Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 8, No. 6, p. 1-13, 2016
http://www.innspub.net

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RESEARCH PAPER
OPEN ACCESS

Effect of biochar amendment on soil microbial biomass, abundance and enzyme activity in the mash bean field

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Article published on June 11, 2016

Key words: Biochar, Microbial biomass, Enzyme, Legume.

Abstract

Biochar is being evaluated globally as a means to improve soil fertility, ecosystem services and sequester carbon. The present study was conducted in the arid zone agricultural region of Pakistan to investigate the impact of biochar on the soil microbial biomass, abundance, and activity in the rhizosphere of mash bean crop. For this, pyrolyzed biochar of sugarcane bagasse was prepared and applied at rates of 0, 0.25 and 0.5%-C (C-equivalent basis) with and without NPK fertilization (23 N, 45 P and 25 K kg ha^{-1}). Biochar treatments were applied before sowing of mash bean, and the soil samples were taken from each treatment plot at crop maturity. Bacterial 16S rRNA gene copy numbers were significantly increased with biochar (132%) and NPK fertilization (27%) in mash bean, while 18S rRNA was significantly decreased with biochar application by 22%. 18S abundance was increased (20%) when biochar was applied along with chemical fertilizer. Microbial biomass carbon and nitrogen increased by 19% and 67% with biochar amended at 0.5%-C. Urease and dehydrogenase activities significantly increased with biochar applied at 0.5%-C and NPK fertilization. The results suggest that the application of sugarcane bagasse-derived biochar can be useful in improving the legume yield and soil functions in the calcareous soil of the arid area.

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Introduction

The production and application of pyrogenic biomass derived-black carbon or biochar to soils have emerged as a viable tool for the stable and long-term storage of carbon in terrestrial ecosystems (Lehmann and Joseph, 2009). High stability of biochar arises from the change in the chemical structure of the cellulose, hemicellulose and lignin which take place at > 300 ℃. Recalcitrant carbon of biochar is resistant to microbial attack and eventually less carbon dioxide is released back to the atmosphere (Shackley *et al*., 2009). Biochar yields between 2-35% by weight of the biomass as biochar and various studies demonstrated the Mean Residence Time (MRT) of biochar from 100s to 1000s of years (Verheijen *et al*., 2009). The useful effects of biochar addition in soil include: nutrient retention (Liang *et al*., 2006) or change in soil pH (Rousk *et al*., 2010), soil water retention, reduction in greenhouse gases emission and nitrate leaching, adsorption of toxic metals and agrochemicals (Spokas *et al*., 2009; Sohi *et al*., 2010) which ultimately leads to increase the productivity of soil (Zwieten *et al.,* 2010). The soil microbial communities composition and abundance also change with the biochar addition (Pietikäinen *et al*., 2000; Yin *et al.,* 2000; Kim *et al.,* 2007; O'Neill *et al.,* 2009; Liang *et al.,* 2010; Grossman *et al.,* 2010; Jin, 2010). These changes affect microbial structures (Rillig and Mummey, 2006) and nutrient cycling (Steiner *et al*., 2008) that indirectly affects the plant development (Warnock *et al.,* 2007).

Biochar effects on soil biological processes are not well understood (Lehmann *et al.,* 2011) due to high variability in the response of soil microbial biomass to biochar additions reported (O'Neill *et al*., 2009; Khodadad *et al.,* 2011). Biochar amendments have been shown to increase microbial biomass due to the presence of labile C fractions and un-pyrolysed feedstock (Bruun *et al.,* 2011; Zimmerman et al., 2011; Luo *et al.,* 2013). Other studies have reported that biochar has no effect on soil microbial biomass (Castaldi *et al.,* 2011) as a result of its recalcitrance (Kuzyakov *et al.,* 2009). Dempster *et al.* (2012a) reported that biochar amendments reduced soil microbial biomass induced by a toxicity effect. Biochar application rates and soil type also affected the response of soil microbial biomass (Lehmann *et al*., 2011). Explanations for soil microbial biomass change in response to addition of biochars include enhanced soil nutrients availability (DOC, P, Ca and K), adsorption of toxic compounds and improved soil water and pH status, all of these factors influence the activity of soil microorganisms (Lehmann *et al.,* 2011). The internal porosity of biochars may help soil microorganisms avoid grazers (Pietikäinen *et al.,* 2000) and to store C substrates and mineral nutrients (Saito and Muramoto, 2002; Warnock *et al*., 2007).

