



Effects of biochar and NPK on soil microbial biomass and enzyme activity during 2 years of application in the arid region

Muhammad Azeem^{1,2} · Rifat Hayat² · Qaiser Hussain² · Muhammad Ibrahim Tahir³ · Muhammad Imran⁴ · Zafar Abbas⁵ · Muhammad Sajid³ · Abdul Latif⁶ · Muhammad Irfan²

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Abstract

The studies regarding interaction of biochar and fertilizer and their effect on soil microbial biomass and enzyme activity are scarce. This study centers at analysis of soil microbial biomass carbon (MBC), nitrogen (MBN), and phosphorous (MBP) over a period of 2 years under mash bean-wheat cropping system. Overall, six treatments were performed which included (1) B0F0: no biochar no fertilizer; (2) B1F0: 5 tons ha⁻¹ (hectare) biochar with no fertilizer; (3) B2F0: 10 tons ha⁻¹ biochar (B2F0) with no fertilizer; (4) B0F1: no biochar with recommended N:P:K fertilizer (i.e., 23:45:25 kg ha⁻¹ respectively); (5) B1F1: 5 tons ha⁻¹ biochar with recommended fertilizer; (6) B2F1: 10 tons ha⁻¹ biochar with recommended fertilizer. Results elucidated increased soil MBC which increased with increasing biochar concentration. Enzyme activity and microbial biomass were higher initially but decreased in the second year of biochar amendment (both with and without fertilizer) which may be attributed to positive priming effect at the start of the experiment. MBN was observed lowest with fertilizer application but increased with biochar application alone. Seasonal variation in MBC was less in biochar-amended soils suggesting that biochar induced a less extreme environment for microbes throughout the season. The biochar treatment (i.e., B2F0) enhanced the soil microbial biomass, phosphorus, and phosphatase activities while biochar with fertilizer treatment (B2F1) improved urease, dehydrogenase, and alkaline phosphatase activity significantly ($P > 0.05$) in mash bean crop. In wheat however, this treatment decreased urease activity while dehydrogenase activity remained unchanged. A positive correlation was observed between MBN and dehydrogenase activity with soil organic carbon, dissolved organic carbon, and soil moisture content. These outcomes imply that bagasse biochar improves soil conditions for microbes thereby plummeting temporal variations in carbon dynamics.

Keywords Biochar · Soil microbial biomass · Enzyme · Mash bean-wheat · NPK

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✉ Muhammad Azeem
cmazeem78@gmail.com

✉ Rifat Hayat
hayat@uaar.edu.pk

- ¹ College of Natural Resources and Environment, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China
- ² Department of Soil Science and SWC, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi 46300, Pakistan
- ³ Department of Agriculture, Hazara University, Mansehra, Khyber Pakhtunkhwa 21300, Pakistan
- ⁴ Directorates of Agriculture, Pest Warning & Quality Control of Pesticides, Lahore, Punjab 54000, Pakistan
- ⁵ Soil and Water Testing Laboratory for Research, Bahawalpur, Punjab 63100, Pakistan
- ⁶ School of Resource and Environment, Anhui Agriculture University, Hefei 230036, Anhui, China

Introduction

Biochar is a carbon (C)-rich solid residue produced by thermal degradation of organic materials under oxygen (O₂) limited conditions for use specifically as an amendment to benefit soils (Lehmann and Joseph 2009). Amendments of biochar to soil have improved soil quality (Sohi et al. 2010; Sohi 2012; Kamran et al. 2018) and enhanced crop yields by improving soil physico-chemical properties including nutrient retention and increased water content (Glaser et al. 2002; Steiner et al. 2007), pH, and C levels (Chan et al. 2008) and by enhancing nutrient use efficiency (Van Zwieten et al. 2010; Ippolito et al. 2012). Various studies have reported that biochar application have boosted the soil biological activity (Anderson et al. 2011; Lehmann et al. 2011).

The effects of biochar application on soil biological processes varied with respect to feedstocks and its mechanism in various soils (Lehmann et al. 2011), which ensured high

variability in response of soil microbial biomass to biochar additions reported by various studies (O'Neill et al. 2009; Grossman et al. 2010; Khodadad et al. 2011). Microbial biomass increased with biochar additions due to the presence of labile C fractions and unpyrolyzed feedstocks (Steinbeiss et al. 2009; Bruun et al. 2011; Zimmerman et al. 2011; Luo et al. 2013). In contrast, various studies reported no effect of biochar on soil microbial biomass (Castaldi et al. 2011; Zavalloni et al. 2011) as it is recalcitrant in nature (Kuzuyakov et al. 2009). Dempster et al. (2012a) found that biochar amendments reduced soil microbial biomass due to toxic effect. Biochar application rates and soil type also affected response to soil microbial biomass (Lehmann et al. 2011).

Explanations for soil microbial biomass variation in response to biochar additions include increased available soil nutrients (dissolved organic matter—phosphorous (P), calcium (Ca), and potassium (K)—adsorption of toxic compounds, and improved soil pH and water content, all of which can affect the activity of soil microbes (Lehmann et al. 2011). The inner porosity of biochar might help soil microbes to evade grazers (Pietikäinen et al. 2000) and store carbon substrates and mineral nutrients (Saito and Muramoto 2002; Warnock et al. 2007).

Soil biological processes such as organic matter degradation, mineralization, and nutrient cycling are affected by the enzymes present in the soil due to microbial activities (Marx et al. 2001). Enzyme substrates are adsorbed by the biochar surfaces, thus enhancing the enzyme reactions leading to increased soil enzyme activity. Also the addition of biochar improves soil properties that indirectly increase soil enzyme activities (Zhang et al. 2016). Alkaline phosphatase has significant role in P cycling and is dominant in alkaline soils (Eivazi and Tabatabai 1977). Phosphatase enzyme liberates inorganic phosphates by hydrolysis of ester phosphate bonds which are assimilated by plants and microorganisms (Nannipieri et al. 2012). It converts organic P compounds to different inorganic forms. Urease enzyme is known for breaking the carbon-nitrogen bonds of some amides and urea (Bremner and Mulvaney 1978). Wu et al. (2012) reported increased urease activity in wheat straw biochar-amended soil and activity increased with increasing biochar rates. Further, the variation in urease activity is known to be effected considerably by soil pH and soil texture (Makoi and Ndakidemi 2008). The use of dehydrogenase activity as an index of overall microbial activity has been suggested by Palanisamy et al. (2017). The dehydrogenase activity indicates positive priming effect of biochar. Due to large surface area and porosity of biochar, it can stabilize native soil organic carbon (SOC) through direct sorption of dissolved organic matter and microbial enzymes on biochar surfaces and within pore spaces, which results in limiting soil enzyme activity (Bailey et al. 2011; Keith et al. 2011; Lammirato et al. 2011). Many other studies found immediate effect of biochar application on

dehydrogenase activity which increased initially but decreased at the end of the experiment (Kuzuyakov et al. 2009; Farrell et al. 2013; Bhaduri et al. 2016).

