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The influence of added biochar on soil microbial biomass in a less fertile alkaline calcareous soil under different management practices

Tasneem Shah*, Muhammad Tariq and Dost Muhammad
Department of Soil and Environmental Sciences, The University of Agriculture, Peshawar-Pakistan
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Abstract

Biochar can be used as a strategy to enhance soil microbial biomass (SMB) and improve soil health. The influence of added biochar was examined on soil microbial biomass in soil under different management practices including two cropping systems and two mineral fertilizer rates. The cumulative amount of biochar added both to summer and winter crops at different annual rates over the last six years to four biochar treatments were 0, 70, 105 and 140 t ha^{-1} by summer 2019. The soil microbial biomass-C (MBC) and –N (MBN) were significantly ($p<0.05$) greater for biochar treatment relative to control during both 2018 and 2019 years. Both MBC and MBN increased with increasing levels of biochar. The rate of CO_2 evolution followed similar pattern of response to added biochar as of MBC and MBN during 2019. The rate of CO_2 evolution per mg MBC increased from 252 µg in the control to 330 µg g^{-1} soil d^{-1} in the treatment receiving 140 t biochar ha^{-1} during 2019. Moreover, all microbial attributes were significantly greater for full than half of recommended NPK doses. The effects of cropping systems on all the studied microbial attributes were non-significant during both the years. Moreover, the interactive effects of biochar and mineral fertilizers were significant for all the microbial attributes. The correlations of MBC were positive with MBN and CO_2 evolution but negative with C/N ratio in SMB suggesting that microbial activities were likely the functions of SMB and can be utilized as a predictor of microbial activity in soil. These results suggest that continuous application of biochar improves the SMB and their activity. Thus, regular application of biochar could be used as a strategy to improve soil health of a less fertile alkaline calcareous soil.

Keywords: Biochar, microbial biomass, microbial activity, soil fertility, soil health

Introduction

Biochar, which is derived from organic materials through a pyrolysis process, can be used as a strategy to enhance soil microbial biomass (SMB), microbial activity and improve soil health of degraded lands. Less fertile soils lack the necessary organic C for microorganisms and are mostly low in microbial biomass. One of the reasons of low organic C in degraded lands could be the prevailing high temperature in the arid and semi-arid climates. Organic matter decomposes quickly and cannot retain in soil for longer time where the temperature remains higher for longer period of time during the year. As a result, such soils remain low in organic matter and subsequently low in microbial biomass due to limited C supply as energy source. In order to maintain reasonable level of organic matter in soils of the arid and semi-arid regions, application of stable kind of organic materials may be encouraged. Among organic sources, biochar is rich in stable C and resistant to decomposition and persist in soil for longer even under high temperature zones. Its application is likely to continuously provide organic C to soil microorganisms and keep them active to play their role in supporting essential soil microbial processes as well as maintain good level of microbial biomass in soil. Application of biochar has been reported to enhance C sequestration in soil by mitigating the emission of CO_2 (Lehmann, 2007). Lehmann *et al*. (2011) reported that application of biochar enhanced SMB and improved nutrient cycling, which resulted in improved plant growth. Strong positive effect of biochar was also observed on soil microbial biomass-C by Zhang *et al*. (2014) in a 4-year long field experiment, but such effect was negligible on soil microbial biomass-N. Moreover, the application of biochar significantly enhanced the carbon-to-nitrogen ratio in SMB suggesting that biochar might have decreased the mineralization of biomass N.

*Email: tasneemshah92@gmail.com

However, contrasting results have been reported for the effects of biochar on SMB. The effect of biochar application on soil microbial biomass was positive and significant in some studies (e.g., Lehmann *et al*., 2011 and others), non-significant in others (Zavalloni *et al*., 2011; Castaldi *et al*., 2011) and negative in some studies (Dempster *et al*., 2012). Moreover, Steiner *et al*. (2008) reported that the size of microbial biomass was positively correlated with the amount of biochar used. Biochar type is however responsible for variable effects on soil microbial biomass and microbial activity (e.g., Steinbeiss *et al*., 2009).