In order to sustain long-term productivity of the fallow-wheat cropping pattern, an efficient management of natural resources need to be emphasized. In many parts of Pakistan, there is a hot summer period during the cereal-based cropping cycle (between the harvest of wheat and the sowing of maize or other crop), which takes about 70-80 days of the last week of April to mid-July. The short duration legume crops such as mash bean (*Vigna mungo*) can be grown in this "summer gap" as part of a legume-cereal rotation (Arif *et al*., 2015), which can enhance the farm productivity by providing additional pulse and oilseed grain legumes and valued fodder or green manure (Shah *et al*., 2003), and by assisting symbiotic biological N_2-fixation can improve soil fertility for subsequent rotation crops (Aslam *et al*., 2003). The objectives of the study were to investigate the effect of biochar with or without chemical fertilizer on soil microbial abundance and activity and function in the rhizosphere of mash bean. Nevertheless, the information on the soil microbial response and crop productivity in response to biochar-C storage under legume fields are scarce. Therefore, assessment of the role of soil biological properties and carbon storage for higher productivity of legume in the biochar amended field will provide valuable information that can assist policy makers in implementing the environmental friendly interventions like biochar to ensure food security in arid regions of Pakistan.

Materials and methods

Site description

The present study was conducted on the research farm of PMAS-Arid Agriculture University Rawalpindi, Chakwal road (33° 1′ N to 36° 6′ N, 73° 30′ E to73° 45′ E). The soil texture is sandy loam; neutral to alkaline pH with varying moisture contents depends on rainfall**.** The average soil organic carbon is less than 1%. The climate of the site is semi-arid to sub-tropical continental, sub-humid and has a bimodal rainfall occurrence pattern, with two maxima in winter-spring periods and late summer. Rainfall is erratic, about 60-70% of the rainfall usually occurs during the monsoon season (mid-June to mid-September) (Shafiq *et al*., 2005).

Biochar

Biochar was produced by the pyrolysis of bagasse (sugarcane) in the conventional pyrolysis tank. Bagasse was air-dried and pyrolysis performed in the airtight vessel consisting of two metal barrels at 250 °C. The space between the barrels was ignited through the natural gas (one hour) while the produced charcoal was left to cool for an hour converting approximately 50% of the biomass into biochar (Gunther, 2009). For the field application, the biochar mass was crushed to pass through a 2 mm sieve, and mixed with the soil mass (Pan *et al*., 2011).

Field experiment

The field experiment was conducted with biochar soil amendment (BSA) on the carbon-equivalent basis. Three treatments of biochar were amended with and without chemical fertilizer i.e. Biochar @ 0% C ha^{-1} (B0F0); Biochar @ 0.25% C ha^{-1} (B1F0); Biochar @ 0.5% C ha^{-1} (B2F0); Biochar @ 0% C ha^{-1} + NPK (B0F1); Biochar @ 0.25% C ha^{-1} + NPK (B1F1) and Biochar @ 0.5% C ha^{-1}+ NPK (B2F1). The chemical fertilizer was applied @ of 23 kg N, 45 kg P, and 25 kg K per hectare. Treatments were assigned to field plots (1.5 m × 4.5 m) using a randomized complete block design (RCBD). Before sowing of mash bean, biochar was spread on the soil surface, thoroughly mixed with soil with a wooden rake, and then tilled to a 12 cm depth. The biochar was applied on June 27, 2013 and mash bean was planted after a week i.e., July 4, 2013. Each treatment was carried out in triplicate plots, and individual plots were separated by border rows 0.5 m in width.

