Research Article

Biochar Application Improves Soil Respiration and Nitrogen Mineralization in Alkaline Calcareous Soil under Two Cropping Systems

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Abstract | Biochar can be used as a strategy to facilitate C and N sequestration in less fertile soil. Surface soil samples from a 4 years long field experiment were collected to assess the effect of added biochar (0, 50, 90 and 130 t ha^{-1}) and chemical fertilizers (viz., urea for N, single superphosphate for P and potassium chloride for K) on soil respiration and N mineralization during lab incubation experiment under wheat-mungbean and wheat-maize cropping systems. The rate of CO_2 evolution increased significantly (P<0.05) with increasing level of added biochar during each incubation period. At day 2, the rate of CO_2 evolution increased from 142 µg CO_2 g^{-1} soil d^{-1} in the control to 259 µg CO_2 g^{-1} soil d^{-1} in the treatment receiving 130 t biochar ha^{-1}. The same trend continued during 4, 8 and 16 days of incubation periods. However, the rate of CO_2 evolution declined over time in each treatment. The effect of mineral fertilizers and cropping systems on CO_2 evolution were statistically non-significant (P<0.05). The response of cumulative CO_2 production to biochar, mineral fertilizers and cropping systems was almost similar to rate of CO_2 production. The data on net N mineralization revealed that maximum N mineralization was observed for the treatment which had received biochar at 90 t ha^{-1} over the last few years whereas the differences between the control (no biochar), lower biochar (50 t ha^{-1}) treatments and highest biochar (130 t ha^{-1}) treatment were statistically non-significant during 7, 14 and 28 days of incubation periods. Nitrogen mineralization did not vary significantly among the mineral fertilizers or cropping systems treatments. Moreover, the interactive effects of cropping systems x biochar, and cropping systems x mineral fertilizers were significant for CO_2 efflux and N mineralization at least during the first 2 days of incubation period. These results suggest that biochar amendment enhanced CO_2 evolution during short-term incubation period (i.e., 16 days) suggesting the availability of labile C pool in the added biochar which serve as a source of energy for promotion of microbial activity in the soil. The low net N mineralization in the high biochar treatment implies that biochar could be used as a strategy to minimize N losses from the easily available labile N pools through greater N retention in soil.

Introduction

Soil respiration and nitrogen mineralization are important microbiological processes and are the key indicators of soil health. Soil respiration is

a microbiological process where microorganisms decomposes organic matter and releases carbon in the form of carbon dioxide (CO_2). Soil respiration produces a major flux of CO_2, emitting over 10 times more CO_2 per year to the atmosphere than combustion

of fossil fuel as reported in Phillips and Nickers (2015). Soil respiration thus contribute substantially to the green house gases in the atmosphere in the form CO_2. It also indicates the level of soil fertility and microbial activity occurring in the soil. More CO_2 evolution means more organic matter and its decomposition in the soil. Thus, soils with greater soil respiration is an indicator where the soils are more fertile and active in microbial action and more supportive to plant growth and all kinds of soil life.

Nitrogen mineralization is another important process where unavailable organic nitrogen is converted into plant available nitrogen as ammonium (NH_4) and nitrate (NO_3) through the action of soil microorganisms. Since plants can only absorb nitrogen in the form of NH_4 and NO_3, N mineralization contributes significantly to support crop growth. Large amount of nitrogen in soil is present in organic form (organic matter, crop residues, manures etc.) but become available only after mineralization. Depending on various factors, the release of mineral N from organic N may be predicted. It is therefore important to understand such prediction in a given growing season as it is necessary to calculate correct doses of fertilizers application. Knowing the rate of nitrogen mineralization from organic amendments including manures, crop residues, organic wastes and soil organic matters during a given growing season would enable you to predict correct dose of fertilizer N application. In this way over or lower application of fertilizer N can be avoided. According to a report in Barker (2011), N mineralization may contribute from 15-50 kg N ha^{-1} in the top 0-15 cm of soil during a typical growing season. For soils with high organic matter contents under no-till and following legumes based crop system, the contribution of N from mineralization could be much greater than 50 kg ha^{-1}.

