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S. I. BIOCHAR

Impact of biochar prepared from leaves of *Populous euphratica* on soil microbial activity and mung bean (*Vigna radiata*) growth

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Abstract

In Pakistan, a lot of waste is generated from the falling leaves of Populous trees every season and dumped as unused waste material. A study was conducted at Pir Mehr Ali Shah-Arid Agriculture University Rawalpindi, Pakistan, to investigate the effect of biochar prepared from populous trees on soil microbial biomass, soil enzymes activity, microbial population, and growth of mung bean. Biochar was prepared from leaves of *Populous euphratica* at 350 °C for 3 h. Eighteen plastic pots were filled with 5.0-kg soil in each to accommodate six treatments with three replications. The treatments were biochar at 50 g kg^{-1}, N at 0.0216 g kg^{-1}, P at 0.0652 g kg^{-1}, biochar (50 g kg^{-1}) plus N (0.0216 g kg^{-1}), biochar (50 g kg^{-1}) plus P (0.0652 g kg^{-1}), and control (no application). Mung bean (*Vigna radiata*) plants were grown in each pot for 60 days. Soil samples were collected at crop maturity and analyzed for microbial indices like enzymatic activity and microbial population, and plant nutrients. The results indicated that highest microbial biomass carbon (MBC) was observed in biochar + phosphorus (BC + P) treatment which was 65% higher than control. Similarly, highest microbial biomass nitrogen (MBN) was observed in BC + N treatment which was 38% higher than control. For enzyme urease and alkaline phosphatase, highest activity was observed in treatment BC + N and BC + P respectively, which was 4% and 24% higher than control. Highest population of bacteria, fungi, and actinomycetes was observed in treatment where biochar and BC + P were applied, respectively. The study concluded that biochar prepared from populous leaves can be used effectively along with N and P fertilizers to improve microbial activity of soil and consequently crop growth.

Keywords Soil · Biochar · Populous leaves · Microorganism · Mung beans

Introduction

Increasing global human population, diminishing food reserves, and climate change is growing concern. It has been predicted that within few decades, the yields of primary foods crops like maize, rice, and wheat will decrease to a great extent as a result of warmer and drier climatic conditions mainly in semi-arid areas. This degradation and low soil fertility are major problems of agricultural soils (Glover 2009). Nutrient deficiency is a common problem in crop production. As a means of addressing these problems, the application of biochar to soils has been brought forward in an attempt to sustainably amend low nutrient-holding soils (Lehmann and Joseph 2009). Biochar is a new product which makes the nutrients available to plants for their growth. Biochar can be produced from different organic waste materials like vegetable waste, fast-food waste, wheat straw, rice husk, poultry and farmyard manure, sewage sludge, and many other products and materials (Chan et al. 2008) under different pyrolytic conditions, and thus having different nutrient contents. The burning process of organic materials in limited or no oxygen is termed as pyrolysis, which produces carbon-rich char that is extremely resistant to decomposition. The properties of biochar are markedly influenced by temperature condition and type of pyrolysis, which in addition to soil affect its different properties (Brown 2009). Like the total nitrogen and