Similar to soil microbial biomass, the effect of biochar on CO_2 evolution was also inconsistent. Bruun *et al*. (2012) and Yin *et al*. (2014) reported greater CO_2 efflux with the application of biochar. Deng *et al*. (2017) also found that CO_2 evolution (soil respiration) increased with increasing doses of biochar but this happened during a short (30 days) incubation period. On the other hand, Fatima *et al*. (2020) reported that the biochar-only treatments produced lower or similar CO_2 evolution relative to control treatment. CO_2 evolution exhibited inverse relationship to the biochar levels. Several researchers including Teutscherova *et al*. (2017) have reported a slow break down of added biochar in soil. Hardy *et al*. (2019) reported that the effect of biochar accumulation was negligible on soil respiration (CO_2 emission). Sagrilo *et al*. (2014) reported that CO_2 emissions was considerably increased with the additions of large amount of biochar to soil, but a low input of biochar did not significantly affect CO_2 emissions. In another study, Sagrilo *et al*. (2014) reported that the application of large amount of biochar during a longer incubation period (>200 days) rather negatively affected the average CO_2 evolution. The contrasting effects of biochar could be associated with its amount and quality, and on soil properties (Lehmann *et al*., 2011). Biochar rich in protein and sugars (prepared at low pyrolysis temperature) exhibited greater mineralization rates and CO_2 emission, and that produced at higher pyrolysis temperature showed lower C mineralization and CO_2 emissions (Fabbri *et al*., 2012).

Variable rates of biochar breakdown therefore could also be associated with the pyrolysis temperatures of biochar preparation. A low pyrolysis temperature-biochar produced greater CO_2 evolution in soil (Fatima *et al*., 2020) while that produced at high pyrolysis temperature are relatively stable and produce low CO_2 evolution (Hailegnaw *et al*., 2019). Nevertheless, the effects of biochar on soil microbial biomass dynamics is still unclear. This paper reports the effects of biochar on soil MBC, MBN and CO_2 emission measured once before sowing of summer crops in July 2018 and second after harvest of summer crops in September 2019 within a 6-year long rotation experiment on an alkaline calcareous less fertile soil.

Materials and Methods

Site characterization and experimental details

The effect of added biochar on soil microbial biomass and microbial activity was examined within a 6-year long crop rotation experiment under different management practices at the research farm of Agriculture University, Peshawar, Pakistan (34.01° N, 71.50° E). Cropping systems chosen for this study were maize-wheat and mungbean-wheat. Mineral fertilizer treatments were full and half NPK. Full NPK means the one which received full recommended doses of NPK fertilizers by each crop in each season whereas half NPK means receiving half of the recommended doses of NPK. Recommended doses of NPK used for wheat were 120:90:60 and maize were 150:100:60 kg ha^{-1}. There were four biochar treatments receiving different levels of biochar in each season. The cumulative amount of biochar received by summer 2019 were 0 (T1), 70 (T2), 105 (T3) and 140 (T4) t ha^{-1}. The trial was organized in a RCB design with split-split plot arrangement having four replications and 64 treatment plots altogether. Cropping systems were placed to main plots, mineral fertilizers to sub-plots and biochar levels to sub-sub-plots in the experiment. Mineral fertilizers used were urea for N, single superphosphate for P and potassium chloride for K in each season. Biochar used in the study was prepared from *Acacia* sp using a pyrolysis temperature of about 400 ^{o}C and was obtained from the market. The biochar used in the experiment had pH, 7.1; EC (dS m^{-1}), 2.1; total N (g kg^{-1}), 11.2; total P (g kg^{-1}), 12.1; Ca (g kg^{-1}), 2.7 and Mg (g kg^{-1}), 12.0.

The soil of the experimental site was silty clay loam, alkaline (pH 8.2), strongly calcareous (17.2%), non-saline (<1.98 dS m^{-1}), and low in soil fertility (OM <1.0%; TN, 0.07%; extractable P, 3.0 mg kg^{-1} soil) and classified as Pirsabak soil series (Shafi *et al*., 2007). The mean annual rainfall in the region is about 380 mm year^{-1} and classified as semi-arid agro-climatic conditions. The rainfall however varies considerably from season to season. February and September are wet months with peak rainfall mostly occurs in February to April and in July to September. October to January is mostly dry. The hot season normally ranges from mid-May to mid-September with a mean daily high temperature over 97 ^{o}F (36 ^{o}C). The cool season occurs from early December to early March with a mean daily high temperature below 69 ^{o}F (20 ^{o}C).

