

Research Article



Impact of Biochar and Nitrogen Application on Soil Physicochemical Attributes at Various Growth Stages of Maize and Subsequent Wheat Crop

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Abstract | This study aimed to investigate the impact of integrated use of biochar and nitrogen (N) fertilizer on soil physicochemical properties at various growth stages of maize and subsequent wheat crop. Biochar was applied before sowing, while N application was carried out in splits to the crops. The study was conducted in district Buner, Pakistan using randomized complete block design with split-plot arrangement. Biochar was applied at the rates of 0, 5, 10, 15 and 20 ton/ha as the main plot factor. The immediate effect of biochar was assessed on maize crop integrated with four levels of N viz. 0, 100, 150 and 200 kg/ha applied as sub-plot factor. The residual impact of the previously applied biochar levels coupled with fresh applications of N at the rate of 0, 80, 120 and 200 kg/ha was assessed on subsequent wheat crop grown on the same plots. The soil physicochemical properties were evaluated in the beginning, reproductive stage and at maturity of each crop. The average values of soil pH, electrical conductivity (EC), organic matter (OM), N, P and K of the soil before sowing of maize crop were found to be 8.2, 0.37 dS/m, 0.95%, 1.74 mg/kg, 3.45 mg/kg, 61.5 mg/kg, respectively. Biochar and N application significantly ($p \leq 0.05$) increased soil pH whereas soil OM was substantially enhanced only with biochar at the reproductive stage of maize. However, at this stage, the effect of biochar and N application was not significant ($p \geq 0.05$) on soil EC. At maturity stage of maize crop, soil pH, OM and EC were not significantly ($p \geq 0.05$) varied from that at reproductive stage. Residual biochar significantly ($p \leq 0.05$) increased soil pH and OM at reproductive stage of wheat. Similarly, the effect of both residual biochar and different N levels was not significant ($p \geq 0.05$) on soil EC. However, at maturity stage of wheat crop soil OM, was significantly ($p \leq 0.05$) increased with biochar residues and N applications, whereas soil pH was only influenced by residual biochar. The effect of N was not significant ($p \geq 0.05$) on soil pH and EC at maturity stage of wheat crop. As biochar improve the soil physicochemical properties therefore biochar application along with N fertilizer might be helpful in maintaining healthy soil for better crop productivity.

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Introduction

Biochar is a fine ground charcoal having higher carbon content and is prominently sturdy to microbial decay. Biochar is produced through pyrolysis (combustion in low or total absence of oxygen) from organic matter. As a soil amendment biochar applica-

tion has gained a significant importance in improving the quality of degraded and highly weathered soil (Lehmann et al., 2006). Biochar as a soil conditioner improves plant growth by increasing soil nutrients, increases water holding capacity (WHC) of the soil, increases soil bulk density, soil permeability and increases fauna, which lead to higher crop yield (Le-

hmann et al., 2006). Biochar interaction with physical properties of soil along with some other factors like climatic conditions and biochar application rates and types are the determinant of the net effect on soil physicochemical properties. Moreover, biochar is highly obstinate to microbial decomposition and proved to be beneficial for soil fertility on long-term basis (Steiner et al., 2007).

Biochar may also increase the decomposition of the native soil organic matter (positive priming effect), particularly after application of low temperature biochars containing more than 411 g/ kg of volatile matter (Zimmermann, 2010; Zimmermann et al., 2011). The positive priming effect may be caused by enhanced soil microbial activity that results in increased remineralization of soil nutrients and co-metabolism of soil organic matter, e.g. soil humic materials (Zimmermann et al., 2011). However, undesirable effect might be expected that prevail for long-term, as soil organic matter is increasingly adsorbed within biochar pores and onto surfaces where it cannot be exposed to be degraded (Zimmermann et al., 2011).

As a soil amendment application of biochar soil may affects the chemistry of soil by increasing cation exchange capacity (CEC) of the soils linked to increased soil specific area that increase oxidation of biochar which results to produce more ions leading to more cation on biochar surface (Liang et al., 2006; Major et al., 2010).

Biochar efficacy in increasing crop productivity and quality can be improved by integrating N, P and K fertilizers with biochar (Steiner et al., 2007). According to Jose et al. (2013) integrated application of biochar along with inorganic fertilization increased the yield of wheat crop by 20-30% compared to mineral fertilizer alone. The enhanced crop yield was attributed to the availability of adequate amount of P from biochar and N from mineral fertilizer (Albuquerque et al., 2013). Similarly, positive response of biochar was observed when applied in combination with synthetic fertilizer on semi-arid soil in Australia (Chan et al., 2008) and significantly increased the yields of wheat and maize in Indonesia when integrated with N fertilizer (Yamato et al., 2006).