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phosphorus contents are typically higher in biochars produced from feedstocks of animal origin than those of plant origin (Chan and Xu 2009). The knowledge of chemical changes that occur in soils by the application of biochar is very helpful in managing agricultural soils because the use of biochar to soils has shown a number of physico-chemical advantages and disadvantages. For example, many studies have shown that biochar adds basic cations to soils, enhances soil water retention, and has liming potential of acidic soils (Sohi et al. 2010). Although the liming capacity of biochar caused an increase in yield and biomass, it also has some disadvantages like increase in soil pH which cause micronutrient deficiency. Most of the investigations have been carried out in dark black soils of Amazon basin of Brazil with respect to the effect of biochar application (Lehmann et al. 2003) and some other low organic matter content in soils (Novak et al. 2009). Productivity and quality of soils with poor nutrients status can be improved by biochar addition because it adsorbs and exchanges essential plant nutrient and also improves soil physico-chemical properties (Atkinson et al. 2010). However in highly fertile soils, the effect of biochar application is still understudy (Laird et al. 2010). Biochar improves soil health and productivity and also helps in carbon dioxide sequestration in order to mitigate global warming. Different agrochemicals used for crop production are retained by biochar and it also increases nutrient retention and enhances water quality. Biochar has a significant role in mitigation of environmental change. Application of biochar results in improvement of soil quality and plant growth, and also causes considerable decrease in release of greenhouse gases (Van Zwieten et al. 2009). Biochar has excellent capacity to bind heavy metal ions and nutrient, due to this, it can improve productivity and quality of low fertility soils (Atkinson et al. 2010). Biochar is a unique and effective source to enhance soil fertility. It sequesters carbon and prevents its release in atmosphere in the form of carbon dioxide (Sohi et al. 2010). Biochar has several visual effects on soil such as it improves soil fertility through adsorption of ions and cation exchange capacity. In particular, soils amended with different rates of biochar have shown improved soil structure, texture, growth, and yield of various crops (Glaser et al. 2002). It has been reported in literature that quantity of different types of microbes present in soil is highly influenced and enhanced by the addition of biochar in soil (Jin 2010). In addition to some disadvantages and risk for soil microbial community, biochar has so many healthy effects on soil properties. The practical example of biochar effects on soils has been shown in terra preta soils of Amazon basin where the presence of biochar has significantly enhanced soil microbial biomass (Liang et al. 2010). Still, very little knowledge about the effects of biochar application on soil enzyme activities has been reported by the researchers. In Pakistani soils, organic matter is low and these soils also show poor microbial activity; in addition to this, there is a lot of popular (*Populous euphratica*) leaves wastes in the fields every season. So, the purpose of this study was to produce biochar from popular leaves and study its effects on microbial activities such as soil enzymes and biomass, and consequently on crop growth.

Materials and methods

Soil and biochar

Bulk soil was collected from 0–15-cm depth from the research area of the Pir Mehr Ali Shah-Arid Agriculture University Rawalpindi. The soil sub-sample was analyzed for basic soil properties (Table 1). Popular (*Populous euphratica*) leaves were collected and used for biochar production. Plant leaves were air-dried, ground, and were placed in a muffle furnace for 3 h at 350 °C. After 3 h, biochar was collected and stored in bottles. The prepared biochar was analyzed for basic properties given in Table 1. The treatments (biochar at 50 g kg^{-1}, N at 0.0216 g kg^{-1}, P at 0.0652 g kg^{-1}, biochar (50 g kg^{-1}) plus N (0.0216 g kg^{-1}), biochar (50 g kg^{-1}) plus P (0.0652 g kg^{-1}), and control (no application) were applied in plastic pots. Mung bean (*Vigna radiata*) seeds were sown, and after thinning, three plants were maintained in each pot. Plant were harvested after 2 months and soil samples were collected to analyze soil microbial biomass (carbon, nitrogen), enzyme activities (urease, alkaline phosphatase), and microbial population (bacteria, fungi, and actinomycetes). Plant samples were dried and analyzed shoot N and grain P. Shoot length, plant biomass, and the number of pods per pot were also calculated.

Table 1 Physico-chemical analysis of soil and chemical composition of biochar used in the study

Characteristics	Soil	Biochar
Soil texture	Sandy loam	–
Clay	3%	–
Sand	62%	–
Silt	35%	–
pH	7.78	8.20
EC	0.27 dS m^{-1}	1.32 dS m^{-1}
Available P	6.75 mg kg^{-1}	–
Total organic carbon	3.37 g kg^{-1}	670 g kg^{-1}
Nitrate N	3.83 mg kg^{-1}	–
Total N	–	12.45 g kg^{-1}
Total P	–	5.38 g kg^{-1}

Analytical methods

Soil microbial biomass carbon was determined through the fumigation extraction method. Ten-gram soil was fumigated at 25 °C for 24 h with ethanol-free chloroform. After fumigation, the soil sample was used for extraction with 50 mL solution of 0.5 M K_2SO_4 and then filtered after shaking for 30 min through filter paper. Ten-gram non-fumigated soil was treated in the same way and filtrate was collected. The titration method was used to determine total organic carbon, and for this purpose, 4-mL extract was taken in a digestion tube along with 1 mL of 0.0667 M $K_2Cr_2O_7$. Five-milliliter concentrated sulfuric acid was added in digestion tubes. Digestion tubes were heated at 50 °C for 30 min. After cooling, 4 drops of indicator solution (1:10-phenanthroline indicator) were added and mixed. Titration of the sample was done with acidified ferrous ammonium sulfate solution. Microbial biomass carbon was calculated by the following formula (Vance et al. 1987):

$$\text{Microbial biomass C} = \left(\text{extracted } C_{\text{fumigated}} - \text{extracted } C_{\text{unfumigated}}\right) \times 2.64$$