Soil respiration and N mineralization are generally limited in soils low in organic matter. Application of organic amendments promotes both the processes. Among organic amendments, biochar contain carbon which is resistant to decomposition and can provide a long-term support to soil microorganisms. Biochar is thus promoted as a soil amendment to enhance soil microbial processes necessary for maintaining and improving soil quality. Biochar is also seen as a source of long-term sequestration of carbon that help increasing soil quality and reduce CO_2 evolution to the atmosphere (Laird, 2008; Novak et al., 2010). The

biochar can influence C and N losses by controlling their cycles and can improve physicochemical properties of soils (Lehmann et al., 2011). Ameloot et al. (2013) reported that mineralization activity was higher during the first few days after the biochar amendment after which the respiration rates become similar to non-amended soil. The effect of biochar however varies with the type of biochar depending on how biochar is prepared (Amonette et al., 2008). Although biochar application to soils has a significant influence on soil respiration and emission of other gases (N_2O and CH_4), there have been no consistent results regarding the response of such processes to biochar application. Biochar amendments resulted in enhanced evolution of CO_2 in some studies as of Kammann et al. (2012), Zheng et al. (2012), Shah et al. (2017) and others. The results of Rondon et al. (2005) and several others (Liu et al., 2011; Dempster et al., 2012; Fabbri et al., 2013; Malghani et al., 2013) revealed that biochar amendments decreased CO_2 emission from soil. In addition to CO_2 evolution, biochar can also influence N transformations (Anderson et al., 2011). Nelissen et al. (2012) reported increase in net mineralization of N in soil after biochar amendment. In other studies, biochar application increased the process of nitrification (e.g. Song et al., 2014) and denitrification in soil (Cayuela et al., 2013).

Although the potential benefits of biochar application was observed in previous studies for soil fertility and crop growth, the reports have been inconsistent depending on type of biochar and soil and environmental conditions. Moreover, there is a lack of understanding of the interactions of biochar application with different microbiological processes in the soil. This study was therefore, undertaken to improve our understanding regarding the effect of biochar application on soil respiration (CO_2) and N mineralization in an alkaline calcareous soil in the semi arid environment.

Materials and Methods

Site characterization

The experiment was conducted at the University of Agriculture, Peshawar, Pakistan (34.01° N, 71.50° E). The surface soil of the experimental site was classified as silty clay loam and fine mixed, hypothermic, alkaline astochrepths belong to the Pirsabak soil series. The soil was non-saline (<2.00 dS m^{-1}), alkaline (pH 8.0), strongly calcareous (17.5%) and low in soil fertility

(organic matter <1.0%, total N 0.07%, AB-DTPA extractable P 3.0 mg kg^{-1} soil) (Shafi *et al.*, 2007). The agroclimatic conditions are semi-arid climate with a mean rainfall in the region is about 380 mm year^{-1} and that vary considerably from season to season. The wet season is between February and September with peak rainfall mostly occurs in February-April and July-September. October through January are mostly the dry months. The hot season lasts for about 4 months from mid May to mid September, with an average daily high temperature above 97 °F (36 °C). The cool season last for 3 months, from early December to early March, with an average daily high temperature below 69 °F (20 °C).

Trial description
This study was conducted in a field trial which was started back in November 2014 with wheat cropping. The aim of the field was to determine the effects of biochar application on crop yields and soil properties under different cropping systems in the long term. Randomized complete block design with split-split plot arrangements was used in the trial. There were four replications. The experiment was comprised of two cropping systems, two chemical fertilizer treatment, four biochar levels and four replications, thus having 64 treatment plots altogether. The main plots were assigned to cropping systems, sub-plots to chemical fertilizers (full recommended NPK and ½ of recommended NPK) and sub-sub-plots to biochar levels. Chemical fertilizers were applied in the form of urea (N), single superphosphate (P) and potassium chloride (K). So far, the experiment had received various levels of biochar (t ha^{-1}) viz., 0 (T1), 50 (T2), 90 (T3) and 130 (T4) over the past four years. Biochar used in the experiment was derived from *Acacia* sp. through pyrolysis temperature over 400 °C and obtained from the market. For this study, the surface 0-15 cm soil samples were sampled from all treatment plots after harvest of wheat crop, when the field was being prepared for summer crops (maize, mungbean) but before application of any amendments in the first week of July 2018. Two cropping systems viz., wheat-mungbean and wheat-maize were selected for this study. Wheat was sown in the second week of November and harvested in third week of May every year. Maize and mungbean were planted in second week of July and harvested in second and third week of September each year. Composite soil samples of each treatment plot were processed immediately after collection. After removing stones and large plant residues, the soil samples were sieved <2-mm sieve) while still moist. The soil samples after proper homogenization of individual treatments were stored in a safe place until before the incubation experiments.