Soil sampling

Rhizosphere samples were collected at crop maturity. For rhizosphere sampling, plants of each crop with root-soil systems were randomly excavated 10 cm deep from the same replicate plot. One cm thick soils tightly attached to the root system of plants were considered rhizosphere enriched soil (Butler *et al*., 2003; Liu *et al*., 2008). The samples were preserved in polythene bags and shipped to the laboratory within 3 hours after sampling. Soil samples were sieved (< 2 mm) and stored at 4 °C prior analysis. Soil samples for microbial biomass and enzyme activity were not sieved and directly stored in ice/freezer till isolation.

Biochar and soil characteristics

The water content of soil and biochar water content was determined gravimetrically (Gardner *et al*., 1991). The electrical conductivity in a saturated paste extract of soil was measured by a conductivity meter (Rhoades, 1996) and soil pH was analyzed in 1 N potassium chloride (KCl) ratio of 1:1 soil suspension (Thomas, 1996). pH and EC of biochar were measured in a 1:10 (w:v) water-soluble extracts (Cayuela *et al*., 2013). The organic carbon contents of the biochar samples were burnt to ashes in the muffle furnace at 500°C for 4 hours and calculated by using the formula described by Brake (1992).

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

Soil organic carbon (SOC) was measured by the wet digestion process by 1 N potassium dichromate ($K_2Cr_2O_7$) solution and concentrated sulphuric (H_2SO_4) acid (Nelson and Sommers 1982). For total nitrogen (TN), the digestion was carried out with sulphuric acid (H_2SO_4) and allowed to distillation process with the addition of boric acid and NaOH in the distillation chamber. Nitrogen in the distillate was

analyzed by titration against 0.01 N H_2SO_4 till the color changed from green to pink (Van Schouwenberg and Walinge, 1973).

Soil microbial abundance

Microbial structure and abundance analysis was done using a culture- independent molecular technique. Three DNA extractions of each soil sample (0.5 g) from the same replicated field plot were made using Power Soil DNA extraction kit according to the manufacturer's instructions. Relative bacterial and fungal abundances were estimated using real-time PCR (qPCR) using bacterial (16S rRNA) and fungal (ITS rRNA) primers (Bustin *et al*., 2009). The DNA concentration was measured by using nanodrop. Each qPCR reaction was carried out in a 25 µL volume having 10 ng of DNA, 0.2 mg/mL BSA, 0.2 µmol of each primer and 12.5 µL of SYBR premix EX TaqTM. The size of the PCR product was confirmed by melting curve analysis and electrophoresis in a 1.5% agarose. A plasmid having a target region of bacterial (16S rRNA) and fungal (ITS rRNA) gene was used to construct a standard curve (Fierer *et al*., 2005).

Microbial biomass

Microbial biomass carbon (MBC) was determined by the fumigation-extraction technique. Ten grams of soil was fumigated for 24 hr at 25°C with ethanol-free chloroform ($CHCl_3$), and samples were extracted with 50 mL 0.5 M K_2SO_4 for 30 min on a horizontal shaker at 200 rev min^{-1} and filtered through paper (Whatman No. 42). Similarly, 10 g soil was extracted for non-fumigation at the same time (Brookes *et al*., 1985). SOC in the extracts was measured by the titration method. Then MBC was calculated as:

Microbial biomass C = ($C_{fumigated}$ - $C_{unfumigated}$) x 2.64

Microbial biomass nitrogen (MBN) was also estimated by the fumigation extraction technique. Total N in the K_2SO_4 extract was measured after Kajeldahl digestion. After cooling, one gram of a digestion mixture ($FeSO_4$ 10: $CuSO_4$ 1: Se 0.1) and 4.5 mL of concentrated H_2SO_4 was added to each digestion tube and refluxed the mixture for 3 hours. After cooling, 20 mL of distilled water was added to the digestion tube. Then the contents were mixed thoroughly after the addition of 25 mL 10 M NaOH. The digest was moved into the steam distillation chamber of Kajeldahl by using 10 M NaOH and 2% H_3BO_3. The 40 mL of distillate collected and titrated to bluish red end point with 50 mM H_2SO_4 (Wu *et al*., 1990). The soil MBN was calculated as: Microbial biomass N = ($N_{fumigated}$ – $N_{unfumigated}$) x 1.46