Biochar additions improve the ecosystem and its functioning (Lehmann et al. 2011; Biederman and Harpole 2013), including soil productivity and soil nutrient contents (Ding et al. 2016). The effect of biochar varies with the type or feed stock of biochar (Al-Wabel et al. 2018), soil, and climate. However, the present knowledge on soil microbial biomass dynamics due to biochar amendment is mostly based on the assessment of biochar application and no biochar treatment without considering the co-use of biochar and NPK specifically in legume-cereal cropping system and dearth in long-term field experimental data. The present field study with sequential biochar application and NPK lasted nearly 2 years in a mash bean-wheat cropping system, with whole inputs of biochar at 0%, 0.25%, and 0.5% biochar-C (carbon-equivalent). The aim of the present study is to study the effects of sugarcane bagasse biochar and NPK application on the seasonal variation in microbial biomass carbon (MBC), nitrogen (MBN), phosphorous (MBP), and different enzyme activities measured within a mash bean-wheat growing season during 2 years. The study helps to explore the microbial biomass dynamics with co-use of biochar and NPK application for enhanced carbon storage and higher crop yield in carbon-deficient soils of arid region.

Material and methods

Field experiment

Field experiment was carried out at Pir Mehr Ali Shah (PMAS)-Arid Agriculture University Rawalpindi Koont research farm (33° 1' N to 36° 6' N, 73° 30' E to 73° 45' E). The treatments were applied in randomized complete block design (RCBD). The soil of experimental area was sandy loam with pH 8.5 and moisture contents depend mainly on rainfall received in monsoon season. The average soil organic carbon (SOC) is less than 1%. The climate of the site is semi-arid to sub-tropical continental and sub-humid and has a bimodal rainfall incidence pattern, with two maxima in winter spring periods and late summer. Rainfall is erratic, around 60–70% of the entire rainfall usually occurred during the monsoon wet season (June to September) (Shafiq et al. 2005). Intense rain and undulating landscape are the major sources of soil erosion.

Biochar preparation and application

Sugarcane bagasse was used as a feedstock for biochar preparation. For this, bagasse was air-dried and pyrolysis was done in the sealed vessel entailing two metal barrels at 350 °C. The space between the barrels was ignited with natural gas. The

process took 1 h, while the produced biochar was left to cool for an hour converting approximately 50% of the biomass into biochar (Gunther 2009). In the field, biochar mass was crushed to powder to pass through a 2-mm sieve to get a fine granular uniformity (Pan et al. 2011).

In the field, three treatments of biochar (0, 5, and 10 tons ha⁻¹) were applied with and without chemical fertilizer, i.e., biochar at 0 tons ha⁻¹ (B0F0), biochar at 5 tons ha⁻¹ (B1F0), biochar at 10 tons ha⁻¹ (B2F0), biochar at 0 tons ha⁻¹ + NPK (B0F1), biochar at 5 tons ha⁻¹ + NPK (B1F1), and biochar at 10 tons ha⁻¹ + NPK (B2F1). The NPK was applied at 23 kg nitrogen (N), 45 kg phosphorous (P), and 25 kg potassium (K) per hectare, and treatments were assigned to field plots (1.5 m × 4.5 m) using RCBD. Biochar was applied one time before sowing of mash bean to the soil surface, thoroughly mixed, and then tilled to a depth of 12 cm, while fertilizer was applied to both crops during 2 years as recommended for both crops. Biochar treatments were applied to the mash bean crop, after which residual effects of the treatments on microbial biomass and activity were tested on the wheat crop in two growing cycles. The basic characteristics of the studied topsoil (0–15 cm) and biochar are given in Table 1.

Rhizosphere soil sampling

The rhizosphere soil samples were collected at crop maturity. Plants were randomly excavated at a depth of 10 cm from the replicate plots and tightly adhered soil attached to the plant root system was collected (Butler et al. 2003; Liu et al. 2008). The soil samples were collected in polyethylene bags and were transferred to the laboratory within 2 h of sampling. The samples were sieved (< 2 mm) and were stored at 4 °C. Soil samples for assessing microbial communities were not sieved and directly stored in freezer until isolation.

Biochar and soil characteristics

Biochar and soil moisture content (SMC) were determined gravimetrically. Soil and biochar samples were weighed, oven-dried, cooled, and then reweighed (Gardner et al. 1991). Electrical conductivity (EC) of soil from saturated paste was determined by a conductivity meter (Rhoades 1996) and soil pH was examined in 1 N KCl (potassium chloride) ratio of 1:1 soil suspension (Thomas 1996). EC and pH of biochar were analyzed in a 1:10 (w:v) water soluble extracts (Cayuela et al. 2013). Elemental composition (C/H/N/S) of the biochar was determined using a CHN Elemental Analyzer (Varian EL III) and the oxygen content was determined by weight difference. Proximate analysis including volatile matter and ash content was conducted according to the ASTM3174 (2011) and ASTM 3175 (2007), respectively, and fixed carbon content was determined by mass difference. Surface area and porosity of the biochar were determined via N₂ adsorption using an

ASAP 2020 instrument (Micromeritics Instrument). Prior to the measurement, the samples were passed through a 0.15-mm sieve and outgassed at 250 °C under vacuum for 4 h. Four data points, between relative pressures of 0.05 and 0.3, were used to construct the monolayer adsorption capacity. The total pore volume (V_{total}) was estimated from a single N₂ adsorbed point at a N₂ relative pressure of 0.99.