Microbial biomass nitrogen of soil was determined through the fumigation extraction method. The Kjeldahl method was used to determine total nitrogen in the K_2SO_4 extract. Twenty-milliliter extract was taken in each digestion tubes (100 mL capacity) along with 3.5 g of digestion mixture containing selenium powder, potassium sulfate, and sulfuric acid. Digestion tubes were placed on block digester at 420 °C for 70 min. Post-digestion 50-mL distilled water was added in each tube and after that, 55 mL of 35% NaOH was added. Each tube was run on Kjeldahl auto-DTP-3 apparatus containing boric acid solution. The soil MBN was calculated by the following formula (Brooks et al. 1985):

$$\text{Microbial biomass N} = \left(\text{extracted } N_{\text{fumigated}} - \text{extracted } N_{\text{unfumigated}}\right) \times 1.46$$

For Alkaline phosphatase activity, 1.0-g soil was taken in a flask and then, 0.25-mL toluene was poured into the flask. After this, 4 mL of modified universal buffer solution composed of 12 g Tris, 11.6 g maleic acid, 14 g citric acid, and 6.3 g boric acid was dissolved in 500 mL NaOH and diluted to 1000 ml with distilled water, and 1.00 mL solution of p-nitrophenyl phosphate made in the same buffer was added in the flask. Then, contents were incubated for 1 h at 37 °C. After incubation, 1.00 mL solution of $CaCl_2$ (0.5 M) and 4 mL solution NaOH (0.5 M) was added in the same flask and mixed very well. After, mixing filtrate was taken. Then, spectrophotometer was used to take the readings at 400 nm. For soil urease activity, 5 g of moist soil was taken in a flask. After this, 2.5 mL solution of urea was added and incubated at 37 °C for 2 h. Then, 50 mL KCl solution was poured in the flask and shaken for 30 min. The resulting suspension was filtered and the filtrate was analyzed for ammonium content. For this, 1.0 ml of the clear filtrate was taken and 9 mL of distilled water was added along with 5 mL of Na Salicylate/NaOH. Two milliliters of dichloroisocyanide solution was added and cooled at room temperature. After cooling, absorbance was measured at 690 nm (Kandeler and Gerber 1988).

For examination of soil microbial population, isolations were carried out on dextrose nitrate agar medium for fungi and actinomycetes while soil extract agar medium was used for bacteria. One-gram soil from each treatment was taken under sterile conditions in test tubes along with 9-mL sterile water and serial dilution was made. Bacteria, fungi, and actinomycetes were isolated by using a dilution of 10^{-3} and 10^{-4}. The medium that had been inoculated was placed in an incubator at 28 °C for 2 days and the number of colonies on medium was counted using colony counter.

Results and discussion

Effect of treatments on soil microbial biomass

The data regarding the effect of treatments on microbial biomass carbon is presented in Fig 1. Maximum MBC was recorded in treatment BC + P which was 65.8% higher than control. Similarly, MBC was 59.1%, 46.3%, 39.6%, and 14.80% higher in treatments BC + N, BC, P, and N respectively as compared with control. When biochar was used in combination with N and P, then MBC was 13.3% and 8.7% higher as compared with biochar only. The data indicated that means of biochar and biochar along with N and P treatments are statistically non-significant while these means are highly significant as compared with control (CK). Han et al. (2013) used biochar prepared from corncob by pyrolysis at 350–550 °C at the rate of 0, 12.5, and 25 t ha^{-1} in the rhizosphere of spinach. They observed that MBC in treatments applied with 12.5 and 25 t ha^{-1} was significantly higher than control which was 1.56- and 2.50-fold of control. Shenbagavalli and Mahimamairaja (2012) in an incubation experiment using biochar at the rate of 0, 1, 2, 3, 4, 5, and 6% of soil observed highest value of MBC in treatment (5% biochar) at 30 (2.57 g kg^{-1}) and 60 days (2.61 g kg^{-1}). The increase in MBC might be due to high carbon contents of biochar. Prabha et al. (2013) conducted a pot experiment for rice crop by using biochar and other organic amendments and observed maximum MBC (1.98 mg CO_2-C ha^{-1}) in treatment receiving 35-g biochar.