Soil enzymes analysis

Dehydrogenase activity (DA) in the soils was measured by the reduction of TTC (2, 3, 5-triphenyltetrazolium chloride) into TPF (triphenyl formazan). After filtration, the optical density of the soil extract was analyzed at 546 nm wavelength on a spectrophotometer. The dehydrogenase activity (TPF µg g^{-1} dwt soil) was calculated as TPF (µg ml^{-1}) x 45/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 for the optical density of 690 nm (Kandeler and Gerber, 1988).

Statistical analysis

Differences among treatments comparing the effects of biochar, fertilization, and their interaction were analyzed using a two-way analysis of variance (ANOVA) (Statistix 8.1). The significance of difference was tested using LSD test at a level of 0.05 (Steel and Torrie, 1997).

Results

Physico-chemical properties

Biochar application significantly affects the soil physical and chemical properties with and without fertilizer (Table 1). In treatments without NPK, SOC, TN and soil moisture was increased by 23%, 27% and 55% under biochar amendment at 0.5% C (B2F0) and by 9%, 14% and 37% under biochar application at 0.25% C (B1F0) as compared to no biochar application (B0F0), respectively. However, in treatments with NPK, SOC, TN and soil moisture were enhanced by 17%, 2% and 19% under biochar application at 0.5% C (B2F1) and by 5.4%, 2% and

14% under biochar application at 0.25% C (B1F1) as compared to no biochar application (B0F1), respectively. In treatments without NPK, DOC was increased by 11% and 4% under biochar amendment at 0.5% C (B2F0) and 0.25% C (B1F0) as compared to no biochar application (B0F0), respectively. However, biochar application had no effect on soil pH and only a minimal effect on EC was observed.

Table 1. The physico-chemical properties of biochar and soil (influenced by different treatments of biochar with and without fertilizer application).

Treatments	pH	EC (dS m^{-1})	SOC (g kg^{-1})	DOC (g kg^{-1})	TN (%)	Bulk density (g cm^{-3})	Gravimetric soil moisture (%)
Biochar	6.68	0.3	497	0.46	1.4	-	-
B0F0	8.25a	0.53a	6.07b	0.46f	3.03c	1.46a	9.35b
B1F0	8.22a	0.54a	6.59ab	0.48d	3.44bc	1.41bc	12.90ab
B2F0	8.11a	0.56a	7.47a	0.51b	3.86a	1.39c	14.57a
B0F1	8.09a	0.52a	6.20b	0.47e	3.70ab	1.45ab	10.55b
B1F1	8.18a	0.56a	6.54ab	0.49cb	3.79ab	1.42abc	11.99b
B2F1	8.29a	0.54a	7.27a	0.52a	3.77ab	1.39c	12.53ab

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).

Letters in a single column indicate a statistical difference among the treatments at $P<0.05$.

EC-Electrical Conductivity, SOC-Soil Organic Carbon, DOC-Dissolved Organic Carbon, TN-Total Nitrogen.

Bacterial (16s rRNA) and fungal (18s rRNA) gene abundance

The results of biochar application with and without fertilizer application on the total bacterial gene abundance are presented in the fig. 1. The results revealed that biochar application significantly increased the 16S rRNA gene abundance in the mash bean crop. However, the fertilizer application significantly decreased the 16S rRNA gene abundance, whereas, the interactive effect of biochar and fertilizer revealed a significant increase in the gene abundance. The maximum gene abundance (4.81×10^{10}) was observed in B2F0 indicating a 132% increase, followed by B1F0 (3.30×10^{10}) showing a 59% increase as compared to B0F0.