The ability of biochar to provide nutrients and ions, improved water holding capacity of soil, increased the growth of microorganism as well as controlled pH in

such a way to provide an environment supportive for root growth. Similarly, increased root growth has also been reported when biochar was amended to a peat-based substrate (O'Hara, 2013). Biochar might have influenced the plant growth by an increase in nutrient supply. Similarly, biochar application increased the availability of N and P to plant roots (Prendergast-Miller et al., 2014). Based on the role and function of biochar as a soil amendment to maintain and improve the physical, chemical and biological properties of soil, this study was undertaken to evaluate the effect of biochar integrated with N fertilizer on soil physicochemical attributes at various growth stages of maize and successive wheat crop under field condition.

Materials and Methods

Experimental site

The field trials were carried out in district Buner (34° 30'41" N 72° 29' 02" E), Khyber Pakhtunkhwa province of Pakistan during 2014-15. Weather data for the crops growing season are shown in Figure 1.

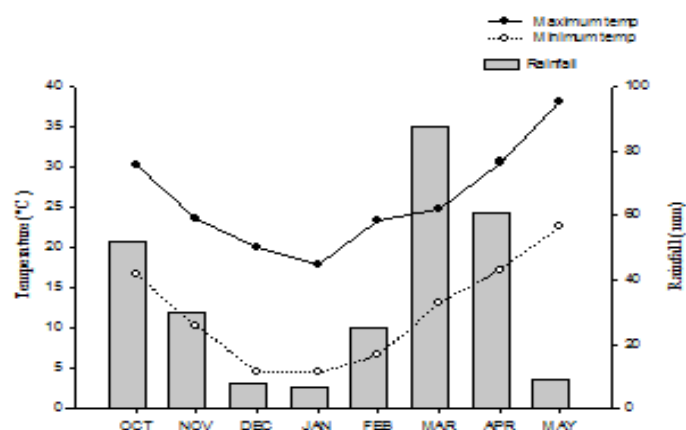


Figure 1: Meteorological data of district Buner during the Maize-Wheat cropping season (2014-2015).

Experimental design and land preparation

The study was designed in randomized complete block design (RCBD) with split-plot arrangement having three replications with net plot size 3 × 3 m. A one year experiment was carried out following Maize-wheat cropping pattern. Biochar levels i.e. 0, 5, 10, 15 and 20 ton/ha were assigned to the main plots. The immediate effect of biochar amendment was assessed on maize crop integrated with different N levels i.e. 0, 100, 150 and 200 kg ha⁻¹ applied as sub-plot factor. The residual effect of biochar was examined on subsequent wheat crop grown on the same plots after harvesting the maize crop. However, nitro-

gen was applied at dose rates of 0, 80, 120 and 200 kg ha⁻¹. All standard agricultural practices were followed throughout the experimental period. The experimental treatments are presented in Table 1.

Table 1: *Treatment combinations of biochar (t/ha) and nitrogen (kg/ha) for maize-wheat trial*

Treatments	Maize		Wheat	
	Biochar * (ton ha ⁻¹)	Nitrogen ** (Kg ha ⁻¹)	Biochar * (ton ha ⁻¹)	Nitrogen ** (Kg ha ⁻¹)
1	0	0	0	0
2	0	100	0	80
3	0	150	0	120
4	0	200	0	200
5	5	0	5	0
6	5	100	5	80
7	5	150	5	120
8	5	200	5	200
9	10	0	10	0
10	10	100	10	80
11	10	150	10	120
12	10	200	10	200
13	15	0	15	0
14	15	100	15	80
15	15	150	15	120
16	15	200	15	200
17	20	0	20	0
18	20	100	20	80
19	20	150	20	120
20	20	200	20	200

* Immediate (maize) and residual (wheat) effects of biochar; ** Nitrogen was applied to maize and wheat through urea fertilizer in two equal split doses. It was applied to maize at sowing and thirty days of growth whereas to wheat it was applied at sowing and boot stage.

Soil sampling and analysis

Composite soil samples were collected randomly from three different locations of each experimental unit before sowing, at reproductive and at maturity stages of maize and subsequent wheat crop. Sampling was carried out at a depth of 15 cm using the manual agar. All the samples were collected in clean plastic bags, air dried in lab and ground before physicochemical evaluation.