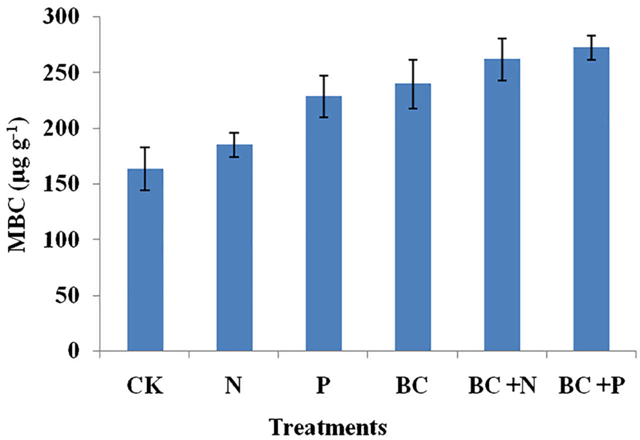

Fig. 1 Effect of treatments on soil microbial biomass carbon (MBC)

The data regarding microbial biomass nitrogen is presented in Fig. 2. The MBN was 13.4, 15.1, 16.5, 16.8, 18.6, and 15.6 mg kg^{-1} for treatments CK, N, P, BC, BC + N, and BC + P respectively. Maximum MBN was observed in treatment BC + N which was 38.8% higher than control. MBN was 12.1%, 2.3%, 25.3%, and 16.4% higher in treatments CK, N, P, BC, and BC + P as compared with control. The data indicated that means of treatment BC, BC + N, and BC + P are statistically non-significant while means of treatments BC and BC + N are highly significant as compared with control. These results are in contrast to the investigation of Dempster et al. (2012); they used biochar prepared from *Eucalyptus marginata* for wheat crop at the rate of 0, 5, and 25 t ha^{-1}. They observed no change in the MBN by the application of either level of biochar. According to Pietikainen et al. (2000), biochar acts as a habitat for microorganism which involved in the transformation of N, P, and S.

Effect of treatments on soil enzyme activities

The data regarding the effect of biochar, N, P, BC + N, and BC + P on soil urease activity is presented in Fig. 3. Different treatments affected the urease activity as highest urease activity was observed in treatment BC + N which was 4.90% higher as compared with control (CK). While in N, P, BC, and BC + P treatments, urease activity was 2.6%, 2.1%, 3.5%, and 4.2% higher than control. The treatments (in which biochar and combination of biochar along with N and P fertilizer were used) were non-significant while these treatments are highly significant as compared with control. Jindo et al. (2012) studied the effect of 2% (v/v) addition of biochar on quality of a composting mixture produced from poultry manure and other organic materials. By evaluating the quality of compost, they observed 30–40% increase in urease activity. The results of current study are in contrast to the study of Wu et al. (2012). They used wheat straw and its biochar in a 100-day incubation study and observed that urease activity was decreased as compared with control, while biochar increased urease activity as compared with its straw. The result of our study is supported by the study of Lee et al. (2011); they conducted an incubation experiment using biochar at the rates of 0, 1, 3, 5 10, 20, and 30% of soil and found that 30% biochar application had highest urease activity (13.17-fold) as compared with control.

Data regarding the effect of treatments on alkaline phosphatase activity is presented in Fig. 4. Maximum phosphatase activity was recorded in treatment biochar plus P, which was 23.9% higher than control. Similarly, phosphatase activity is 16.3%, 9.3%, 9.0%, and 6.1% higher in treatments BC + N, BC, P, and N respectively as compared with control. It was observed that BC + P increased the phosphatase activity 13.3% higher than biochar only. The treatment in which biochar is used in combination with phosphorus is statistically significant as compared with control while all other treatments are non-significant with control. Yang et al. (2013) conducted a paddy field experiment by using biochar prepared from a wheat straw at 350–550 °C at the rate of 0, 5, 10, 20, and 40 t ha^{-1} and reported 29.7–193% increase in phosphatase activity. Jindo et al. (2012) studied the effect of 2% (v/v) addition of biochar on quality of a composting mixture

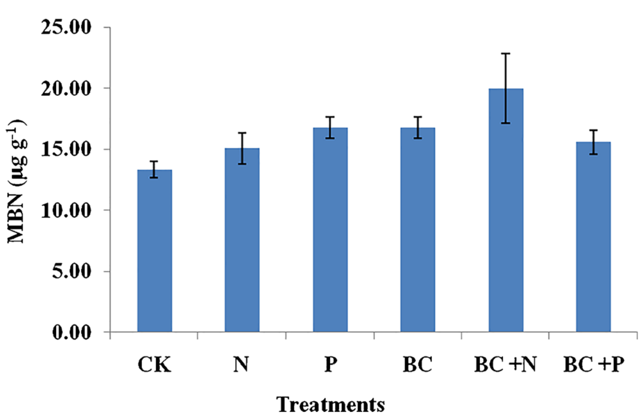

Fig. 2 Effect of treatments on soil microbial biomass nitrogen (MBN)