The results of total fungal gene abundance demonstrated that biochar application significantly decreased the 18S rRNA gene abundance in mash bean crop (Fig. 1). However, the fertilizer application and the interactive effect of biochar and fertilizer significantly increased the 18S rRNA gene abundance. The lowest gene abundance (2.36×10^7) was observed in B2F0 indicating a 22% decrease without NPK fertilization as compared to B0F0. However, the highest gene abundance (3.63×10^7) was recorded in B2F1 indicating a 10% increase with NPK fertilization as compared to B0F1 (3.29×10^7).

Soil microbial activity

The biochar and fertilization interaction had a significant increase in soil urease activity in the mash bean soil (Fig. 2). The maximum UE activity (475 µg NH_4-N g^{-1} dwt $2h^{-1}$) was observed in B2F0 showing a 13% increase, followed by B1F0 (456 µg NH_4-N g^{-1} dwt. $2h^{-1}$), indicating a 9% increase without NPK fertilization over control. Similarly, maximum urease activity (501 µg NH_4-N g^{-1} dwt $2h^{-1}$) was recorded in B2F1 indicating a 7% increase followed by B1F1 (490 µg NH_4-N g^{-1} dwt $2h^{-1}$) illustrating 4% increase with NPK fertilization respectively, as compared to B0F1. Biochar application significantly increased the dehydrogenase (DE) activity in the mash bean field. The biochar and fertilization interaction had a significant effect on DE activity (Fig. 2). However, maximum DE activity (129 mg TPF kg^{-1} $24h^{-1}$) was observed in B2F0 indicating a 19% increase, followed by B1F1 (mg TPF kg^{-1} $24h^{-1}$) designating a 16% increase without NPK fertilization, as compared to B0F0.

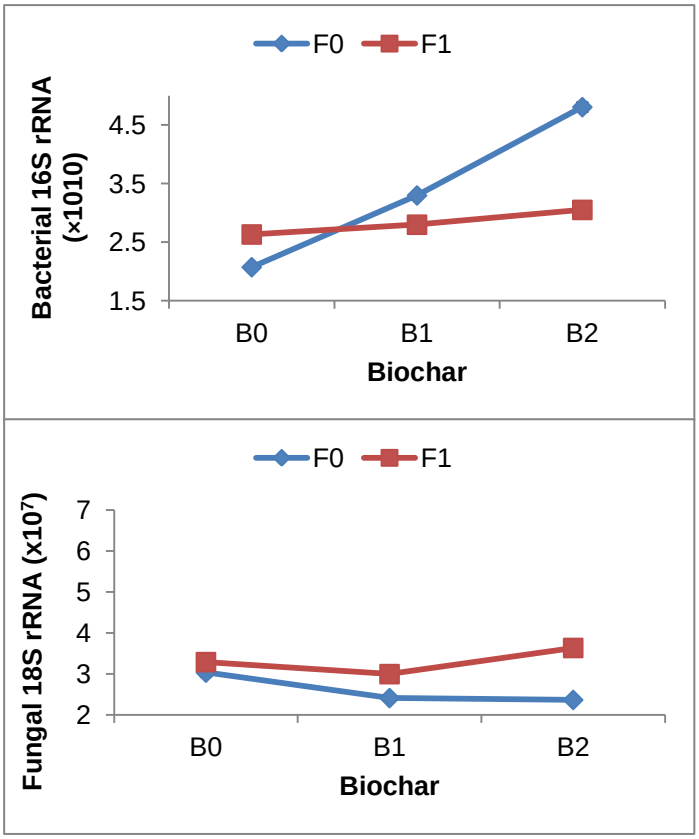

Fig. 1. Effect of biochar on total bacterial 16S rRNA (x10^{10}) and total fungal 18S rRNA (x10^{7}) in mash bean; Biochar amendment at 0, 0.25 %C and 0.5 %C ha^{-1} (B0, B1 and B2) respectively, with (F1) and without NPK fertilization (F0).