Soil pH (1:5)

pH of the soil was determined by the procedure adopted by Rhoads (1996) using pH meter (DOCU-PH/P10 Sartorius Corporation, NY 11716, USA).

Soil sample (10g) along with 50 ml distilled water were taken in a conical flask to make 1:5 soil-water suspensions. The soil-water suspension was shaken on mechanical shaker for 30 min and then filtered through Whatman No.42 filter paper. The filtrate was subjected to determination of pH using standards of pH 7 and 10.01 for pH meter calibration.

Soil electrical conductivity

Soil electrical conductivity (EC) was determined by the procedure of Rhoads (1996) using EC meter (WTW, Germany).

Soil organic matter

The procedure of Walkley-Black described by Nelson and Sommers (1996) was applied for the determination of organic matter (OM) of the experimental soil samples. Soil sample (1g) was treated with 1 N K₂Cr₂O₇ (10 ml) and concentrated H₂SO₄ (20 ml) in order to make suspension. After the addition of 20 ml distilled water, the suspension was left to cool down. After filtration, the suspension was treated with 0.5 N FeSO₄·7H₂O solution. Few drops of O-phenolphthalein were added as indicator that produced maroon color at the end-point of the reaction.

Grain yield

Two central rows in each crop were harvested, sun-dried and threshed. Thereafter, the samples were separately weighed and converted into kg/ha.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using the statistical package Statistix 8.1 and the significant differences between treatment means were determined using Least Significant Difference (LSD) test for main as well as interaction effect (Steel et al., 1996). All the means were calculated from triplicate values.

Results

Biochar analysis

The basic chemical characteristics of biochar is presented in Table 2. Biochar was analyzed for pH, EC, carbon (C), phosphorus (P) and nitrogen (N). The pH value was 7.35 and EC was 1.25 dS/m. The average concentrations of C, P and N in biochar were found to be 568 (g/kg), 10.8 (g/kg) and 9.5 (g/kg), respectively.

Soil physicochemical properties of the experimental site

Table 3 represents the physiochemical properties of

Table 2: Basic physicochemical characteristics of biochar

Composition	Biochar
pH	7.35
EC (dS/m)	1.25
C (g/kg)	568
P (g/kg)	10.8
N (g/kg)	9.5

the experimental site before the start of the experiment. The experimental site was slightly alkaline in nature having 7.97 to 8.35 pH and electrical conductivity from 0.3 to 0.43 dS/m. The average organic matter was found to be 0.95%. The NPK contents of the soil were 1.74, 3.45 and 61.5 mg/ kg, respectively.

Table 3: Soil physicochemical properties of the experimental site before the start of the experiment

Property	Mean
pH	8.2
Electrical conductivity (dSm ⁻¹)	0.37
Organic matter (%)	0.95
N (mg/ kg)	1.74
P (mg/kg)	3.45
K (mg/ kg)	61.5

Table 4: Soil properties at reproductive stage of maize as affected by different biochar (B) and nitrogen (N) levels

Biochar (t/ ha)	Soil pH	Soil EC (dS/m)	Soil organic matter (%)
0	7.82 ab	0.29	0.57 c
5	7.79 bc	0.27	0.69 b
10	7.76 c	0.28	0.79 a
15	7.86 a	0.30	0.57 c
20	7.86 a	0.29	0.60 c
LSD (0.05)	0.05	NS	0.05
Nitrogen (kg/ha)			
0	7.81 ab	0.29	0.65
100	7.86 a	0.28	0.64
150	7.84 a	0.30	0.62
200	7.78 b	0.28	0.65
LSD (0.05)	0.05	NS	NS
Interaction			
B x N	NS	NS	**

Means followed by different letters within each category are statistically different at $p \leq 0.05$; NS= non-significant