Surface area was measured via the Brunauer, Emmett, and Teller (BET) method that measures N₂ gas sorption (0.162 nm²) at 77 K. Approximately 200 mg of ground sample (bagasse biochar) was out gassed at 120 °C for 16.5 h and then analyzed on an Autosorb-1 Surface Area Analyzer (Quantachrome Instruments). Five data points, with relative pressures of 0.05–0.3, were used to calculate the surface area (Brunauer et al. 1938). For the determination of soil organic carbon (SOC) contents, biochar samples were burnt to ashes in the muffle furnace at 500 °C for 4 h and calculated by using the formula described by Brake (1992).

$$\text{Organic C (\%)} = \frac{100 - (\text{Ash\%})}{1.8}$$

SOC was analyzed by the wet digestion process as described by Nelson and Sommers (1982). Wet digestion was performed with concentrated sulfuric (H₂SO₄) acid and 1 N K₂Cr₂O₇ (potassium dichromate) solution. After digestion, the titration was carried out with 0.5 N ferrous ammonium sulfate solution. For total nitrogen (TN), the digestion was carried out with sulfuric acid (H₂SO₄) and allowed to distillation process with the addition of boric acid and NaOH. Nitrogen in the distillate was analyzed by the method given by Van-Schouwenberg and Walinge 1973. Dissolved organic carbon (DOC) was analyzed using total organic carbon (TOC)-V analyzer (Shimadzu Corp., Kyoto, Japan).

Microbial biomass analysis

MBC was measured by fumigation extraction method (Brookes et al. 1985). Ten-gram soil sample was fumigated for 24 h at 25 °C with ethanol-free chloroform (CHCl₃) and extraction was performed with 0.5 M K₂SO₄. The similar process was carried out for non-fumigated soil samples. After the titration of extracts, MBC was calculated from the given formula: $MBC = (\text{Carbon}_{\text{fumigated}} - \text{Carbon}_{\text{unfumigated}}) \times 2.64$.

For MBN, TN in the potassium sulfate (K₂SO₄) extract was determined after Kjeldahl-digestion. One gram of a digestion mixture (FeSO₄ 10: CuSO₄-1: Se-0.1) and conc. H₂SO₄ (4.5 ml) was added after cooling, and digestion took 3 h. After cooling, the contents were mixed thoroughly after the addition of water (20 ml) and 10 M NaOH (25 ml). The digested material was moved into the steam distillation chamber of Kjeldahl by using 10 M NaOH and 2% H₃BO₃. Then, 40 ml of distillate collected and titrated to bluish red end point

with 50 mM H₂SO₄ (Wu et al. 1990). The soil MBN was determined by using the equation; $MBN = (N_{\text{fumigated}} - N_{\text{unfumigated}}) \times 1.46$.

For MBP (microbial biomass phosphorous), 2.5 g oven-dried soil sample was taken in duplicate. One was fumigated with alcohol-free CHCl₃ (for 24 h at 25 °C), while second portion was incubated aerobically at 25 °C for 24 h at the same time. Both the portions were extracted by shaking for 30 min with 0.5 M NaHCO₃ (adjusted to pH 8.5). Ten milliliter aliquot of clear extract was taken into a 50 ml volumetric flask and 8 ml of ascorbic acid solution and ammonium molybdate solution (color developing reagent). After 15 min of color development, reading was noted on spectrophotometer at 882-nm wavelength. Phosphorus standards (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 ppm) were prepared using KH₂PO₄ (Brookes et al. 1982; Joergensen et al. 1995). The MBP was calculated by using the following equation:

$$MBP = (P_{\text{fumigated}} - P_{\text{unfumigated}}) \times 2.5$$

Enzyme activity

Dehydrogenase activity (DA) in the soil was measured by the reduction of TTC (2,3,5-triphenyl tetrazolium chloride) into TPF (triphenyl formazan). After filtration, the soil extract was analyzed at 546-nm wavelength on a spectrophotometer. The dehydrogenase activity (TPF $\mu\text{g g}^{-1}$ dry weight (dwt) soil) was calculated as $\text{TPF } (\mu\text{g ml}^{-1}) \times 45/\text{dwt}/5$ (Alef 1995). For urease activity (UA), the soil extract was collected by using 50 ml KCl solution. After filtration, the ammonium content in the filtrate was analyzed by measuring the optical density at 690 nm (Kandeler and Gerber 1988).

For APA (alkaline phosphatase activity), 10 g moist soil was added in 100-ml volumetric flask and incubated (for 3 h at 37 °C) after addition of toluene (1.5 ml), disodium phenyl phosphate (10 ml), and buffer (20 ml). After incubation diluted to 100 ml and filtered 5 ml buffer and 1–8 ml of filtrate were transferred in 100-ml volumetric flask and again diluted to 25 ml. After dilution, added 2,6-dibromo quinonechloromide and incubated for 20–30 min at room temperature. Finally diluted with distilled water to 100 ml and analyzed the optical density (600 nm). Calibration curve was prepared by using 0–20 ml standard phenol solution. APA was expressed as phenol ($\mu\text{g g}^{-1}$ dwt 2 h⁻¹) (Alef 1995).

Statistical analysis

To determine the differences among treatments, two-way ANOVA (analysis of variance) was performed (Steel and Torrie 1997). Canonical correspondence analysis (CCA) was carried out to determine the correlation of the environmental variables with all parameters studied.

Table 1 Soil (0–15 cm) and biochar basic properties

Property	Biochar	Soil
pH (H ₂ O)	6.68	8.25
EC (dS m ⁻¹)	0.30	0.53
SOC (%)	49.7	0.51
DOC (%)	0.47	0.05
TN (%)	0.51	1.0
H (%)	5.01	/
O (%)	25.5	/
S (%)	0.24	/
Surface area (m ² g ⁻¹)	3.90	/
Ash content (%)	10.50	/
Bulk density (g cm ⁻³)	/	1.46
Gravimetric moisture content (%)	/	9.35

EC electrical conductivity, SOC soil organic carbon, DOC dissolved organic carbon, TN total nitrogen, H hydrogen, O oxygen, S sulfur

Results

Soil properties affected with biochar and NPK

The biochar amendment with and without fertilizer affected the soil properties. Significant variations in the SOC, DOC, TN, and soil moisture were recorded which increased with increasing biochar level (Table 2). Soil bulk density decreased significantly in both crops but improved SOC and TN in biochar treatments with fertilizer. In treatment of biochar without fertilizer (B2F0), SOC, TN, and SMC increased by 23%, 27%, and 56%, respectively in the mash bean crop. In the second year, similar trend was observed in mash bean crop. In wheat crop, the treatment of biochar with fertilizer (B2F1), SOC and SMC improved 32% and 39% as compared to control in the first year and the increase was 34% and 72% in the second year, respectively. DOC of soil increased in the mash bean crop while it significantly decreased in wheat crop. However, biochar application had no effect on soil EC and pH during both growing years.