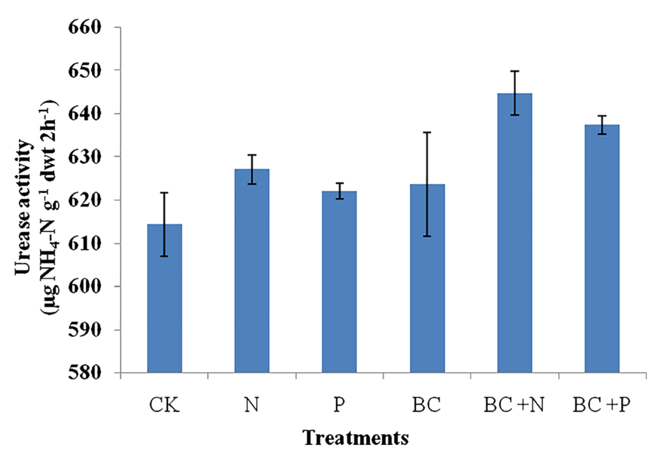

Fig. 3 Effect of treatments on soil urease activity

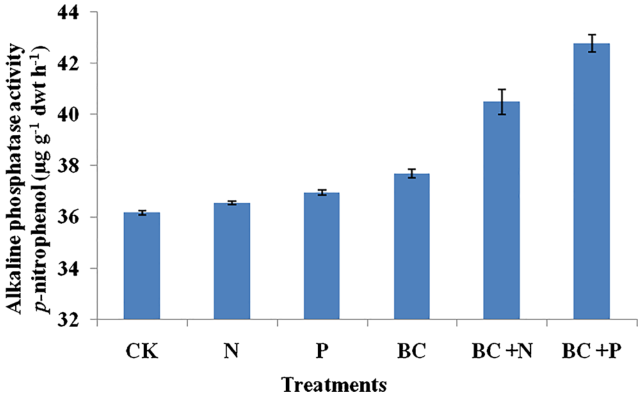

Fig. 4 Effect of treatments on soil alkaline phosphatase activity

produced from poultry manure and other organic wastes. By evaluating the quality of compost, they observed 30–40% increase in phosphatase activity. Lee et al. (2011) conducted an incubation experiment using biochar at the rate of 0, 1, 3, 5 10, 20, and 30% (weight basis of soil) and observed that by the application of 30% biochar had highest alkaline phosphatase (54-fold). The increase in phosphatase activity could be due to high pH of soil.

Effect of treatments on soil microbial population

The data regarding the effect of various treatments on the quantity of soil microbial population is presented in Fig. 5. The results indicated that significant increase in bacterial quantity was observed by the application of various treatments. The maximum bacterial quantity was observed in treatment BC which was 141% higher as compared with control (CK). Similarly, 105%, 71%, and 42% increase of bacterial quantity was in treatments BC + N, BC + P, P, and N respectively as compared with control; however, when biochar was used in combination with N and P, then bacterial quantity was decreased. Han et al. (2013) used biochar prepared from corncob by pyrolysis at 350–550 °C at the rate of 0, 12.5, and 25 t ha^{-1} in the rhizosphere of spinach and observed about 1.06–1.25-fold increase in bacterial quantity.

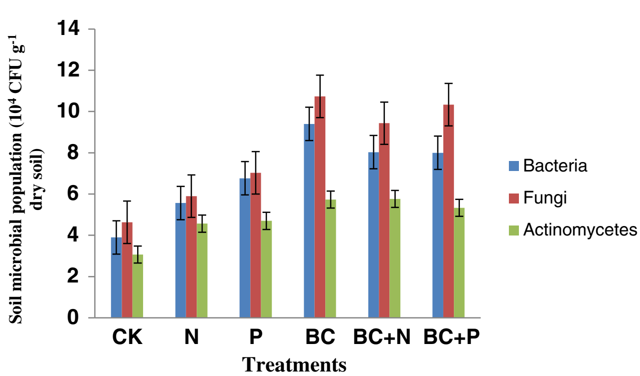

Fig. 5 Effect of treatments on soil microbial population (10^4 CFU g^{-1} dry soil)