Soil physicochemical properties at reproductive stage of maize crop

Table 4 shows data regarding soil physicochemical

properties i.e. soil pH, EC and organic matter (OM) in response to different levels of biochar and nitrogen at reproductive stage of maize. It was observed that the individual effect of biochar as well as N was significant ($p \leq 0.05$) on soil pH whereas their interactive effect was non-significant ($p \geq 0.05$). Similarly, different biochar levels as well as their interactions with different N levels also showed significant ($p \leq 0.05$) effect on soil organic matter. Whereas, the effect of different N levels was not significant ($p \leq 0.05$). Both biochar and N showed non-significant ($p \leq 0.05$) result for soil EC. Mean values of soil pH revealed that soil pH was highest (7.86) with 20 t/ha which was statistically similar with 15 t/ha biochar. The soil pH was lowest (7.76) with 10 t/ha biochar application. Among the different nitrogen levels, soil pH was highest (7.86) with 100 kg/ha N application rate which was followed by 150 kg/ ha. Nitrogen application at the rate of 200 kg/ha indicated lowest soil pH i.e. 7.78. The soil organic matter was highest (0.79 %) with biochar application rate of 10 t/ha which was followed by 5 t/ha and lowest (0.57 %) with no biochar application. The EC values non-significantly varied from 0.27 to 0.30 dS/m among various treatments.

Table 5: Soil properties at harvest stage of maize as affected by different biochar (B) and nitrogen (N) levels.

Biochar (t/ha)	Soil pH	Soil EC (dS/m)	Soil organic matter (%)
0	7.79	0.29	0.54
5	7.74	0.27	0.50
10	7.73	0.28	0.49
15	7.81	0.30	0.51
20	7.80	0.29	0.52
LSD (0.05)	NS	NS	NS
Nitrogen (kg/ha)			
0	7.74	0.29	0.50
100	7.80	0.28	0.52
150	7.80	0.30	0.53
200	7.75	0.28	0.50
LSD (0.05)	NS	NS	NS
Biochar x Nitrogen	NS	NS	**

Means followed by different letters within each category are statistically different at $p \leq 0.05$; NS= non-significant

Soil physicochemical properties at maturity stage of maize crop

Table 5 represents soil physiochemical properties at maturity stage of maize crop as affected by various

biochar and nitrogen levels. Both the treatments biochar and different N levels as well as their interaction showed non-significant ($p \geq 0.05$) effect on all the physiochemical properties i.e. pH, EC and organic matter at maturity stage of maize crop.

Table 6: Soil properties at reproductive stage of wheat as affected by different residual biochar and nitrogen (N) levels

Biochar (t/ha)	Soil pH	Soil EC (dS/m)	Soil organic matter (%)
0	7.75 a	0.29	0.64 b
5	7.69 b	0.28	0.74 a
10	7.68 b	0.27	0.81 a
15	7.74 a	0.30	0.63 b
20	7.77 a	0.29	0.64 b
LSD (0.05)	0.04	NS	0.00
Nitrogen (kg/ha)			
0	7.69	0.28	0.71
80	7.75	0.30	0.69
120	7.75	0.29	0.66
200	7.71	0.27	0.71
LSD (0.05)	NS	NS	NS
Interaction			
B x N	NS	NS	**

Means followed by different letters within each category are statistically different at $p \leq 0.05$; NS= non-significant

Soil physicochemical properties at reproductive stage of wheat crop

Table 6 indicates the soil properties at reproductive stage of wheat as affected by residual biochar levels along with fresh N applications. Analysis of the data revealed significant ($p \leq 0.05$) result for residual biochar and non-significant ($p > 0.05$) result for freshly applied N on soil pH and soil organic matter. The interactive effect of both the treatment was significant ($p \leq 0.05$) for organic matter while non-significant ($p \geq 0.05$) for soil pH. Both biochar and freshly applied different N levels as well as their interaction showed non-significant ($p \geq 0.05$) result for soil EC. Mean values for soil pH indicated that residual biochar at 20 t/ha resulted in the highest pH (7.77) followed by 15 t/ha. Soil pH was lowest (7.68) at 10 t/ha. The soil organic matter was highest (0.81 %) with 10 t/ha residual biochar previously applied to maize crop which was followed by 5 t/ha. The lowest soil organic matter (0.63 %) was recorded with 15 t/ha biochar application.

Soil physicochemical properties at maturity stage of wheat

crop

Soil physicochemical properties at maturity stage of wheat crop as influenced by different biochar with fresh application of different nitrogen levels is given in Table 7. Findings of the results showed significant ($p \leq 0.05$) effect of biochar residue on pH. Similarly, freshly applied different N levels as well as their interaction with residual biochar showed non-significant result on pH. Both residual biochar and freshly applied N levels had no significant result on soil EC. Both biochar and different N levels as well as their interaction significantly ($p \leq 0.05$) affected the organic matter of soil at maturity stage of wheat crop. Mean values of soil pH indicated highest soil pH value of 7.77 with the application of 20 t/ha followed by the control plot. Similarly, the lowest (7.68) soil pH was recorded with previously applied biochar to maize crop at 10 t/ha. Regarding organic matter of the soil, highest value (0.47 %) was recorded with the addition of 20 or 15 t/ha and the lowest (0.38 %) soil organic matter was recorded with 10 t/ha biochar applied to earlier maize crop. Application of nitrogen at the rate of 120 kg/ha exhibited maximum (0.47 %) soil organic matter and the lowest (0.43%) was shown by 80 kg N/ha.

