	**222**	**282**
N120	**B0**	**126**	**210**	**11**	**18**	**132**	**119**
	B10	**136**	**229**	**20**	**24**	**139**	**134**
	B20	**173**	**249**	**23**	**28**	**157**	**175**
	B40	**181**	**302**	**29**	**31**	**193**	**433**
N150	**B0**	**128**	**233**	**11**	**18**	**127**	**134**
	B10	**135**	**252**	**14**	**22**	**151**	**187**
	B20	**153**	**289**	**20**	**27**	**174**	**203**
	B40	**195**	**319**	**22**	**29**	**245**	**358**
LSD (P<0.05)		**B=4.4** **N=4.9** **B x N=9.8**	**B=5.4** **N=6.0** **B x N=12**	**B=1.0** **N=1.1** **B x N=2.3**	**B=0.8** **N=0.9** **B x N=1.8**	**B=9.0** **N=10.0** **B x N=20**	**B=16.0** **N=18.0** **B x N=36**

4. Discussion

4.1. Effect of biochar on crop biomass

Crop productivity is often reported to increase with biochar application to soils but not always consistently (Jeffery *et al*. 2011; Subedi *et al.* 2016). In the present study, wheat biomass varied from 82 to 99 g pot^{-1} in the unamended soils, which was not significantly increased following application of different rates of rice residue biochar alone or in combination with N (ranged from 78 to 98 g pot^{-1}). However, the results showed beneficial effect of residual biochar on the subsequent maize biomass with application rate of 10 and 20 t ha^{-1} in both soils (LS and SCL). Similar biochar-induced delayed responses to yield improvements, with negative or no impact on the first crop followed by yield increases in subsequent crops, have been reported in the literature (Haefele *et al*. 2011; Carvalho *et al*. 2016). Possible reasons for the non-significant effect of biochar on wheat biomass may be related to lower biochemical processes in winter, in the presence or absence of biochar, plus slower biochar degradation and its interaction with soil and consequently delaying its beneficial effects on soil properties and plant productivity (Fang *et al*., 2015; Verheijen *et al*. 2010). Another possibility could be that biochar may have increased soil microbial biomass soon after its application in soil (Singh and Cowie 2014), possibly through the release of its labile organic matter, which may have caused immobilisation of N (van Zwieten *et al.* 2010). As we measured available N only at the end of one year of the cropping sequence (i.e. after maize harvest), we could not verify dynamics of N immobilization in our study. Nevertheless, the positive effects of biochar on the biomass yield of the subsequent maize crop in the current study may be hypothesized to be due to the ageing of biochar in the soils, which would have slowly released available nutrients for the next crop (Verheijen *et al*. 2010; Haefele *et al* 2011). Overall, the increased oxidisable OC and available nutrient levels in the biochar amended treatments at the end of cropping season were consistent with positive effects on the maize biomass. This was also confirmed by significantly positive relationships between maize biomass and measured soil properties in the biochar amended soils after one year of cropping (Figure 2). At the same time, reductions in crop yield following application of biochar have been reported especially on a short-term basis (Kloss *et al.* 2014; Reibe *et al.* 2015), possibly due to nutritional imbalances and toxicity (Ippolito *et al*. 2012). Similarly, in the present study, decreased crop biomass at the highest rate of biochar addition (40 t ha^{-1}), may have been due to availability of some phyto-toxic compounds above critical levels. According to EPA (2008), toxicity to soil organisms is directly proportional to the critical amounts of available poly-aromatic hydrocarbons (PAHs). However, in our study, all tested PAH compounds were below the detection limits and thus at such low concentrations, we do not expect that the decreased crop biomass could be attributed solely to the supply of toxic levels of PAHs via biochar application in soil. Consistent with our results, Zheng *et al*. (2013) also suggested that biochar addition greater than 1% (w/w) may not lead to higher crop biomass. Thus, the optimal level of biochar for different environments should be determined to get the desired crop and soil functionality benefits.

On the other hand, application of N (up to 120 kg N ha^{-1}) along with biochar increased maize crop biomass because N application was responsible for increased dry matter production, as compared with N0 treatments. In addition, enhanced MBC and available nutrients in the treatments with combined application of biochar and N (one year after biochar application) confirmed the favourable role of N and biochar in enhancing soil fertility and crop yield (Figure 1 and 4; Table 2 and 4).

4.2. Effect of biochar on soil biochemical properties

Soil pH and EC.

The rice residue biochar contains high concentrations of soluble oxides, hydroxides and carbonates of Ca, Mg and K (Table 1), which may have contributed to the increase in soil pH, as observed in our study (Table 2). Similar increase in soil pH were reported by Laird *et al.* (2010), where biochar with high ash content (14-56%), similar to present study (36% ash), were used. Application of biochar in combination with N showed no change in soil pH, relative to the biochar only treatment (Table 2), possibly due to the high buffering capacity of biochar (Muhammad *et al.* 2017).

Further, our results showed a significant increase in EC with increasing rate of rice residue biochar, with high EC (12.5 dSm^{-1}) and presence of soluble salts (Table 4), after one year of wheat-maize cropping. In addition, combined application of biochar and N may also enhance plant C input and release organic acids that react with sparingly soluble salts already present in soil and convert them either into soluble salts or increase their solubility (Sarwar *et al.* 2008). Furthermore, in both soils, the EC values increased when both biochar and N were applied together, particularly at higher addition rates, possibly due to the exchange of ammonium (NH^{4+}) with Ca^{2+}, magnesium (Mg^{2+}) and K^+ ions in the soils and biochar, thus increasing the concentration of salts in the solution (Wu *et al.* 2014).