Measurement of soil respiration (CO$_2$ evolution)
The soil samples while still moist were analyzed for CO$_2$ evolution following the alkali trapping procedure (Shah *et al.*,2010). For determination of CO$_2$ evolution, 50 g moist soil sample, in duplicate, was transferred to a clean 500 mL conical flask and a vial with 5 ml of 0.3 M NaOH solution was suspended in the flask. After proper sealing using rubber bungs, the samples were incubated at 25 °C in the incubator on 23 July 2018. After 2 days on 25 July, the flasks were taken out and the NaOH solution of the vial was titrated with 0.1 N HCl in the presence of barium chloride solution (1 M 10 ml) and phenolphthalein indicator till reaching the end point. The amount HCl used was taken as a measure of CO$_2$ evolved during the given incubation period. The vials were refilled with fresh 5 ml 0.3 M NaOH solution and suspended in the flask, and the samples were incubated again under similar conditions. The process of CO$_2$ measurement was repeated similarly after 4 d (27th July), 8 d (31st July) and 16 d (8th August 2018) of incubation. The CO$_2$ produced was expressed both as rate (μg CO$_2$ g^{-1} soil day^{-1}) as well as cumulative amount for the given incubation periods (μg CO$_2$ g^{-1} soil produced during the given incubation period).

Measurement of N mineralization
Nitrogen mineralization was also measured in same soil samples run for measurement of soil respiration. For measurement of N mineralization, 500 g moist soil samples of each treatment plot were taken in individual incubation pots, in duplicate, and incubated at 25 °C in the incubator on 13 August 2018. The soil samples were analysed for total mineral N at day 0 on 13 August 2018 and subsequently on 20 August after 7 days, 27 August after 14 days and on 10 September 2018 after 28 days of incubation periods. Moisture contents were also measured at each incubation period. Soil samples were regularly stirred and watered when needed to maintain field moisture condition in the incubated samples. Net N mineralization (μg N g^{-1} soil) in soil samples was calculated by removing the mineral N at day 0 from that obtained after incubations i.e., at day 7, 14 and 28.

Determination of total mineral N in soil

Total mineral N in soil samples was determined following the procedure described in Mulvaney (1996). For this, 20 g moist soil sample was aggressively shaken with 100 ml of 1 M KCl in a 250 ml shaking bottles using end-to-end horizontal shaker for one hour. After allowing the sediment to settle down, carefully filtered the suspension. Twenty ml of the filtrate was distilled with 0.2 g each of MgO and Devarda's alloy into 5 ml indicator solution containing boric acid, methyl red and Bromo cresol green indicators for about 10 minutes followed by titration with 0.005 N HCl. The amount of HCl used in titration was taken as a measure of the amount of mineral N (NH_4-N and NO_3-N) in the sample.

Statistical analysis

Statistical analysis of the data was carried out using appropriate statistical package following the principles described in Steel *et al.* (1997). The LSD tests were used to determine statistical differences between treatments at 5% level of probability.