Table 7: Soil properties at harvest stage of wheat as affected by residual biochar and nitrogen (N) levels.

Biochar (t/ha)	Soil pH	Soil EC (dS/m)	Soil organic matter (%)
0	7.75 a	0.29	0.45 ab
5	7.69 b	0.28	0.44 b
10	7.68 b	0.29	0.38 c
15	7.74 a	0.30	0.47 a
20	7.77 a	0.30	0.47 a
LSD (0.05)	0.04	NS	0.03
Nitrogen (kg/ha)			
0	7.69	0.30	0.44 b
80	7.75	0.28	0.43 b
120	7.75	0.31	0.47 a
200	7.71	0.29	0.44 b
LSD (0.05)	NS	NS	0.02
Interaction			
B x N	NS	NS	**

Means followed by different letters within each category are statistically different at $p \leq 0.05$; NS= non-significant

Maize grain yield

Table 8 shows the maize grain yield (kg/ha) as affected by biochar and nitrogen. Statistical analysis of the

data showed that biochar application did not significantly ($p \geq 0.05$) affected the maize grain yield, however, the effect of nitrogen was significant ($p \leq 0.05$) on maize grain yield. Similarly, the interactive effect of both the treatments was also non-significant ($p \geq 0.05$). Maximum grain yield was recorded (4761.8 kg/ha) with the application of N at the rate of 200 kg/ha, followed by 150 kg/ha. Minimum grain yield was observed for control treatment.

Wheat grain yield

Data regarding grain yield of wheat as affected by residual biochar and fresh application of N is shown in Table 8. Analysis of the data showed that both biochar and nitrogen significantly ($p \leq 0.05$) affected the wheat grain yield. The interaction BxN was also found significant ($p \leq 0.05$). Maximum grain yield was recorded (3289 kg/ha) with the application of biochar at the rate of 10 t/ha, followed by 5 t/ha. Minimum grain yield was observed for control treatment. Application of N at the rate of 200 kg/ha showed the highest yield i.e. 3894.2 kg/ha, followed by 120 kg/ha. The lowest grain yield was recorded in the absence of nitrogen i.e. 2449.2 kg/ha. The interactive effect of both the treatment showed that grain yield was highest (3934 kg/ha) at 5 t/ha biochar along with 200 kg/ha N. Similarly, the lowest (2422 kg/ha) grain yield was recorded in the absence of biochar and N.

Table 8: Grain yield of maize and wheat crop as affected by biochar and nitrogen (N) levels

Biochar (t/ha)	Maize grain yield (kg/ha)	Wheat grain yield (kg/ha)
0	4255.7	3183.8bc
5	4255.1	3271.4ab
10	4238.8	3289.0a
15	4312.4	3216.6b
20	4381.1	3200.6bc
LSD (0.05)	NS	72.85
Nitrogen (kg/ha)		
0	3749.8d	2449.2d
80	4123.0c	3060.1c
120	4519.8b	3525.6b
200	4761.8a	3894.2a
LSD (0.05)	98.1	50.45
Interaction		
B x N	NS	**

Discussion

Application of biochar to soil had proven to have a multidimensional effect on soil quality depends upon soil composition as well as on biochar properties. Biochars are highly diverse materials, whose complexity depends upon pyrolysis temperature as well as on starting organic material. It acts as nutrient reservoir, enhance moisture sorption capacity of soil, improves soil biological properties and add recalcitrant C to the soil. Soil pH at all growth stages of both maize and wheat crop were positively influenced with increasing levels of biochar except at the harvest stage of maize crop where no statistical difference was noted in soil pH at various levels of biochar. The presence of inorganic carbonate and organic anions on biochar surface might be the probable reason for increasing soil pH (Yuan et al., 2011). Yuan and Xu (2011) also observed a significant linear correlation between soil pH due to biochar amendment which might be due to the release of base cations and the higher CaCO_3 content of biochar resulted in an increased soil pH. Similarly, the incorporation of coal to soil had increased the pH 6.0–6.8 (Cox et al., 2001). Beraa et al. (2016), also reported increased in the pH value due to biochar application. Application of charcoals from burned biomass had demonstrated well their ability to raise soil pH in previous studies (Mbagwu and Piccolo, 1997). The significant increase in soil pH due to biochar residues is consistent with the earlier report of Yuan and Xu (2010) that used crop residue biochar. This might be due to the reason that soil pH after biochar application was positively correlated with the acid buffering capacity of biochar residue (Yuan and Xu, 2010). According to Wang et al. (2014) presence of biochar residue in the soil is an effective way to increase soil pH and base cations. However, the magnitude of soil pH increase would be reduced at higher biochar application rates, as further addition of biochar simply reduced exchangeable acidity without affecting the overall soil pH.