Carbon dynamics.

Earlier research findings have shown that biochar supports the growth of microorganisms, possibly by providing intrinsic labile organic components for microbial utilisation (Singh and Cowie 2014). Biochar presence had a positive effect on microbial biomass in the present study (Figure 4). Furthermore, studies have suggested that micro-structures within the biochar support its strong affinity for DOC (relatively labile organic matter) and nutrients to support microbial growth (Lehmann *et al.* 2011). Since the biochar used in the present study had large surface area and porous structure, as was evident from SEM images (data not shown), more volumes of larger pores become available with higher addition rates of biochar for protection of micro-organisms from grazers. Similarly, there was also an increase in oxidisable OC concentrations, possibly due to increased plant-derived organic matter inputs across the treatments, as well as the release of relatively simpler forms of organic C in biochar during its degradation in the soils. However, corresponding to input of biochar C in LS (3.8-15.3 t C ha^{-1}) and SCL (3.3-13.4 t C ha^{-1}) at 10-40 t ha^{-1} of biochar addition, increase in oxidisable OC after one year of cropping was lower than the biochar C input in both soils (0.8-7.2 t C ha^{-1} in LS; 1.1-4.5 t C ha^{-1} in SCL). These results indicate that the detectable oxidisable forms of C (by the Walkley-Black method) may have represented less than 30-60% of added biochar-C in the present study. Therefore, our study supports the argument that the Walkley-Black method is inappropriate for the determination of C with high degrees of aromaticity and stability in soils; and it may lead to the under estimation of total C content in such soils (van Zwieten *et al*. 2010). Interestingly, measurement of total C by dry combustion in the biochar amended treatments showed that increases in SOC stocks were higher than input of C through biochar (0.1-2.1 t C ha^{-1} in LS and 1.8-4.8 t C ha^{-1} in SCL). Thus, the estimate of higher SOC stocks in the amended soils (beyond the input from biochar) suggests that the higher biomass production in the biochar amended versus non-amended soils may have contributed towards more belowground root-derived C input.

Furthermore, the belowground root-derived C may have been stabilized through sorption on biochar surfaces and pores, and also possibly due to enhanced soil aggregation and organo-biochar-clay mineral interactions, e.g. via cation bridging and ligand exchange reactions in the biochar-amended soils (Weng *et al.* 2017).

Further, addition of N to soils with or without biochar did not have a significant effect on oxidisable OC values in both soils except at higher rates of N addition (120 and 150 kg N ha^{-1}) in SCL. Greater crop biomass and associated larger input of C via root exudates in high N treatments may have possibly contributed to increased oxidisable OC values in these treatments (Lehmann and Kleber 2015). The possible increase in root C input may have also contributed to higher MBC in treatments where biochar was added in combination with N fertilizer. However, at the highest levels of N addition (120 and 150 kg N ha^{-1}), MBC concentrations were lower irrespective of biochar rates (Figure 4). The negative effect of higher N addition rates on microbial growth can be possibly explained by reactions of surplus ammonium ions with labile and recalcitrant compounds in biochar leading to formation of stable or toxic complexes (Ågren e*t al*. 2001). Additionally, Mavi and Marschner (2013) also reported a decrease in MBC at higher rate of N addition, possibly due to development of toxicity, especially where sufficient N is already available to soil microbes.

Available nutrients (N, P, K).

Biochar presence, at increasing application rates (10, 20 and 40 t ha^{-1}), enhanced available N in both soils possibly due to higher biochar-induced mineralisation of N from native soil organic matter, and also to some extent from microbial mineralisation of organic N in the biochar. As biochar is a relatively high C:N ratio organic matter input in soil, while containing some labile organic matter, this may increase demand for soil N by microorganisms and therefore, the mining of soil organic matter for N may enhance N mineralisation and availability in the soil (Mandal *et al.* 2016). High surface area and a possible increase in cation exchange capacity (CEC) of the biochar during its ageing may also enhance availability and retention of organic matter released N in the soils (Olmo *et al.* 2016).

Similar to N, available P and K increased with increasing rate of biochar application in both soils in the present study. Lashari *et al*. (2015) suggested that biochar might contain significant amounts of soluble P and K, contributing to plant-available nutrient pools upon incorporation in the soil. Although addition of urea N has been reported to enhance soil acidification in the microsites, application of biochar can buffer the N acidification effect and minimise pH changes (Xie *et al*. 2013). On the contrary, higher availability of P and K in the biochar and N amended treatments has been reported by Subedi *et al*. (2016) due to inhibition of P and K adsorption or precipitation after fertilizer additions.

5. Conclusions

Results of the study showed that rice straw derived biochar, when applied at definite rates to contrasting soils, could provide beneficial effects on crop biomass and soil functions. In addition, enhancement of total and soil organic C stocks (beyond the input by biochar that is, by 0.1–2.1 t ha^{-1} in LS and 1.8–4.8 t ha^{-1} in SCL) indicates greater input and stabilization of belowground root-derived C in the biochar amended soils. Further, the greatest positive effect on crop performance was observed in treatments where biochar was applied at 20 t ha^{-1}, along with 60 and 90 kg N ha^{-1} in LS and SCL, respectively. This clearly showed the potential of biochar to be used together with certain rates of N fertilizers to support crop productivity. However, in contrast to relatively short-term greenhouse studies, biochar impacts on soil functions and properties may vary under field conditions that make it imperative to test these findings in long-term field experiments.

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