Soil samples at 0-15 cm depth were collected from all treatment plots of the experiment for this study once just before planting summer crops (maize, mungbean) in July 2018 and second after harvest of summer crops in September 2019. Wheat-mungbean and wheat-maize cropping systems were selected in this study. The sowing and harvesting details of maize, mungbean and wheat crops in crop rotation experiments are given elsewhere (Shah *et al*., 2021). Each treatment plot was randomly sampled from 10-15 spots and samples of the same treatment plot was composited. Soil samples were processed immediately after collection for measurement of microbial biomass and microbial activity. After removing plant residues and other debris, the samples were sieved (2 mm sieve) while still moist. After proper homogenization of soil samples of individual treatment plots, the samples were stored in a safe place until ready to use for measurement of microbial attributes.

Measurement of CO_2 efflux

The CO_2 efflux in soil samples was measured by the technique as described in Shah *et al*. (2010). For this measurement, duplicate moist soil sample of 50 g was taken in a 500-mL conical flask. Five mL of 0.3 *M* NaOH solution was taken in a vial and suspended in the centre of the flask. The flasks were air-tightly sealed using rubber bungs and placed in the incubator at 25 ºC for 2 days. After incubation, the vials were carefully taken out of the incubated flasks and the NaOH solution transferred to another clean conical flask and titrated against 0.1 N HCl in the presence of $BaCl_2$ solution and phenalpthalien indicator till the disappearance of pink colour. The amount of HCl used in the titration was taken as a measure of CO_2 evolved during the incubation period.

Determination of microbial biomass-C and -N

Fumigation technique of Brookes *et al*. (1985) as explained in Horwath and Paul (1994) was used to determine MBC and MBN in soil. For these measurements, soil sample was split in two equal parts. Each part was placed in separate desiccator. One desiccator was fumigated and other un-fumigated. Both fumigated and un-fumigated desiccators were kept in the dark for 24 hrs. After incubation, the fumigated desiccators were thoroughly evacuated of chloroform. Both fumigated and un-fumigated soil samples were run for CO_2 measurement after 10 days of incubation. Microbial biomass-C was calculated by subtracting CO_2 produced in the un-fumigated samples from CO_2 produced in the fumigated samples as described in Horwath and Paul (1994). For microbial biomass-N, the same soil samples were run for total mineral N and microbial biomass-N was calculated as described in Horwath and Paul (1994).

Determination of mineral N in soil samples

For determination of mineral N in soil, soil sample was shaken with KCl in a shaking bottle using horizontal shaker for one hour (Mulvaney, 1996). After filtering the suspension, 20 mL of the filtrate was distilled with 0.2 g each of MgO and Devarda's alloy into mixed indicator solution followed by distillation with standard HCl solution till reaching the end point. The amount of HCl used in titration was used as a measure of the amount of mineral N present in the sample.

Statistical analysis

The data were analysed statistically using a statistical package Statistix 8.1 following the principles described in Steel *et al*. (1997). LSD test was applied to determine statistical differences between different experimental treatments at 5% level of probability.

Results and Discussion

An experiment was conducted to examine the effect of biochar addition, mineral fertilizers and cropping systems on soil microbial biomass in a 6-year long field experiment. The surface soil samples were collected before sowing of summer crops (maize and mungbean) in June 2018 and after harvest of summer crops in September 2019, and analysed for microbial biomass-C and –N. Also, CO_2 efflux evolved per mg biomass-C per day was calculated and the results obtained are presented and discussed below:

Soil microbial biomass-C

The results obtained on soil microbial biomass-C (MBC) as affected by added biochar and other management practices showed that MBC was significantly (p<0.05) greater in biochar than in the control during both 2018 and 2019 (Table 1). The response of MBC to biochar amendment was similar in both years. These results indicated that MBC increased with increasing levels of biochar. The highest MBC of 405 µg g^{-1} soil was obtained with the highest added biochar compared with 254 µg g^{-1} soil in the no biochar treatment. The data further revealed that differences in MBC were also significant (p<0.05) between the full and half NPK treatments during both years (Table 1). The MBC was significantly greater for full NPK treatment compared with half NPK treatment in both years. On an average, the highest MBC of 335 µg g^{-1} soil was obtained for treatment receiving full NPK compared with 314 µg g^{-1} soil in the half NPK treatment.