Results and Discussion

Surface soil samples from a 4 year long field experiment was collected to assess the effect of biochar and chemical fertilizers (N, P and K) on soil respiration and N mineralization during lab incubation experiment under wheat-mungbean and wheat-maize cropping systems. The results obtained are presented and discussed below:

Soil respiration

The results obtained on soil respiration are expressed as rate of CO_2 evolution and cumulative CO_2 production and presented below:

Rate of CO_2 production

It was observed from the data that the rate of CO_2 evolution increased significantly (P<0.05) with increasing level of added biochar during each incubation period as reflected in Table 1. At day 2, the rate of CO_2 evolution increased from 142 µg CO_2 g^{-1} soil d^{-1} in the control to 259 µg CO_2 g^{-1} soil d^{-1} in the treatment receiving 130 t biochar ha^{-1}. The same trend continued during other incubation periods. It was observed that the rate of CO_2 evolution in each treatment was greater during earlier incubation periods and the rate decreased with increasing

incubation period. The rate of CO_2 evolution declined from 142 µg at day 2 to 34 µg CO_2 g^{-1} soil d^{-1} at day 16 in the control, and from 259 µg at day 2 to 44 µg CO_2 g^{-1} soil d^{-1} at day 16 in treatment receiving the highest biochar level. However, differences among treatments were significant at each incubation period. With respect to mineral fertilizer treatments, no significant differences in CO_2 evolution were observed between ½ NPK and full NPK treatments. It was noticed that although non-significant, the rate of CO_2 evolution was consistently greater for full than ½ NPK treatment (Table 1). Similarly, differences in CO_2 evolution between the wheat-mungbean and wheat-maize cropping system were not significant (Table 1). Generally, the rate of CO_2 evolution in the mungbean based cropping system was greater during 2 and 4 days incubation suggesting the presence of easily decomposable organic materials.

Table 1: *Rate of CO_2 production as affected by biochar application and chemical fertilizers under wheat-mung and wheat-maize cropping systems. Biochar levels are cumulative amount added over the last five years. Full NPK means recommended doses of NPK for the given crops while ½ NPK means ½ of the same recommended NPK doses.*

Treatment		CO_2 µg g^{-1} soil d^{-1} during incubation at			
Biochar (BC)	Level (t ha^{-1})	Day 2	Day 4	Day 8	Day 16
	0	142	112	58	34
	50	149	135	57	30
	90	205	198	83	44
	130	259	244	102	44
	Significance	*	*	*	*
Chemical fertilizers (CF)	½ NPK	187	168	71	36
	Full NPK	190	177	79	40
	Significance	ns	ns	ns	ns
Cropping systems (CS)	Wheat-mung	194	180	70	37
	Wheat-maize	183	164	80	39
	Significance	ns	ns	ns	ns
Interactions					
	BC x CF	ns	ns	ns	ns
	CS x CF	*	*	*	*
	CS x BC	Fig. 1	ns	ns	ns
	CS x BC x CF	*	ns	ns	ns

The interactions between cropping systems and mineral fertilizers were significant during all

incubation period (Table 1). It was noticed that the rate of CO_2 evolution was remarkably greater for full NPK relative to ½ NPK treatment in soil taken from wheat-maize cropping system relative to wheat-mungbean cropping system. The same trend continued during 2, 4, 8 and 8 days of incubation period (Table 1). It appears that mineral fertilizer application was more effective in continuous cereal cropping relative to that having legume in the rotation. Moreover, the interaction between cropping system and biochar was also significant during the first 2 days of incubation period (Figure 1). We noticed that the rate of carbon dioxide efflux was greater for mungbean based cropping system in the control or in low biochar treatment. With increasing biochar level, the rate of CO_2 evolution elevated for cereal based cropping system suggesting that biochar become more effective under cereal-cereal cropping system.
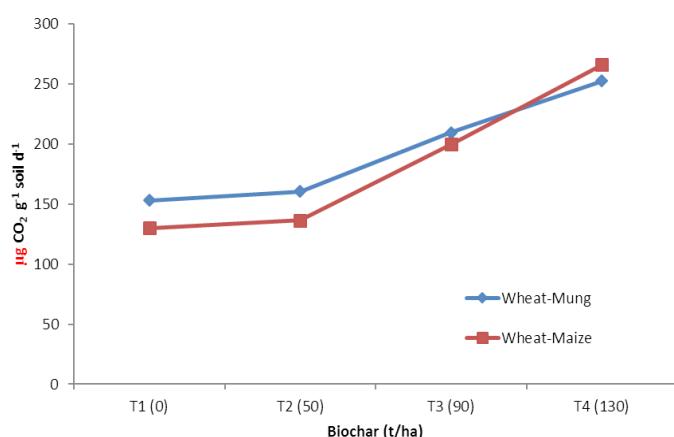