Microbial biomass carbon

In mash bean, MBC significantly increased with increasing biochar level and addition of fertilizer further supplemented it (Table 3). The results elucidated that highest increase was observed with treatment B2F1, i.e., 25% as compared to control during the first year. In second year, the increase in MBC was slightly lower as compared to first year. However, highest MBC was recorded in treatment B2F1, i.e., 8% as compared to respective control. In wheat crop during first year, highest MBC was recorded in treatment B0F1 with 6% increase as compared to control while non-significant difference was recorded in counterparts. In second year, we observed that with

Table 2 The effect of biochar and NPK application on soil properties (mean ± SD, *n* = 3)

Treatments	Crop	Bulk density (g cm ⁻³)	EC (dS m ⁻¹)	pH	SOC (g kg ⁻¹)	DOC (g kg ⁻¹)	TN (%)	Gravimetric soil moisture (%)	
Mash bean	No fertilizer	First year	1.46 ± 0.02a	0.53 ± 0.03a	8.25 ± 0.09a	6.07 ± 0.42b	0.46 ± 0.02f	3.03 ± 0.21c	9.35 ± 1.42b
		Second year	1.42 ± 0.03a	0.52 ± 0.02ab	8.18 ± 0.07a	6.13 ± 0.43b	0.46 ± 0.02b	3.09 ± 0.29c	9.63 ± 1.38d
	B1F0	First year	1.41 ± 0.02bc	0.54 ± 0.02a	8.22 ± 0.1a	6.59 ± 0.05ab	0.48 ± 0.02d	3.44 ± 0.07bc	12.90 ± 1.35ab
		Second year	1.40 ± 0.02ab	0.53 ± 0.01ab	8.20 ± 0.05a	6.83 ± 0.29ab	0.45 ± 0.04c	3.38 ± 0.26bc	12.44 ± 0.98bc
	B2F0	First year	1.39 ± 0.02c	0.56 ± 0.02a	8.11 ± 0.27a	7.47 ± 0.81a	0.51 ± 0.05b	3.86 ± 0.36a	14.57 ± 1.79a
		Second year	1.37 ± 0.01b	0.54 ± 0.01a	8.23 ± 0.06a	7.57 ± 0.85a	0.44 ± 0.03d	3.56 ± 0.27ab	13.59 ± 0.41ab
Wheat	No fertilizer	First year	1.45 ± 0.03ab	0.52 ± 0.02a	8.09 ± 0.06a	6.20 ± 0.60b	0.47 ± 0.03c	3.70 ± 0.22ab	10.55 ± 1.66b
		Second year	1.43 ± 0.02a	0.51 ± 0.01b	8.12 ± 0.13a	6.29 ± 0.62b	0.48 ± 0.03a	3.25 ± 0.30bc	11.25 ± 1.03cd
	B1F1	First year	1.42 ± 0.03abc	0.56 ± 0.02a	8.18 ± 0.15a	6.54 ± 1.01ab	0.49 ± 0.03cb	3.79 ± 0.19ab	11.99 ± 3.07b
		Second year	1.40 ± 0.03ab	0.52 ± 0.02ab	8.14 ± 0.12a	6.67 ± 1.08ab	0.46 ± 0.04b	3.57 ± 0.27ab	12.31 ± 1.41bc
	B2F1	First year	1.39 ± 0.03c	0.54 ± 0.01a	8.29 ± 0.06a	7.27 ± 0.83a	0.52 ± 0.04a	3.77 ± 0.15ab	12.53 ± 1.51ab
		Second year	1.36 ± 0.02b	0.53 ± 0.02ab	8.22 ± 0.03a	7.48 ± 0.70a	0.48 ± 0.03a	3.87 ± 0.43a	14.51 ± 1.06a
NPK fertilizer	No fertilizer	First year	1.45 ± 0.02a	0.53 ± 0.03ab	8.25 ± 0.10a	6.20 ± 0.20b	0.46 ± 0.03c	2.87 ± 0.25c	9.17 ± 1.81c8.7
		Second year	1.41 ± 0.02a	0.54 ± 0.01a	8.20 ± 0.08a	6.20 ± 0.57c	0.47 ± 0.04b	3.19 ± 0.20ab	8.72 ± 0.65e
	B1F0	First year	1.41 ± 0.01ab	0.54 ± 0.02ab	8.21 ± 0.09a	6.97 ± 0.26ab	0.45 ± 0.04d	3.06 ± 0.04c	11.57 ± 1.02ab
		Second year	1.39 ± 0.02ab	0.53 ± 0.01a	8.22 ± 0.04a	7.12 ± 0.53b	0.45 ± 0.03c	2.93 ± 0.23c	12.77 ± 1.19c
	B2F0	First year	1.38 ± 0.02b	0.56 ± 0.01a	8.11 ± 0.27a	8.07 ± 0.53a	0.44 ± 0.04e	3.65 ± 0.40a	12.53 ± 0.65ab
		Second year	1.35 ± 0.02cd	0.55 ± 0.02a	8.14 ± 0.05a	8.13 ± 0.49a	0.44 ± 0.03d	3.17 ± 0.13abc	14.17 ± 0.60ab
NPK fertilizer	No fertilizer	First year	1.42 ± 0.05ab	0.52 ± 0.01b	8.09 ± 0.06a	6.34 ± 0.33b	0.48 ± 0.02b	3.04 ± 0.29c	10.23 ± 1.08bc
		Second year	1.42 ± 0.02a	0.52 ± 0.01a	8.10 ± 0.11a	6.30 ± 0.54c	0.48 ± 0.05a	3.30 ± 0.20a	11.32 ± 0.96d
	B1F1	First year	1.41 ± 0.03ab	0.55 ± 0.02ab	8.18 ± 0.15a	7.80 ± 0.61a	0.47 ± 0.01a	3.18 ± 0.41bc	10.77 ± 0.11abc
		Second year	1.38 ± 0.03bc	0.52 ± 0.01a	8.12 ± 0.11a	7.21 ± 0.48b	0.47 ± 0.04b	2.98 ± 0.14bc	13.51 ± 1.05bc
	B2F1	First year	1.37 ± 0.02b	0.54 ± 0.02ab	8.28 ± 0.06a	8.17 ± 0.59a	0.48 ± 0.03	3.55 ± 0.36ab	12.77 ± 1.76a
		Second year	1.36 ± 0.02d	0.53 ± 0.03a	8.16 ± 0.06a	8.29 ± 0.46a	0.48 ± 0.05a	3.20 ± 0.16ab	15.02 ± 1.28a