Table 1: The influence of added biochar, mineral fertilizers and cropping systems on microbial biomass C measured in soil samples collected once before sowing of summer crops in June 2018 and second after harvest of summer crops in September 2019 from a 5-6 years long field experiment

Treatment			2018	2019	Mean
Biochar (BC)	BC Level[$] (t ha^{-1}) by		Microbial biomass C (μg g^{-1} soil)		
	2018	2019			
	0	0	249d	259d	254
	50	70	298c	320c	309
	90	105	318b	342b	330
	130	140	383a	426a	405
	Significance		*	*	
Mineral fertilizers (MF)	½ NPK[$$]		299b	330b	314
	Full NPK		325a	344a	335
	Significance		*	*	
Cropping systems (CS)	Maize-wheat		299	331	315
	Mung-wheat		325	343	334
	Significance		*	ns	
Interactions					
	BC x MF		*	*	
	BC x CS		ns	ns	
	MF x CS		ns	ns	
	BC x MF x CS		ns	ns	

[$]Biochar levels are cumulative amount added both to summer and winter crops at different annual rates over the last six years; [$$] ½ NPK means half of the recommended dose of NPK while full NPK means full recommended dose of NPK fertilizers for the given crops in each season.

Table 2: The influence of added biochar, mineral fertilizers and cropping systems on microbial biomass N measured in soil samples collected once before sowing of summer crops in June 2018 and second after harvest of summer crops in September 2019 from a 5-6 years long field experiment

Treatment			2018	2019	Mean
Biochar (BC)	BC Level[$] (t ha^{-1}) by		Microbial biomass N (μg g^{-1} soil)		
	2018	2019			
	0	0	13.10c	15.19b	14.15
	50	70	20.41b	25.61a	23.01
	90	105	19.28b	23.61a	21.45
	130	140	28.42a	23.61a	31.04
	Significance		*	*	
Mineral fertilizers (MF)	½ NPK[$$]		19.23b	23.04b	21.14
	Full NPK		21.38a	26.00a	23.70
	Significance		*	*	
Cropping systems (CS)	Maize-wheat		19.98	23.89	21.94
	Mung-wheat		20.38	25.14	22.88
	Significance		Ns	ns	
Interactions					
	BC x MF		*	ns	
	BC x CS		Ns	ns	
	MF x CS		Ns	ns	
	BC x MF x CS		Ns	ns	

[$]Biochar levels are cumulative amount added both to summer and winter crops at different annual rates over the last six years; [$$] ½ NPK means half of the recommended dose of NPK while full NPK means full recommended dose of NPK fertilizers for the given crops in each season

The data revealed that in both years, MBC was greater in soil under mung-wheat than under maize-wheat cropping system (Table 1). However, differences in MBC among the two cropping systems were significant during 2018 but non-significant during 2019. On an average, the maximum MBC of 334 μg g^{-1} soil was obtained for soil under mung-wheat compared with 315 µg g^{-1} soil for treatment under maize-wheat cropping system. The interactive effects of biochar and mineral fertilizers on MBC were significant during both 2018 and 2019 but all other interactions were statistically non-significant (Table 1). It was noticed that the MBC was remarkably greater for full NPK compared to ½ NPK treatment in soil receiving the highest levels of biochar.

Soil microbial biomass-N

Like MBC, the MBN was also significantly (p<0.05) greater in biochar relative to control treatment during both 2018 and 2019 (Table 2). The response of MBN to biochar amendment was almost similar in both years. On an average, the maximum MBN of 31.04 µg g^{-1} soil was obtained in soil receiving the highest level of biochar compared with 14.15 µg g^{-1} soil in the control treatment. However, differences in MBN among various biochar treatments were generally non-significant except in 2018 where significantly greater MBN were recorded for treatment receiving the highest level of biochar (130 t ha^{-1}) compared with other two lower levels of biochar (50 and 90 t ha^{-1}).

The data further revealed that differences in MBN were also significant (p<0.05) between the full and half NPK treatments during both 2018 and 2019 (Table 2). The MBN was significantly greater for full NPK treatment compared with half NPK treatment during both the years. On an average, the maximum MBN of 23.70 µg g^{-1} soil was obtained for treatment receiving full NPK compared with 21.14 µg g^{-1} soil for that receiving half NPK fertilizers. Moreover, the differences in MBN among the two cropping systems (maize-wheat and mung-wheat) were statistically non-significant (p<0.05) during both years (Table 2). It was observed that the average MBN was 23.70 µg g^{-1} soil for treatment under mung-wheat compared with 21.14 µg g^{-1} soil for treatment under maize-wheat cropping system. However, the interactive effect of biochar and mineral fertilizers on MBN was significant only during 2018. All other interactions for MBN were statistically non-significant during both years (Table 2). It was noticed that the MBN in year 2018 was greater for full NPK than ½ NPK treatment in soil receiving the highest levels of biochar.