Figure 1: *Interactive effect of cropping systems and biochar levels on rate of CO_2 production during the first 2 days of incubation period.*

Cumulative CO_2 production

The data revealed that cumulative CO_2 production was affected considerably by the biochar treatment during each incubation period (Table 2). The CO_2 production increased as the level of added biochar increased. Almost the same trend was observed at each incubation period. As evident in Table 2, the total CO_2 production during the first 2 days increased from 283 µg CO_2 g^{-1} soil in the control to 519 µg CO_2 g^{-1} soil in treatment receiving the highest amount of biochar. The corresponding values during 16 days of incubations were 1073 µg CO_2 g^{-1} soil in the control and 1866 µg CO_2 g^{-1} soil in treatment receiving the highest amount of biochar. This correspond to 83% in CO_2 during the first 2 days and 74% increase during 16 days of incubation due to higher doses of biochar application. With respect to influence of mineral fertilizers treatment, CO_2 production was

insignificant but consistently greater for full than ½ NPK treatment during all four incubation periods. However, no remarkable differences between the ½ and full NPK treatments were noticed. We found that CO_2 production was initially high in the mungbean bases cropping system compared with cereal based cropping system. However, differences in CO_2 production between the two cropping systems were statistically non-significant (P<0.05).

Table 2: *Cumulative CO_2 production as affected by biochar application and chemical fertilizers under wheat–mung and wheat–maize cropping systems. Biochar levels are cumulative amount added over the last five years. Full NPK means recommended doses of NPK for the given crops while ½ NPK means ½ of the same recommended NPK doses.*

Treatment		CO_2 µg g^{-1} soil during given incubation period			
Biochar (BC)	Level (t ha^{-1})	Day 2	Day 4	Day 8	Day 16
	0	283	507	797	1073
	50	297	567	854	1098
	90	409	805	1219	1568
	130	519	1007	1516	1866
	Significance	*	*	*	*
Chemical fertilizers (CF)	½ NPK	374	710	1067	1357
	Full NPK	380	733	1125	1445
	Significance	ns	ns	ns	ns
Cropping systems (CS)	Wheat-mung	388	748	1099	1399
	Wheat-maize	366	695	1094	1403
	Significance	ns	ns	ns	ns
Interactions					
	BC x CF	ns	ns	ns	ns
	CS x CF	*	ns	ns	ns
	CS x BC	Fig. 2	*	*	*
	CS x BC x CF	*	ns	ns	ns

The interactive effect of cropping system x biochar on cumulative CO_2 production was significant initially only (2 days incubation period) (Table 2). Furthermore, the cumulative CO_2 production was higher for cropping system having mungbean in the rotation relative to that having continuous cereal in the rotation and where there was nil or receiving lower doses of biochar (Figure 2). At increasing biochar level, CO_2 production get leveled or become higher in wheat-maize than wheat-mungbean cropping system. These results suggest that mungbean residue

decomposes quickly and produces greater CO_2 in the absence of biochar. It looks that most of the CO_2 is coming from biochar under wheat-maize cropping system. The interactions between cropping systems and mineral fertilizers for CO_2 production were found significant during all four incubation periods (Table 2). The differences in CO_2 production were insignificant between ½ and NPK doses under wheat-mungbean cropping system but become highly significant under wheat-maize cropping system during the first 2 days and subsequently same trend was observed during 4, 8 and 16 (Table 4) days of incubation periods. These results suggest that mineral fertilizers application increases CO_2 only under cereal-cereal cropping system.
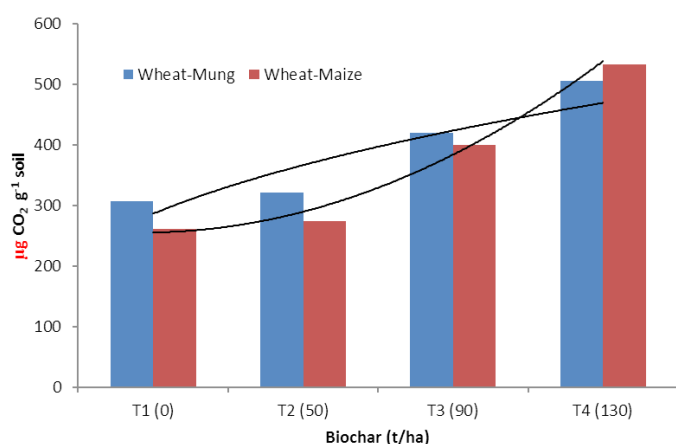