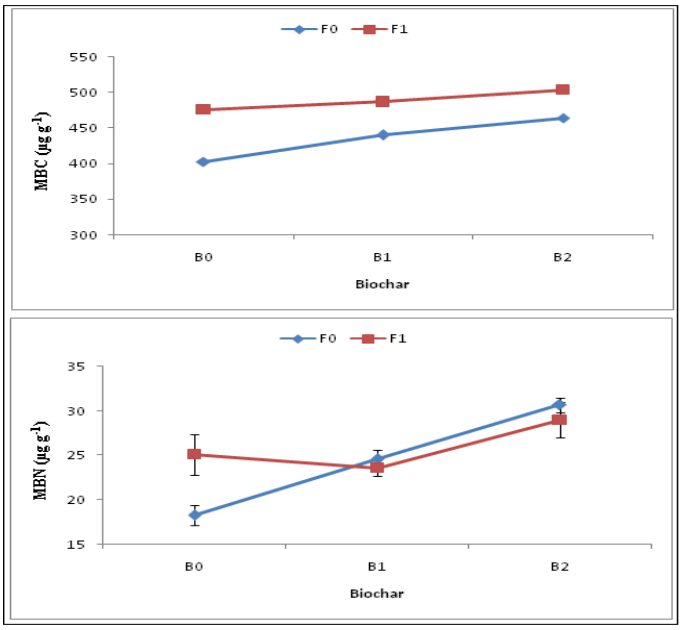

Fig. 2. Effect of biochar on MBC and MBN (µg g^{-1}) in mash bean; Biochar amendment at 0%, 0.25% C and 0.5% C ha^{-1} (B0, B1 and B2) respectively, with (F1) and without NPK fertilization (F0).

Soil microbial biomass

The interactive effect of biochar and fertilization showed significant effect on microbial biomass carbon (MBC) (Fig. 3). The maximum MBC (476 mg kg^{-1} soil) was observed in B2F0 indicating a 19% increase, followed by B1F0 (440 mg Kg^{-1} soil) showing a 9% increase without NPK fertilization, as compared to B0F0. Similarly, maximum MBC (504 mg kg^{-1} soil) was recorded in B2F1 indicating an 8% increase followed by B1F1 (487 mg kg^{-1} soil) with a 5% increase in the MBC with NPK fertilization as compared to B0F1. Similarly, the biochar and fertilizer interaction had a significant effect on the MBN (Fig. 3). The maximum MBN (30.6 µg g^{-1} soil) was observed in B2F0 indicating a 67% increase, followed by B1F0 (24.5 µg g^{-1}soil) illustrating 34% increase without NPK fertilizer as compared to B0F0. Similarly, maximum MBN (28.9 µg g^{-1}soil) was recorded in B2F1 indicating a 15% increase while B1F1 (23.6) caused a 6% decrease in MBN with NPK fertilization, as compared to B0F1.

Discussion

Biochar application has been shown to change soil physical and biochemical properties (Asai *et al.*, 2009; Major *et al.*, 2010). These changes affect soil structures (Rillig and Mummey, 2006), nutrient cycling (Steiner *et al.*, 2008) that indirectly affects the plant development (Warnock *et al.,* 2007). Different biochars have different effects in different soils and climates because biochar feedstocks come from a spectrum of materials (Gaskin *et al.*, 2010; Zwiewten *et al.*, 2010; Haefele et al., 2011). The results of our study revealed that biochar application improves the soil physical and chemical properties (Table 1), similar to the studies of Asai *et al.* (2009) and Major *et al.* (2010). However, biochar application had no effect on soil pH and EC since biochar was near neutral pH. Genesio *et al.* (2012) found that soil physical conditions change with biochar; its dark color alters thermal dynamics and leads to rapid germination, allowing more time for growth compared with controls. Biochar soil application increased the porosity and water holding capacity of soil and decreased the bulk density (Ogawa and Okimori, 2010), which promoted lateral root formation and increased the soil volume that was exploited by plant roots. In contrast, biochar application increased the SOC and TN since it contained 49% C. The higher TN might be due to reduced NO_3 leaching and NH_4 adsorption to biochar particles. Similarly, the enhanced SMC might be due to the large surface area of biochar particles and the pores available to hold water molecules which adhere to biochar particles. The results of a meta-analysis revealed that biochar applied to soil enhanced crop productivity and limited nutrient leaching (Biederman and Harpole, 2013). Blackwell *et al.* (2010) reported that banding biochar at the rate of 1 t ha^{-1} provided beneficial effects in reducing fertilizer requirement and improving crop growth. These effects may be attributed to enhanced essential nutrient and water uptake and crop water supply from increased arbuscular mycorrhizal fungal colonization during dry seasons and in low P soils, rather than through direct nutrient or water supply from biochar.