Biochar amendment at 0, 0.25% C, and 0.5% C ha⁻¹ (B0, B1, and B2, respectively) with (F1) and without NPK fertilization (F0)
 SOC soil organic carbon, DOC dissolved organic carbon, TN total nitrogen

Table 3 The effect of biochar and NPK application on microbial biomass (mean \pm SD, $n = 3$)

		Treatments	Crop	MBC ($\mu\text{g g}^{-1}$)	MBN ($\mu\text{g g}^{-1}$)	MBP ($\mu\text{g g}^{-1}$)
Mash bean	No fertilizer	B0F0	First year	402 \pm 8e	18.3 \pm 1.93d	9.7 \pm 0.37d
			Second year	426 \pm 5b	20.2 \pm 1.12b	10.1 \pm 0.20d
		B1F0	First year	440 \pm 10d	24.6 \pm 1.84c	11.1 \pm 0.64c
			Second year	440 \pm 8ab	23.6 \pm 4.86ab	10.8 \pm 0.43d
		B2F0	First year	465 \pm 10c	30.7 \pm 1.46a	11.7 \pm 0.21bc
			Second year	432 \pm 14b	25.5 \pm 4.38ab	12.2 \pm 0.92c
	NPK fertilizer	B0F1	First year	476 \pm 14bc	25.1 \pm 4.02bc	11.7 \pm 0.56bc
			Second year	462 \pm 9a	24.6 \pm 2.56ab	12.8 \pm 0.63bc
		B1F1	First year	487 \pm 13ab	23.6 \pm 1.52c	12.5 \pm 0.55b
			Second year	450 \pm 14ab	23.1 \pm 1.12ab	13.8 \pm 0.91ab
		B2F1	First year	504 \pm 10a	29.0 \pm 3.45ab	13.6 \pm 0.81a
			Second year	460 \pm 24a	26.0 \pm 3.04a	14.3 \pm 0.61a
Wheat	No fertilizer	B0F0	First year	416 \pm 7bc	16.8 \pm 1.46b	9.9 \pm 0.15e
			Second year	430 \pm 7a	18.5 \pm 1.52ab	10.1 \pm 0.23d
		B1F0	First year	421 \pm 7bc	19.7 \pm 1.46ab	10.6 \pm 0.27d
			Second year	401 \pm 8b	21.4 \pm 0.42ab	10.4 \pm 0.41d
		B2F0	First year	408 \pm 9c	23.6 \pm 2.95ab	11.9 \pm 0.47bc
			Second year	377 \pm 12cd	21.7 \pm 4.75ab	11.3 \pm 0.44c
	NPK fertilizer	B0F1	First year	441 \pm 9a	27.0 \pm 7.62a	11.7 \pm 0.11c
			Second year	444 \pm 8a	26.3 \pm 8.85a	11.3 \pm 0.41c
		B1F1	First year	431 \pm 9ab	22.1 \pm 5.13ab	12.3 \pm 0.26b
			Second year	373 \pm 22d	19.7 \pm 1.26ab	12.0 \pm 0.34b
		B2F1	First year	419 \pm 6bc	26.0 \pm 9.80ab	13.6 \pm 0.28a
			Second year	394 \pm 10bc	16.8 \pm 0.73b	13.0 \pm 0.31a

MBC microbial biomass carbon, MBN microbial biomass nitrogen, MBP microbial biomass phosphate

addition of biochar MBC decreased significantly (i.e., 13%) and lowest MBC was recorded in B1F1.

Microbial biomass nitrogen

MBN results in mash bean elucidated that MBN increased with addition of biochar and fertilizer during first year of study (Table 3). Highest increase (68%) was observed in treatment B2F0 as compared to control. In second year, significant increase (29%) was recorded in the only treatment B2F1 while there was non-significant difference between other treatments. In wheat, we observed non-significant difference in MBN during both the years of study.

Microbial biomass phosphorus

In mash bean during the first year, data of MBP recorded revealed that the highest MBP was found in treatment B2F1 which was 40% higher as compared to control (Table 3). Addition of biochar increased the MBP overall. During the second year, similar trend was observed and the highest MBP was recorded in B2F1. In wheat crop during first year, MBP was found highest in treatment B2F1 which was 37% higher as compared to control. In second year similar trend was recorded and highest value was found in the same treatment with 29% increase.

Urease activity

In mash bean, the analysis of urease enzyme activity was done and data revealed that in first year, biochar addition overall increased urease enzyme activity and highest activity in treatment B2F1 with 20% increase as compared to control (Table 4). However, in second year, there was non-significant difference among the treatments and the control. The only treatment showing significant difference was B0F1 where the highest urease enzyme activity was recorded in second year. In wheat crop, addition of biochar decreased the urease activity overall as compared to control during both the years of study.

Dehydrogenase activity

The results of dehydrogenase activity in mash bean showed that addition of biochar was correlated with dehydrogenase activity (Table 4). The highest dehydrogenase activity was recorded when biochar was applied with fertilizer (B2F1) and 52% increase was recorded as compared to control. However, in second year, the increase in this treatment was only 19% as compared to control and the highest activity was recorded in B0F1 which was found to be 22% higher as compared to control. In wheat crop during first year, there was non-significant difference among the treatments on the account of dehydrogenase activity; however, the highest activity was recorded in treatment B0F1 which was 21% higher as compared to control. In second year, no significant difference was recorded in treatments as compared to control.