Figure 2: *Interactive effect of cropping systems and biochar levels on cumulative CO_2 production during the first 2 days of incubation period.*

Nitrogen mineralization

The data obtained on gross and net N mineralization in soil collected from biochar and mineral fertilizers amended treatments under different cropping systems are presented in Tables 3 and 4, respectively. The data revealed that N mineralization differed significantly among the biochar amended treatments particularly during the first few days of incubation periods. At day 0, the highest mineral N contents were obtained for treatment receiving the highest amount of biochar. Differences between control and low biochar treatments were statistically non-significant during the 0, 7 and 14 days of incubation period. At day 28, no considerable differences in N mineralization were obtained among biochar treatments (Table 3). Moreover, the differences in N mineralization between the ½ and full NPK treatments were not significant, and there was no specific pattern in N mineralization during any of the four incubation periods (Table 3). Similarly, differences in N mineralization between the

wheat-mungbean and wheat-maize cropping systems were not significant during any of the four incubation periods (Table 3). Like mineral fertilizer treatments, no specific pattern in N mineralization was observed during any of the four incubation periods.

Table 3: *Gross N mineralization as affected by biochar application and chemical fertilizers under wheat-mung and wheat-maize cropping systems. Biochar levels are cumulative amount added over the last five years. Full NPK means recommended doses of NPK for the given crops while ½ NPK means ½ of the same recommended NPK doses.*

Treatment		μg N g^{-1} soil during incubation			
Biochar (BC)	Level (t ha^{-1})	Day 0	Day 7	Day 14	Day 28
	0	7.60	10.66	11.32	18.23
	50	8.64	12.47	12.14	19.69
	90	6.40	13.89	14.33	21.58
	130	10.13	13.53	13.84	18.16
	Significance	*	*	*	ns
Chemical fertilizers (CF)	½ NPK	8.86	12.33	12.50	19.87
	Full NPK	7.52	12.94	13.32	18.96
	Significance	ns	ns	ns	ns
Cropping systems (CS)	Wheat-mung	8.65	13.05	10.88	20.31
	Wheat-maize	7.74	12.22	14.93	18.52
	Significance	ns	ns	ns	ns
Interactions					
	BC x CF	ns	ns	ns	ns
	CS x CF	*	*	*	*
	CS x BC	*	*	ns	ns
	CxBCxCF	ns	ns	ns	ns

The interactions between biochar and cropping systems for gross N mineralization were found significant ($P<0.05$) during 0, 7 and 28 days of incubation periods (Table 3). The data indicated that gross N mineralization was greater in soil under wheat-mungbean cropping system when no or lowers doses of biochar were applied during 0 day of incubation period. At higher doses of biochar, N mineralization was greater in soil under wheat-maize cropping system. Exactly the same trend for N mineralization was observed during 7 and 28 days of incubation periods as evident in Table 3. The interactions between cropping systems and mineral fertilizer treatments for gross N mineralization were found significant during the first 3 incubation periods. Table 3 further indicated that gross N mineralization was significantly greater

for full NPK treatment compared with ½ NPK treatment under wheat mungbean cropping system during 0 day incubation period. For wheat-maize cropping system, N mineralization was either leveled or slighter greater in full than ½ NPK treatment during 0 day incubation period. Almost the same trend for N mineralization was observed at day 7 and 14 days of incubation periods. It was visible from the data that N mineralization was remarkably greater for wheat-maize than wheat-mungbean cropping systems during all incubation periods.