The changes in SMB indicates changes in microbial growth and decomposition of soil organic matter. Our results are similar to the findings of Kolb *et al*. (2008), O' Neill *et al*. (2009) and Liang *et al*. (2010) who also observed positive effects of biochar on SMB as in our study where both MBC and MBN increased with increasing levels of biochar amendment. In case of Steiner *et al*. (2008), the magnitude of SMB was positively correlated with the amount of biochar used. In contrary to our results, the

Table 3: The influence of added biochar, mineral fertilizers and cropping systems on carbon-to-nitrogen (C/N) ratio in soil microbial biomass measured in soils collected once before sowing of summer crops in June 2018 and second after harvest of summer crops in September 2019 from a 5-6 years long field experiment

Treatment			**2018**	**2019**	**Mean**
Biochar (BC)	**BC Level$^{\$}$ (t ha^{-1}) by**		**C/N ratio in soil microbial biomass**		
	2018	**2019**			
	0	0	19.2a	17.6a	18.4
	50	70	16.7b	14.9b	15.8
	90	105	13.6d	12.9c	13.3
	130	140	14.9c	12.9c	13.9
	Significance		*	*	
Mineral fertilizers (MF)	½ NPK$^{\$\$}$		16.3	15.3a	15.8
	Full NPK		15.9	13.9b	14.9
	Significance		**ns**	*	
Cropping systems (CS)	Maize-wheat		15.8	14.8	15.3
	Mung-wheat		16.5	14.5	15.5
	Significance		ns	ns	
Interactions					
	BC x MF		ns	ns	
	BC x CS		ns	ns	
	MF x CS		ns	ns	
	BC x MF x CS		ns	ns	

$^{\$}$Biochar levels are cumulative amount added both to summer and winter crops at different annual rates over the last six years; $^{\$\$}$ ½ NPK means half of the recommended dose of NPK while full NPK means full recommended dose of NPK fertilizers for the given crops in each season.

effects of biochar on SMB were either non-significant (Zavalloni *et al*., 2011; Castaldi *et al*., 2011) or even negative (Dempster *et al*., 2012). In other study, the effect of biochar was positive and much stronger on MBC than on MBN (Zhang *et al*., 2014). The variable effects of biochar could be associated with the type of biochar used in the experiment as biochar prepared at low temperature exhibits totally different impact relative to the one produced at high temperature (Steinbeiss *et al*., 2009; Lehmann and Joseph, 2009).

Carbon to nitrogen (C/N) ratio in soil microbial biomass

Our data showed that the C/N ratios in SMB were significantly affected by the added biochar during both 2018 and 2019 (Table 3). During both years, the C/N ratios in SMB decreased with increasing biochar levels. On an average, the lowest C/N ratio in SMB of 13.9 was obtained in soil receiving the highest level of biochar compared with 18.4 in the control treatment.

The data further revealed that differences in C/N ratios of MBC between the full and half NPK treatments were not significant in 2018 but significant in 2019 (Table 3). On an average, the lowest C/N ratio in SMB of 14.9 was obtained for treatment receiving full NPK compared with 15.8 for that receiving half NPK fertilizers. Moreover, the C/N ratios in SMB were not affected significantly by the cropping systems. The interactive effects of biochar, mineral fertilizers and cropping systems or of any two of them on C/N ratios in SMB were statistically non-significant during both years (Table 3).