Table 4 The effect of biochar and NPK application on enzyme activity (mean \pm SD, $n = 3$)

		Treatments	Crop	Urease ($\mu\text{gNH}_4\text{-N g}^{-1}\text{dwt.}$ 2 h^{-1})	Dehydrogenase (mg TPF kg^{-1} 24 h^{-1})	Alkaline phosphatase ($\mu\text{g phenol}$ $\text{g}^{-1} \text{ h}^{-1}$)	
Mash bean	No fertilizer	B0F0	First year	419 \pm 8.6d	109 \pm 6.4d	67.5 \pm 5.9cd	
			Second year	428 \pm 3.1b	105 \pm 8.4b	74.3 \pm 9.1c	
		B1F0	First year	456 \pm 10.5c	126 \pm 5.8c	84.3 \pm 4.0bc	
			Second year	416 \pm 6.3b	118 \pm 2.5a	97.4 \pm 12.3ab	
		NPK fertilizer	B2F0	First year	475 \pm 9.4b	130 \pm 5.3c	103.7 \pm 11.8a
				Second year	426 \pm 8.1b	121 \pm 2.2a	115.3 \pm 10.0a
	NPK fertilizer	B0F1	First year	470 \pm 8.3b	125 \pm 4.7c	63.3 \pm 13.4d	
			Second year	467 \pm 6.1a	128 \pm 5.9a	72.3 \pm 7.9c	
		B1F1	First year	490 \pm 7.2a	144 \pm 8.3b	78.9 \pm 18.1bcd	
			Second year	423 \pm 10.5b	120 \pm 1.9a	79.4 \pm 5.9bc	
		B2F1	First year	501 \pm 14.4a	166 \pm 7.0a	87.9 \pm 3.8ab	
			Second year	125 \pm 6.9a	125 \pm 6.9a	94.7 \pm 9.5b	
Wheat	No fertilizer	B0F0	First year	437 \pm 6.0b	101 \pm 10.3b	75.4 \pm 3.8cd	
			Second year	431 \pm 4.9a	107 \pm 11.0a	80.3 \pm 9.1c	
		B1F0	First year	412 \pm 9.5c	119 \pm 5.1a	92.4 \pm 7.9b	
			Second year	396 \pm 6.0c	108 \pm 6.9a	109.4 \pm 5.9a	
		NPK fertilizer	B2F0	First year	401 \pm 5.6cd	112 \pm 5.1ab	107.4 \pm 11.1a
				Second year	372 \pm 5.5d	111 \pm 4.3a	120.7 \pm 6.8a
	NPK fertilizer	B0F1	First year	453 \pm 3.6a	122 \pm 7.3a	72.2 \pm 8.7d	
			Second year	413 \pm 4.8b	120 \pm 8.6a	82.3 \pm 7.9c	
		B1F1	First year	431 \pm 5.2b	117 \pm 16.2ab	87.1 \pm 9.0bc	
			Second year	410 \pm 4.1b	113 \pm 5.7a	88.4 \pm 3.0bc	
		B2F1	First year	397 \pm 12.9d	111 \pm 12.6ab	82.5 \pm 4.4bcd	
			Second year	417 \pm 8.3b	115 \pm 6.9a	96.7 \pm 11.7b	

Alkaline phosphatase activity

The analysis of alkaline phosphatase enzyme activity during first year in mash bean was found the highest in B2F0 with 54% increase as compared to control (Table 4). In second year, the same treatment was found the highest as compared to control. In wheat crop, similar trend was observed during both the years of study and the highest alkaline phosphatase activity was recorded with B2F0 with 42% increase during first year and 50.3% increase during second year. Other treatments were non-significantly different from the control.

Effect of biochar on biological yield

Biological yield in mash bean elucidated that biological yield (BY) increased with addition of biochar and fertilizer during both years of study (Table 5). The highest increase (37%) was observed in treatment B2F1 as compared to control. In second year, similar trend was noticed in the biochar treatment with significant

difference between other treatments. Similarly, in wheat, we observed significant increase in BY with co-use of biochar and NPK during both the years of study. The highest increase (37%) was observed in treatment B2F1 as compared to control. However, in second year, the increase in this treatment was only 29% as compared to control.

Effect of biochar on grain yield

Mash bean grain yield showed that addition of biochar was correlated with grain yield (GY). The highest GY was recorded when biochar was applied with fertilizer (B2F1) and 23% increase was recorded as compared to control (Table 5). While in second year, 26% increase was observed with B2F1 as compared to control. In wheat crop, similar trend was observed during both the years of study and the highest GY was recorded with B2F1 with 43% increase during first year and 33% increase during second year. Other treatments showed significant differences among each other.

CCA of environmental variable and dependent variables

The CCA results in mash bean revealed that APE and MBN were best correlated with SMC in first axis (51%), while GY and UE were best correlated with BD (Fig. 1). However, in second year, soil BD was the main driver to enhance the MBC and UE in first axis while EC, pH, and SOC were the main drivers to increase APE and MBN. In wheat, UE, APE, MBC, and DE were best correlated with BD in first axis (75%), while APE, MBN, MBP, and DE were best correlated with SMC, SOC, and EC. In second year, MBC, UE, and MBN were best correlated with soil pH and BD in first axis while APE and MBN were best correlated with soil EC (Fig. 2).

Discussion

Soil properties affected with biochar and NPK additions

Physical and chemical properties of soil have been shown to change in various studies with the biochar addition (Asai et al. 2009; Major et al. 2010). Different crops respond differently to the physical and chemical properties of biochar but crops also respond to soil type and climatic conditions (Gaskin et al. 2010; Van Zwieten et al. 2010; Haefele et al. 2011). Our study showed that biochar

amendment improved the physico-chemical properties of soil, similar to the results of Asai et al. (2009) and Major et al. (2010). Biochar soil application increased the porosity and water content of the soil, and decreased the bulk density as reported by Ogawa and Okimori 2010, which promoted lateral root formation and increased the soil volume that was exploited by plant roots. The results of Genesio et al. (2012) study suggested that biochar changes the physical conditions of soil; its dark color alters thermal dynamics and leads to rapid germination, allowing more time for growth compared with controls. Pyrogenic bagasse reduced the soil bulk density and increased the available moisture which was in line with the results of Chen et al. (2010). In this study, biochar application had no effect on soil pH and EC since biochar was near neutral pH. However, the enhanced SMC was observed which might be attributed to the large surface area of biochar particles and the pores available to hold water molecules which adhere to biochar particles.