The effect of N fertilizer on soil pH was significant ($p \leq 0.05$) only at reproductive stage of maize crop, which was significantly decreased with increasing level of N fertilizer. Several studies demonstrated decreased soil pH to a certain extent with different fertilizer treatments (Wang et al., 2010). Similar results were also reported by Zhong et al. (2014). This might be due to the reason that N application by stages increase the soil bulk density which resulted

in decreased soil pH (Wang et al., 2010). Similarly, the decrease in soil pH with increased N rates could also be attributed to the nitrification process in which hydrogen ions are produced and released to the soil solution (Aula et al., 2016). This is consistent with the previous work showing a decrease in soil pH with increased N fertilization (Tuyen et al., 2006; Schroder et al., 2011; Rezig et al., 2013).

Both biochar and nitrogen application showed non-significant results for the electrical conductivity of soil. Similarly, organic matter content of the soil was significantly ($p \leq 0.05$) increased with biochar. Biochar application at the early stages has resulted in obvious changes of OM in the soil. This might be attributed to the organic carbon fractions of biochar that were readily available to the soil for decomposition (Bruun et al., 2008). Analysis of soil at various stage showed that with the passage of time biochar surface is oxidized to form carboxylate group (Laird et al., 2010). These factors might had contributed to the mineralization of biochar that might resulted in the loss of total carbon (Hamer et al., 2004). This lose in carbon with aging might had resulted in low organic matter at the mature stage of wheat crop. Our findings coincide with the previous study of Qadeer et al. (2014).

Similarly, the effect of N fertilizer on soil pH was significant ($p \leq 0.05$) at harvest stage of wheat crop only, which was significantly increased with increasing level of N fertilizer. Our findings are in line with the previous study of Dong et al. (2012). This might be due to the reason that application of chemical fertilizer improves soil aggregation, soil water retention, and reduce bulk density of the soil in the plough layer, promoting crop growth and the return of more root residues to the soil (Hyvonen et al., 2008). Similarly, chemical fertilizers promote biomass production, consequently a higher amount of plant residues, roots and also root exudates which contribute to the soil organic matter pool. This explains the higher soil organic carbon content with fertilizer use (Russel et al., 2009). Similar results were also reported by Six et al. (2002).

Biochar showed to have non-significant ($p \geq 0.05$) effect of maize grain yield, whereas it was significant ($p \leq 0.05$) on wheat grain yield. The significant increase wheat grain yield might be due to the efficacy of biochar to increase plant available water (preventing

leaching of water to the subsoil) and the water vapor sorption as well as nutrient retention by increasing the cation exchange capacity of soil (Cornelissen et al., 2013). Similar results were also reported by Yeboah et al. (2016). Grain yield of both the crops significantly ($p \leq 0.05$) increased with increasing dose of nitrogen fertilizer. The significant increase in grain yield due to N fertilizer might be due to the reason that nitrogen fertilizer improve leaf area index (LAI) as well as leaf area duration (LAD). Highest grain yield of both the crop could be explained by variation of LAI and LAD (Mansouri-Far et al., 2010; Cheema et al., 2010). Workayehu (2000) also reported that grain yield of cereal increase progressively with addition nitrogen fertilizer.

Conclusions

It was concluded that biochar applied at the rate of 15 t/ha significantly improved the physico-chemical properties of soil during maize-wheat cropping season. Application of 200 kg N /ha improved soil physicochemical properties during the growing seasons of both crops. Application of 20 t/ha significantly improve the grain yield of both crop similarly, 200kg/h N also improved the grain yield.

Author's Contribution

Habib Ullah: Wrote the manuscript, designed the study and conducted the experimental work.

Sahib Alam: Supervised the study, provided overall input and conceived the idea of the research.

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