Changes in the C/N ratio of SMB may affect the availability of carbon and nitrogen to microorganisms, as well as the microbial composition in soil. We observed that the C/N ratios in SMB decreased significantly with increasing levels of biochar applied. In contrary to our findings, Zhang *et al*. (2014) observed significantly greater C/N ratio in microbial biomass in biochar treatments relative to control. On the other hand, Dempster *et al*. (2012) observed no significant effect of biochar on microbial biomass C/N ratio. The C/N ratio in soil microbial biomass could be associated with the type of C present in biochar and other organic matters. Nicolardot *et al*. (2001) observed that C/N ratio in soil microbial biomass was positively correlated with the C/N ratio of added organic matter. Kushwaha *et al*. (2000) on the other hand observed a decrease in C/N ratio of soil microbial biomass with the addition of organic material with wide C/N ratio. However, Kallenbach and Grandy (2011) found that the effect of addition of organic C on C/N ratio in soil microbial biomass was not significant. This suggests that organic amendments

Table 4: The influence of added biochar, mineral fertilizers and cropping systems on rate of CO_2 efflux measured in soils collected once before sowing of summer crops in June 2018 and second after harvest of summer crops in September 2019 from a 5-6 years long field experiment

Treatment			2018	2019	Mean
Biochar (BC)	**BC Level[$] ($t\ ha^{-1}$) by**		$\mu g\ CO_2\ g^{-1}\ soil\ d^{-1}$		
	2018	**2019**			
	0	0	259a	66c	163
	50	70	205b	97b	151
	90	105	149c	104b	126
	130	140	142c	139a	141
	Significance		*	*	
Mineral fertilizers (MF)	½ NPK[$$]		187	88b	138
	Full NPK		190	115a	153
	Significance		**ns**	*	
Cropping systems (CS)	Maize-wheat		183	96	140
	Mung-wheat		194	106	150
	Significance		ns	ns	
Interactions					
	BC x MF		ns	ns	
	BC x CS		ns	ns	
	MF x CS		ns	ns	
	BC x MF x CS		ns	ns	

[$]Biochar levels are cumulative amount added both to summer and winter crops at different annual rates over the last six years; [$$] ½ NPK means half of the recommended dose of NPK while full NPK means full recommended dose of NPK fertilizers for the given crops in each season.

may not be solely responsible for increase or decrease in the C/N ratio of SMB. Lehmann *et al*. (2011) and several others observed that biochar application caused remarkable change in soil microbial composition and their activities. The lower C/N ratios in SMB with biochar application suggests greater availability of nitrogen for microorganisms in soil. Zhang *et al*. (2014), however, reported that biochar application significantly increased the microbial biomass C/N ratios in SMB which is contrary to our results. The variable effects of biochar as indicated earlier could be associated with the type of biochar used in different studies.

CO_2 efflux

The results obtained on CO_2 efflux from soil under maize-wheat and mung-wheat cropping systems receiving different levels of biochar and mineral fertilizers in a crop rotation experiment during the last 5-6 years are presented in Table 4. The results on CO_2 efflux with respect to response of biochar were inconsistent during 2018 and 2019. The data showed that during 2018, the CO_2 evolution was significantly ($p<0.05$) greater in the control treatment relative to the biochar treatments, and the lowest amount of CO_2 was recorded for treatment receiving the highest amount of biochar. In contrary, in 2019, the highest amount of CO_2 was produced in treatment receiving the highest level of biochar whereas the lowest amount of CO_2 was recorded in the control treatment. The CO_2 produced in all biochar treatments was significantly ($p<0.05$) greater than in the control treatment during 2019. The reason for this controversy could not be explained. There seems no logic of greater CO_2 in the control treatment which received no biochar and produced lowest crop biomass, we therefore focused on the results of 2019 in this paper. There is a clear trend in CO_2 production during 2019 i.e., increased with increasing levels of added biochar. In 2019, the CO_2 production increased from 66 µg in the control to 139 µg g^{-1} soil d^{-1} with the cumulative amount of 140 t ha^{-1} biochar.

The data further revealed that differences in CO_2 evolution between the full and half NPK treatments were statistically non-significant during 2018 but significant during 2019 (Table 4). The CO_2 evolution was greater for full NPK than half NPK treatment during both years but the differences were significant only in 2019. On an average, the maximum CO_2 evolution of 153 µg g^{-1} soil d^{-1} was obtained for treatment receiving full NPK compared with 138 µg g^{-1} soil d^{-1} for that receiving half NPK fertilizers.

The data revealed that differences in CO_2 evolution among the two cropping systems (maize-wheat and mung-wheat) were statistically non-significant ($p<0.05$) during both the years (Table 4). It was observed that the average CO_2 evolution was 150 µg g^{-1} soil d^{-1} for treatment under mung-wheat compared with 140 µg g^{-1} soil d^{-1} for treatment under maize-wheat cropping system. The interactive effects of biochar, mineral fertilizers and cropping systems, or of any two of them on CO_2 evolution were statistically non-significant during both 2018 and 2019.