Effect of biochar on soil microbial biomass

Microbial biomass reflects the microbial turnover in the soil ecosystem which includes the growth, death, and organic matter decomposition. In the present study, MBC and MBN were increased significantly with biochar additions suggesting that biochar addition accelerates microbial growth. The results of MBC and MBN indicate a

Table 5 Biochar and NPK impacts on biological (BY) and grain yield (GY) (kg ha⁻¹)

		Treatments	Season	BY (kg g ⁻¹)	GY (kg g ⁻¹)	
Mash bean	No fertilizer	B0F0	First year	2649 ± 165e	612 ± 38d	
			Second year	2625 ± 171e	622 ± 19e	
		B1F0	First year	3030 ± 164d	636 ± 7cd	
			Second year	2987 ± 153d	659 ± 24de	
		NPK fertilizer	B2F0	First year	3272 ± 156c	655 ± 39cd
				Second year	3160 ± 144cd	681 ± 30cd
	B0F1		First year	3415 ± 129bc	690 ± 20bc	
			Second year	3305 ± 160bc	715 ± 23bc	
	Wheat	No fertilizer	B1F1	First year	3485 ± 172ab	721 ± 30ab
				Second year	3417 ± 157ab	750 ± 30ab
			B2F1	First year	3609 ± 144a	751 ± 27a
				Second year	3531 ± 135a	782 ± 19a
NPK fertilizer			B0F0	First year	6124 ± 294e	2178 ± 75e
				Second year	6195 ± 301d	2062 ± 257c
	B1F0	First year	6374 ± 239d	2312 ± 162de		
		Second year	6154 ± 192d	2202 ± 234c		
Wheat	NPK fertilizer	B2F0	First year	6488 ± 283d	2443 ± 83cd	
			Second year	6549 ± 366c	2519 ± 257b	
		B0F1	First year	6826 ± 258c	2617 ± 72bc	
			Second year	6826 ± 306c	2716 ± 271b	
		B1F1	First year	7984 ± 293b	2763 ± 86ab	
			Second year	7547 ± 412b	2561 ± 237b	
B2F1	First year	8375 ± 285a	2894 ± 189a			
	Second year	8003 ± 402a	2954 ± 357a			

BY biological yield, GY grain yield

possible increase in microbial carbon use efficiency and a decrease in C turnover in response to biochar addition (Table 3). The enhanced microbial biomass with biochar addition may attribute to positive priming effect of biochar (data unpublished). Similarly, biochar contained labile organic carbon at the start of the experiment indicated enhanced microbial growth and activity. DOC was significantly higher in the mash bean crop when fresh biochar was applied; however, it decreased at the end of the experiment which could be linked with the microbial biomass reduction or remain unchanged over time. The small amounts of organic substrates of low molecular weight, such as glucose, amino acids, and root exudates, might trigger microbial activity and biomass render “apparent” or “real” priming effects. However, reported effects of biochar additions to microbial biomass are pretty inconsistent. Biochar may contain trace quantities of water soluble low molecular organic compounds among predominantly complex C substrates, which could trigger priming effects. In a short-term study of peanut, biochar application results in an increase of MBC due to increase of SOC (Bhaduri et al. 2016). Though, biochar has the potential to reduce microbial biomass as it contains volatile compounds (Deenik et al. 2010). Girvan et al. (2005) demonstrated that benzene concentrations of 40 mg kg^{-1} or higher can decrease the microbial biomass. Dempster et al. (2012b) found that biochar application induced toxic effects which hinder the soil microbial biomass.

MBP represents the immobilization of P by microbes, providing a mechanism of P retention in soils, and is an important contributor to the soil P pool (Liptzin and Silver 2009). Our finding of an increased MBP in high

biochar-amended soil is more likely due to an enhanced nutrient availability, soil moisture, and microbial abundance and therefore increased enzyme levels in response to biochar addition. Our results suggest a greater availability of nutrients for microbe and plant use due to increased phosphatase activity in response to biochar addition. This is consistent with the findings of Biederman and Harpole (2013), whose meta-analysis found that, on average, biochar amendment resulted in increased soil MBP and P availability. Similarly, results of CCA confirmed the positive correlation of the enhanced MBP with SMC, SOC, and DOC (axis 2) in both crops.

Likewise, results of CCA revealed the soil BD was the main driver to enhance the microbial biomass in the mash bean crop. Biochar contains small pore spaces which may provide suitable habitat to microbes which caused increase microbial growth and activity. The internal porosity of biochar may help soil microbes to avoid grazers (Pietikäinen et al. 2000) and store C substrates and mineral nutrients (Saito and Muramoto 2002; Warnock et al. 2007). Various studies suggested that the increase in soil microbial activity in terms of soil MBC in biochar-treated soils (Lehmann et al. 2011; Mastro et al. 2013) was mostly due to improvement of physico-chemical characteristics of the soil (Maestrini et al. 2015). In contrast, other studies reported that biochar has no effect on soil microbial biomass (Castaldi et al. 2011) due to its recalcitrance property (Kuzuyakov et al. 2009). Biochar additions rates and soil type also affected response of soil microbial biomass (Lehmann et al. 2011). An explanation for soil microbial biomass change in response to additions of biochar includes enhanced availability of soil nutrients (i.e.,

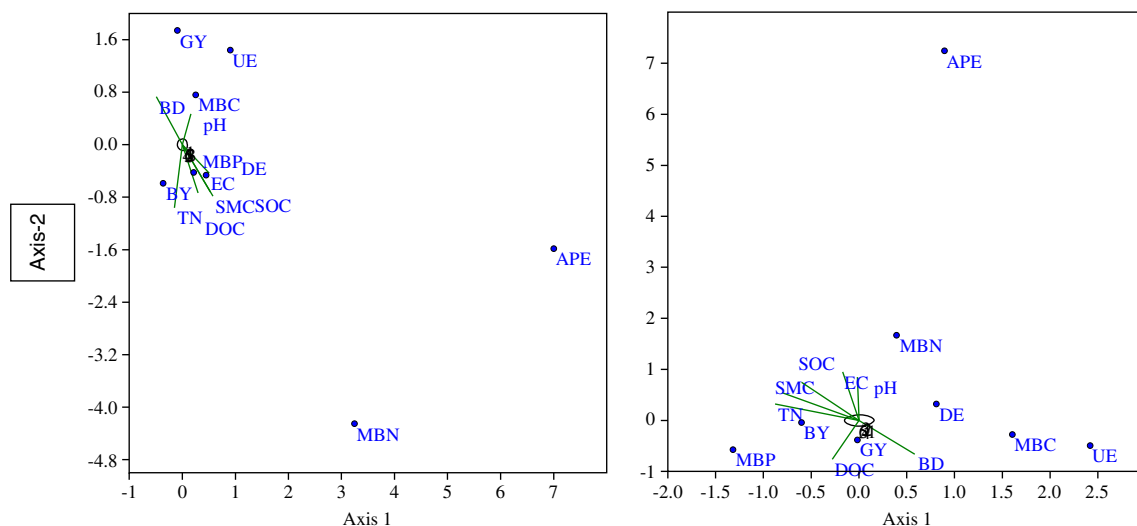


Fig. 1 Canonical correspondence analysis (CCA) of environmental and response variables of mash bean crop during 2 years. EC electrical conductivity, SMC soil moisture content, BD bulk density, SOC soil organic carbon, DOC dissolved organic carbon, TN total nitrogen, MBC

microbial biomass carbon, MBN microbial biomass nitrogen, MBP microbial biomass phosphate, UE urease activity, DE dehydrogenase activity, APE alkaline phosphatase activity