Biochar may provide a physically diverse habitat for microorganisms in soils lacking the organic matter and nutrients that are also the characteristics of the soil used in this study. The micropores of biochar may protect the microbes especially bacteria from grazing due to the smaller size (0.15-1 µm) than fungi (3-8 µm), this could play an important role in improving the soil as a microbial habitat, somewhat analogous to aggregation in more structured soils (Lehmann *et al.*, 2011). The results of MBC and MBN indicate a possible increase in microbial carbon use efficiency and a decrease in C turnover in response to biochar addition (Fig 2). The microbial biomass was higher in our study when fresh biochar was applied in mash bean crop. Few studies have revealed that even small amounts organic substrates of low molecular weight such as glucose, amino acids, root exudates might prompt a trigger response to enhance microbial activity and biomass and ,therefore, prompt 'apparent' or 'real' positive priming effects. Biochar

may contain trace quantities of water soluble low molecular organic compounds among predominantly complex C substrates, which could induce microbe's activity. However, volatile compounds present on biochar have the potential to decrease microbial biomass (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) reported that biochar amendments reduced soil microbial biomass induced by a toxicity effect.

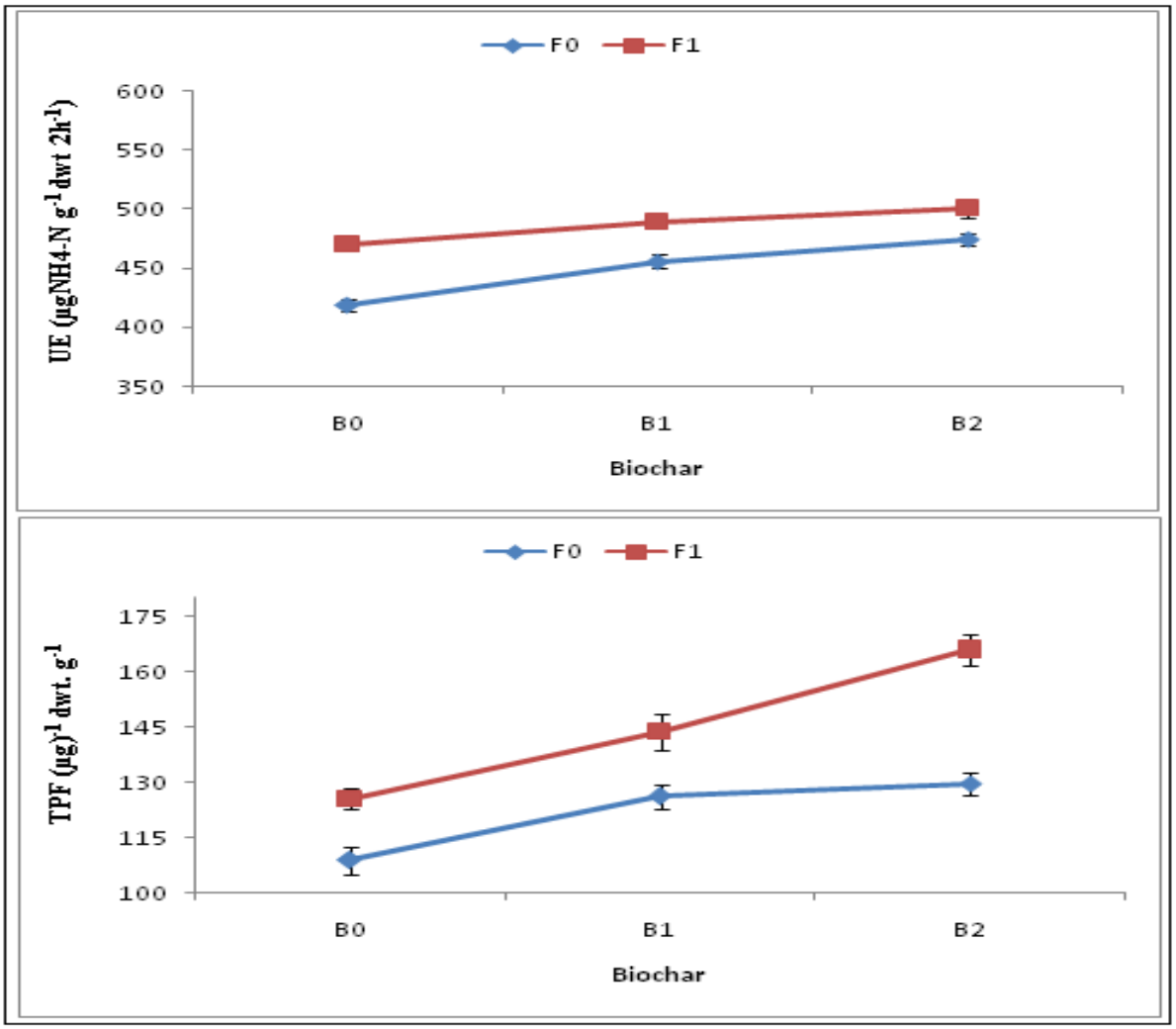

Fig. 3. Effect of biochar on urease (µgNH_4-N g^{-1} dwt. $2h^{-1}$) and dehydrogenase (mg TPF kg^{-1} $24h^{-1}$) activity in mash bean; Biochar amendment at 0, 0.25 %C and 0.5 %C ha^{-1} (B0, B1 and B2) respectively, with (F1) and without NPK fertilization (F0).