This study has shown that the evolution of CO_2 was greater for treatments receiving higher doses of biochar which could be due to the presence of more C input in the form of biochar. Bruun *et al*. (2012) and Yin *et al*. (2014) also reported greater CO_2 efflux associated mainly to mineralization of C content of biochar. Our results were also in line with the results of Deng *et al*. (2017) and Shah *et al*. (2017) who found that CO_2 evolution (soil respiration) increased with increasing doses of biochar but this happened during a short incubation period. Contrary to our results, Fatima *et al*. (2020) suggested that the biochar-only treatments produced lower or similar CO_2 evolution compared with the control treatment. Furthermore, CO_2 evolution exhibited inverse relationship to the biochar levels. Several researchers including Teutscherova *et al*. (2017) have reported a slow break down of added biochar in soil. Hardy *et al*. (2019) also reported that biochar had limited influence on soil respiration (CO_2 emission). On the other hand, Sagrilo *et al*. (2014) reported that CO_2 emissions was considerably increased with the additions of large amount of biochar to soil. However, application of low level of biochar did not significantly affect CO_2 emissions relative to control. Lehmann *et al*. (2011) reported that the effect of biochar application on soil microbiological properties depends on the amount and quality of biochar used as well as on the soil properties. Biochars rich in protein and sugars prepared at low pyrolysis temperature exhibited greater mineralization rates and CO_2 emission, and that produced at higher pyrolysis temperature showed lower C mineralization and CO_2 emissions (Fabbri *et al*., 2012). However, Sagrilo *et al*. (2014) suggested that the effect of biochar on CO_2 emission is for the short-term as in the longer incubation period, the effect of biochar on CO_2 emission was either negligible or depressive.

Variable rates of biochar breakdown therefore could be associated with the pyrolysis temperatures of biochar preparation. A low pyrolysis temperature biochar was used in this study and could be responsible for greater CO_2 evolution. Fatima *et al*. (2020) also observed similar results obtaining greater CO_2 evolution with biochar amendments. Hailegnaw *et al*. (2019) reported that biochars produced at high pyrolysis temperature are relatively stable and the C is not readily available to microbes resulting in slow organic matter decomposition and low CO_2 evolution. We further observed that the influence of biochar on CO_2 evolution varied with the cropping systems. In the control or at low biochar treatment, the evolution of CO_2 was greater in soil which was under mungbean relative to maize crop, but at high biochar dose, the CO_2 evolution was greater with maize compared with mungbean crop. This shift in CO_2 evolution with biochar doses could be associated with different labile pools of C in mung and maize residues. The greater CO_2 evolution in soil under mungbean based cropping system could be due to the presence of greater amount of labile C in the mungbean crop residues relative to maize crop residues.

Table 5: The influence of added biochar, mineral fertilizers and cropping systems on ratio of CO_2 efflux and microbial biomass C (MBC) in soil samples taken before sowing of summer crops in June 2018 and after harvest of summer crops in September 2019 from a 5-6 year long field experiment

Treatment			2018	2019	Mean
Biochar (BC)	**BC Level$ (t ha^{-1}) by**		**µg CO_2 mg^{-1} MBC d^{-1}**		
	2018	**2019**			
	0	0	1056a	252b	654
	50	70	689b	305a	497
	90	105	467c	307a	387
	130	140	372d	330a	351
	Significance		*	*	
Mineral fertilizers (MF)	½ NPK$$		671	265b	468
	Full NPK		621	331a	476
	Significance		**ns**	*	
Cropping systems (CS)	Maize-wheat		662	290	476
	Mung-wheat		630	307	469
	Significance		ns	ns	
Interactions					
	BC x MF		*	ns	
	BC x CS		*	ns	
	MF x CS		ns	ns	
	BC x MF x CS		ns	ns	

$Biochar levels are cumulative amount added both to summer and winter crops at different annual rates over the last six years; $$ ½ NPK means half of the recommended dose of NPK while full NPK means full recommended dose of NPK fertilizers for the given crops in each season.