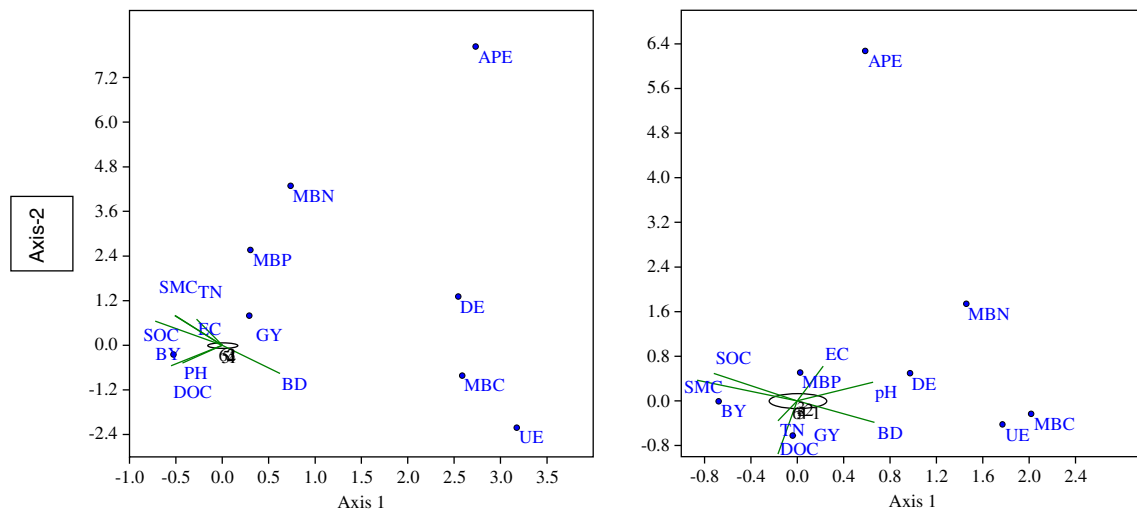


Fig. 2 Canonical correspondence analysis (CCA) of environmental and response variables of wheat crop during 2 years. EC electrical conductivity, SMC soil moisture content, BD bulk density, SOC soil organic carbon, DOC dissolved organic carbon, TN total nitrogen, MBC microbial

biomass carbon, MBN microbial biomass nitrogen, MBP microbial biomass phosphate, UE urease activity, DE dehydrogenase activity, APE alkaline phosphatase activity

DOC, P, Ca, and K), adsorption of toxic compounds, and improved soil water and pH status. All these changes have an impact on the activity of soil microorganisms (Lehmann et al. 2011).

Effect of biochar on soil enzyme activity

Similarly, the enzyme activity was boosted when biochar was applied, but it decreased at the end of the experiment, especially in case of urease, while the dehydrogenase activity remained unchanged. The dehydrogenase activity gives an indication of positive priming effect of biochar. Due to large surface area and porosity of biochar, it can stabilize native SOC through direct sorption of dissolved organic matter and microbial enzymes on biochar surfaces and within pore spaces, which results in hindering the activity of the soil enzymes (Bailey et al. 2011; Keith et al. 2011; Lammirato et al. 2011). Various other studies found immediate effect of biochar application resulting in an increase in dehydrogenase activity at initial phase while it suppressed at the end of the experiment (Kuzyakov et al. 2009; Farrell et al. 2013; Bhaduri et al. 2016). In the present study, biochar application suppressed native SOC mineralization rapidly. This could result from sorption of labile soil organic matter onto biochar particles, inhibitory effect on microbial activity of biochar-associated volatile organic compounds, and/or a brief shift to more labile substrates in biochar, relative to those in the low-C clay soil (Guenet et al. 2010; Lehmann et al. 2011; Spokas et al. 2011). In contrast, alkaline phosphatase activity increased gradually over the course of the experiment which suggested that biochar provides suitable habitat for the phytase-producing microbes. CCA results correlate that the SMC were the main drivers in controlling APA activity. In addition, microbial

phosphatases release the organic bound phosphorous into inorganic P which could be available to plants (Azeem et al. 2015). As soils contain very large amount of organic phosphorus specially in alkaline soils (calcium and magnesium bound P), so microbes mounted or living in the pores of biochar particles may release the biochar-P and also from the organic matter which enhanced the activity of the enzyme which ultimately enhanced the crop growth and yield.

Plant growth is also affected by the biochar-induced variations in soil physical and chemical properties predominantly SMC, BD, nutrient cycling, and carbon turnover (Dempster et al. 2012a, b; Taghizadeh-Toosi et al. 2012). Biochar improves the soil water holding capacity (Laird et al. 2010) and facilitates biomass gain (Kammann et al. 2011). Increased crop yield with biochar additions was reported in various studies which were attributed to improve soil health and nutrient uptake. Alburquerque et al. (2013) found 20–30% increase in wheat yield with the application of wheat straw and olive tree pruning biochar in a loamy sand-textured soil, attributed to enhanced nutrient availability. Availability of water content, reduction in soil bulk density, enhanced microbial growth, and carbon turnover may be attributed to enhanced crop biomass and yield in the arid climate. The results of our study presented that plant-based (bagasse) biochar with chemical fertilizer can be a better option to improve the soil health of dry areas.

Conclusion

The current study enhanced the understanding of the microbial biomass and enzyme activity as affected by the co-use of biochar and NPK additions in mash bean-wheat cropping

system of the dry land of Pakistan. Biochar applications increased the SOC, TN, and crop yield, and decreased the soil bulk density. Seasonal fluctuation in MBC was less for biochar-amended soils than for control and NPK treatments, suggesting that biochar induced a less extreme environment for microorganisms throughout the season. The levels of soil microbial biomass phosphorus and phosphatase activities were most significantly increased with B2F0 treatment. The current study suggests that the use of bagasse biochar as a soil amendment could be adopted as an effective measure to achieve simultaneously high grain yield and lower carbon loss in mash bean and wheat crop lands of calcareous soil poor in organic carbon.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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