Other studies have reported that biochar has no effect on soil microbial biomass (Castaldi *et al.,* 2011) as a result of its recalcitrance (Kuzyakov *et al.,* 2009). Biochar application rates and soil type also affected response, soil microbial biomass (Lehmann *et al.*, 2011). Explanations for soil microbial biomass change in response to additions of biochar includes enhanced the availability of soil nutrients (DOC, P, Ca and K), adsorption of toxic compounds and improved soil, water and pH status, all of which influence the activity of soil microorganisms (Lehmann *et al.,* 2011). The internal porosity of biochars may help soil microorganisms avoid grazers (Pietikäinen *et al.,* 2000) and store C substrates and mineral nutrients (Saito and Muramoto, 2002; Warnock *et al.*, 2007). Similarly, the enzyme activity was boosted when

biochar was applied (Fig. 3). The dehydrogenase activity gives an indication of the positive priming effect of biochar. The results also propose that biochar contains more labile substrates which enhance the activity of soil microbes (Guenet *et al*., 2010). Our research indicates that sugarcane bagasse biochar had a significant effect on soil microbial biomass and activity in the mash bean field.

Conclusion

In conclusion, biochar application to the legume crop in arid area revealed a significant increase in the soil microbial biomass and microbial activity. Biochar application without chemical fertilizer significantly increased the bacterial 16S rRNA gene copy numbers while 18S rRNA gene copy numbers enhanced when biochar was applied in combination with chemical fertilizer. Urease and dehydrogenase activity was significantly increased with biochar applied at 0.5%-C plus NPK fertilization. The results of the study indicate that bagasse biochar application in organic carbon depleted arid soils has the potential to improve the soil function by revitalizing the microbial biomass and activity.

Acknowledgement

The research article is a part of Ph.D. research work, and the first author profoundly acknowledges the full cooperation of supervisory committee and their valuable suggestions during the whole experimental durations. Financial support from the Higher Education Commission Pakistan (PhD. Indigenous Scholarship) is also greatly acknowledged.

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