Table 6: Pearson correlations among various microbial attributes measured in surface soil (0-15 cm depth) collected once before sowing of summer crops in June 2018 and second after harvest of summer crops in September 2019 from a 5-6 year long field experiment under different management practices (n= 64). Management practices include cropping systems, mineral fertilizers and biochar amendments

	MBC	MBN	C/N ratio in MB
MBN	0.87		
C/N ratio in MB	-0.52	-0.84	
CO_2 evolution	0.52	0.54	-0.39

Note: MC = microbial biomass C; MN = microbial biomass N; CN = C/N ratio in microbial biomass; CO_2 = ug CO_2 g^{-1} soil d^{-1}.

CO_2 efflux in relation to MBC

The results obtained on amount of CO_2 produced per mg of MBC in soil under maize-wheat and mung-wheat cropping systems receiving different levels of biochar and mineral fertilizers in a crop rotation experiment during the last 5-6 years are presented in Table 5. Like total CO_2 efflux (Table 4), the amount of CO_2 produced per mg of MBC with respect to response of biochar were also inconsistent during 2018 and 2019. The data showed that during 2018, the CO_2 evolution per mg of MBC was significantly ($p<0.05$) greater in the control treatment relative to the biochar treatments, and the lowest amount of CO_2 was recorded for treatment receiving the highest amount of biochar. In contrary, in 2019, the highest amount of CO_2 per mg of MBC was produced in treatment receiving the highest level of biochar whereas the lowest amount of CO_2 was recorded in the control treatment. The CO_2 produced per mg of MBC in all biochar treatments was significantly ($p<0.05$) greater than in the control treatment during 2019. The reason for this controversy was hard to explain, so we focussed more on the results of 2019. Like total CO_2 efflux (Table 4), the daily CO_2 production per mg of MBC during 2019 increased with increasing levels of added biochar. In

2019, the daily CO_2 production per MBC increased from 252 µg g^{-1} soil d^{-1} in the control to 330 µg g^{-1} soil d^{-1} with the cumulative amount of 140 t ha^{-1} biochar.

The data further revealed that differences in CO_2 evolution per mg of MBC between the full and half NPK treatments were statistically non-significant (p<0.05) during 2018 but significant during 2019 (Table 5). The CO_2 evolution per MBC was greater for full NPK than half NPK treatment during both the years but the differences were significant only during 2019. On an average, the maximum CO_2 evolution per MBC of 476 µg g^{-1} soil d^{-1} was obtained for treatment receiving full NPK compared with 468 µg g^{-1} soil d^{-1} for that receiving half NPK fertilizers.

The data revealed that differences in CO_2 evolution per mg MBC among the two cropping systems (maize-wheat and mung-wheat) were statistically non-significant (p<0.05) during both the years (Table 5). It was observed that the average CO_2 evolution per mg of MBC was 469 µg g^{-1} soil d^{-1} for treatment under mung-wheat compared with 476 µg g^{-1} soil d^{-1} for treatment under maize-wheat cropping system. The interactions between biochar and mineral fertilizers or biochar and cropping systems for CO_2 evolution per mg of MBC were statistically significant during 2018. All other interactions were statistically non-significant.

Correlations among soil microbial attributes

The data obtained on Pearson correlations among various soil microbial attributes are presented in Table 6. The data exhibited that soil microbial biomass C was positively correlated with microbial biomass N (r = 0.87) and CO_2 evolution (r = 0.52). It appeared that the correlation between MBC and MBN were much stronger than between MBC and CO_2 evolution. The data further revealed that correlation between MBN and C/N ratios in SMB were negative (r = -0.84) and that between MBN and CO_2 evolution were positive (r = 0.54).

These results suggested that microbial activities (CO_2 evolution) were likely the functions of soil microbial biomass. Thus, soil microbial biomass can be utilized as a predictor of microbial activity in soil. Stromberger *et al.* (2011) however, suggested that microbial biomass cannot be considered as the sole predictor of microbial activity in soil. Sakamoto and Oba (1994) found positive correlation between CO_2 evolution and total SMB. However, Sato and Seto (1999) observed no correlation between the rates of CO_2 evolution and the amount of microbial biomass-C, but the rates of CO_2 evolution were highly correlated with the amounts of dissolved organic C.

Conclusion

Our study has shown that after 6 consecutive years of application, biochar addition significantly increased the soil microbial biomass-C and -N as well as CO_2 emission compared to the control treatment. The effect of biochar application increased with increasing level of biochar addition. Biochar treatments exhibited the lowest value of soil microbial biomass C/N ratio suggesting that biochar could have increased the fraction of biomass N mineralized.

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