Table 4: *Net N mineralization as affected by biochar application and chemical fertilizers under wheat-mung and wheat-maize cropping systems. Biochar levels are cumulative amount added over the last five years. Full NPK means recommended doses of NPK for the given crops while ½ NPK means ½ of the same recommended NPK doses.*

Treatment		µg N g^{-1} soil during incubation		
Biochar (BC)	Level (t ha^{-1})	Day 7	Day 14	Day 28
	0	3.06b	3.72b	10.63b
	50	3.83b	3.50b	11.05b
	90	7.49a	7.93a	15.18a
	130	3.40b	3.71b	8.03b
	Significance	*	*	*
Chemical fertilizers (CF)	½ NPK	3.47	3.63	11.00
	Full NPK	5.43	5.80	11.44
	Significance	ns	ns	ns
Cropping systems (CS)	Wheat-mung	4.41	2.24	11.66
	Wheat-maize	4.48	7.19	10.78
	Significance	ns	*	ns
Interactions				
	BC x CF	ns	ns	ns
	CS x CF	*	*	*
	CS x BC	*	Fig. 2	ns
	CSxBCxCF	ns	ns	ns

The data on net N mineralization revealed that maximum N mineralization was obtained where the level of added was 95 t ha^{-1}. The differences between the control (no biochar), lower biochar (50 t ha^{-1}) treatments and the treatment receiving the highest biochar (130 t ha^{-1}) were statistically non-significant during 7, 14 and 28 days of incubation periods (Table 4). The reason for lower net N mineralization in the high biochar treatment could be that the turnover rate of N may be greater in this treatment due to higher

microbial activity as evident in Table 1, causing lower accumulation of net mineral N in soil. The differences in net N mineralization were not remarkable between the ½ and full NPK treatments during all incubation periods. Similarly, the net N mineralizations were not significant between the wheat-mungbean and wheat-maize cropping systems during 7 and 28 days of incubation periods. At day 14, the net N mineralization appeared greater in soil under wheat-maize than wheat-mungbean cropping system.

The interactions between biochar and cropping system were significant for net N mineralization during 7, 14 and 28 days of incubation periods (Table 4). The data revealed that net N mineralization was greater for wheat-mungbean cropping system where no or lower (50 t ha^{-1}) biochar was applied. At higher biochar doses (90 and 130 t ha^{-1}), the net N mineralization become greater for wheat-maize than wheat-mungbean cropping systems, and this trend was consistent during 7, 14 and 28 days of incubation periods. The interactive effects of cropping systems x mineral fertilizer treatments on net N mineralization were significant during 7 and 14 days of incubation periods (Table 4). The effect of full NPK treatment on net N mineralization did not vary considerably under both cropping systems but the effect of ½ NPK treatment was remarkable during both 7 and 14 days of incubation periods.

The pattern of CO_2 efflux over the 16 days of incubation period showed that the rate of organic matter decomposition was high initially and then declined afterwards. This implies the availability of labile C in native organic matter and added biochar for microorganisms initially during organic matter decomposition and vanished soon during early days of incubation. Similar pattern in C mineralization and CO_2 efflux has been reported earlier by other researchers such as Fatima *et al*. (2020) and several others such as Kuzyakov (2011), Zimmerman *et al*. (2011) and Mason-Jones and Kuzyakov (2017). The results of this study suggest that biochar amendment enhanced CO_2 evolution during short-term incubation period (i.e., 16 days). This clearly suggests the availability of labile C pool in the added biochar which serve as a source of energy that promote microbial activity in the soil. Similar results were also reported by Bruun *et al*. (2012) and Yin *et al*. (2014) where CO_2 efflux were associated to mineralization of labile C content of biochar. Wu *et al*. (2016) and

Teutscherova et al. (2017) reported that combined application of biochar and compost exerted positive effect on C mineralization and soil organic matter decomposition. Similar to these results, Deng et al. (2017) also found that soil respiration increased with increasing doses of biochar at least during the first 30 days of incubation period. As in this study, Deng et al. (2017) further showed a decreasing trend in CO_2 evolution over time. Fatima et al. (2020) on the other hand found that the biochar-only treatments produced lower or similar CO_2 efflux compared with the control treatment and observed inverse relationship to the biochar rates. The biochar and control treatments exhibited similar rate of C mineralization. Teutscherova et al. (2017) and several other researches have reported low rates of biochar decomposition. These controversies are associated mainly with the pyrolysis temperature. The biochar used in this study was prepared at low pyrolysis temperature and would contain greater amount of labile fraction of C which could be responsible for greater CO_2 evolution than the control. The same was the case with Fatima et al. (2020). It is now clear that biochar which is prepared at temperature contain higher amount of labile C. When such biochar is added to soil, it quickly promotes microbial activity resulting in higher organic matter decomposition (for example, Guo et al., 2020). On the other hand, biochar produced at high pyrolysis temperature are highly stable and the C is not readily available to microbes resulting in slow organic matter decomposition (for example, Hailegnaw et al., 2019).

It was further observed that the effect of biochar on CO_2 efflux varied with the cropping systems. At low biochar (or control) treatment, the CO_2 evolution was greater for treatment having mungbean in crop rotation compared with the one having continuous cereals in the rotation, but at high biochar dose, the CO_2 evolution was greater in the later than the former crop rotation. This shift in CO_2 evolution could be associated with different labile pools of C in the crop residues. The greater CO_2 evolution in the absence of added biochar from mungbean based cropping system could be due to presence of greater amount of labile C in the mung crop residues.

In the current study, net N mineralization in the high biochar treatment was lower than the low biochar treatment. As it was observed that microbial activity was higher in the high biochar treatment that could

have assimilated most of the mineralized N leaving behind little mineral N to accumulate in soil (Prayogo et al., 2014). Reduced net N mineralization was also observed on other studies (e.g., Tsai and Chang, 2020) which was associated mainly with high C/N ratio in biochar. In addition to microbial assimilation of mineral N, biochar may retain the inorganic N such as ammonium-N and nitrate-N on its functional sites (Sanford et al., 2019). Our results suggest that biochar could be used as a strategy to minimize N losses from the easily available labile N pools (i.e., NH_4^+-N, NO_3^--N) through greater retention in soil. Several researchers have shown the ability of biochar to minimize N losses which happens as NH_3 volatilization, leaching of NO_3 and emission as N_2O (e.g., Li et al., 2020).

In this study, the effect of biochar on net N mineralization varied with the cropping system. At low biochar dose, the net N mineralization was greater for wheat-mungbean cropping system. At high biochar level, the net N mineralization was greater for wheat-maize cropping system. This variation in net N mineralization could be associated with different C/N ratios in crop residues. The C/N ratio in mungbean crop residue is generally lower than in maize/ wheat crop residues. The mungbean based cropping system therefore led to greater N mineralization in the absence of nil or low dose of biochar, whereas maize crop residues with wider C/N ratio led to N immobilization. Kuzyako et al. (2000) reported that N immobilization dominate over mineralization when the C/N ratio of organic matter become higher than 32. Fatima et al. (2020) also reported that higher C/N ratio of biochar and wheat straw led to increased N immobilization.

Conclusions and Recommendations

This study has shown that biochar amendment enhanced CO_2 evolution during short-term incubation period (i.e., 16 days) suggesting the availability of labile C pool in the added biochar which serve as a source of energy for promotion of microbial activity in the soil. The low net N mineralization in the high biochar treatment implies that biochar could be used as a strategy to minimize N losses from the easily available labile N pools through greater N retention in soil. In light of results of this study, biochar application is recommended to increase C and N sequestration in agricultural soils.

Novelty Statement

Biochar can be used as a strategy to improve microbial activity and N mineralization in a low fertile alkaline calcareous soil.

Author's Contribution

Tasneem Shah planned and conducted the experiment, and prepared the paper. Prof. Dr. Muhammad Tariq assisted the author in planning and conducting of experiments and helped in paper write up. Dr. Dost Muhammad assisted the author in lab experiments, lab analysis, statistical analysis of the data and in paper preparation.

Conflict of interest
The authors have declared no conflict of interest.

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