Prabha et al. (2013) observed maximum heterotrophic bacterial count (39 × 10^6 cfu) in treatment where 35-g biochar was used for 5-kg soil as compared with control (14 × 10^6 cfu). These observations support that biochar and organic amendments can increase the soil microbial community by providing suitable substrates. The application of biochar can also cause changes in soil biological community composition and quantity (Gaskin et al. 2008). Biochar provides a suitable medium because it has porous structure and high internal surface area, and it has the ability to adsorb nutrients, gases, and for microbes to colonize, grow, and reproduce, particularly, for bacteria. Treatments have significant effect on quantity of fungi over control. The maximum population of fungi was observed in treatment BC which was 131% higher than control (CK). Similarly, 103%, 123%, 51%, and 27% increase of quantity was observed in BC + N, BC + P, P, and N treatments as compared with control. Our results indicated that biochar can increase fungal growth and population. Warnock et al. (2007) had shown that use of biochar could promote colonization of mycorrhizal fungi on plant roots and increase abundance of mycorrhizal fungi. The abundance of fungi mainly depends upon type of biochar and soil. Steinbeiss et al. (2009) observed that yeast-derived biochar increased fungal biomass up to 16% in both arable and forest soils. Treatments showed significant increase in actinomycetes quantity. The maximum bacterial quantity was observed in treatment BC + N which was 88% higher as compared with control (CK). Similarly, 87%, 74%, 53%, and 49% increase of bacterial quantity was in treatments BC, BC + P, P, and N respectively as compared with control. Hamer et al. (2004) reported that certain microorganisms use black carbon as the only carbon source for their survival which explained that biochar addition could promote the growth of certain type of microorganism that could improve soil fertility for long term. The present study indicated that application of biochar in soil could significantly increase quantity of actinomycetes.

Effect of treatments on plant parameters

The result regarding the effect of treatments on total nitrogen of shoots is presented in Fig. 6. The results indicated significant differences among treatments. Maximum total nitrogen was recorded in treatment BC + P which was 32% higher than control. Similarly, total nitrogen is 30%, 26%, 21%, and 13% higher in treatments BC + N, BC, P, and N respectively as compared with control. When biochar was used in combination with N and P, then total nitrogen was 3% and 4% higher as compared with biochar only. Sika (2012) conducted 12-week wheat experiment using biochar along with water-soluble fertilizers. She found that fertilized treatments amended with 10% biochar had higher N % in plant tissues as compared with control. The possible increase in N % in plant tissues might be due to high uptake of nitrogen because biochar enhances the N uptake. Van Zwieten et al. (2009) observed same effect of biochar on nitrogen uptake and concluded that biochar application can significantly increase plant N uptake. The data regarding the effect of various treatments on nitrogen of grains indicated that maximum increase in total nitrogen over control was observed in BC + P treatment which was 21% higher than control. Similarly, 18%, 18%, 14%, and 11% increase in total nitrogen was observed in treatments BC + N, BC, P, and N respectively as compared with control. Statistical analysis indicated that means of all treatments (N, P, BC, BC + N, BC + P) are highly significant as compared with control. Results of present study are in contrast to the findings of Sika (2012) who observed a decrease in nitrogen contents of wheat grain in biochar-amended treatment. This decrease occurred because plant invested more energy on vegetative growth and as crop was approaching to maturity soil became depleted of nutrients and therefore did not have sufficient N for grain production. In the present study, we observed maximum nitrogen contents in biochar-amended treatments. This could be due to higher uptake of N from soil due to biochar. Chan et al. (2008) also observed higher nitrogen uptake in radish plants sown in biochar-applied soil.

Data regarding the effect of different treatment on total phosphorus of grains is presented in Fig. 7. Results indicated increase in total phosphorus contents by the application of various treatments over control. Maximum total phosphorus contents were observed in BC + P treatment which was 78% higher than control. Similarly, 78%, 67%, 31%, and 20% increase in total phosphorus was recorded in treatments BC + N, BC, P, and N as compared with control. Sika (2012) observed maximum P % in wheat grain in a treatment in which 10% biochar was used along with inorganic fertilizer. In our study, maximum P % was recorded in biochar-amended treatments; it might be due to higher P uptake due to more availability of phosphorus from soil because of biochar. Maximum total phosphorus was observed in treatment BC + N which was 43% higher over control. Total phosphorus was 31%, 36%,

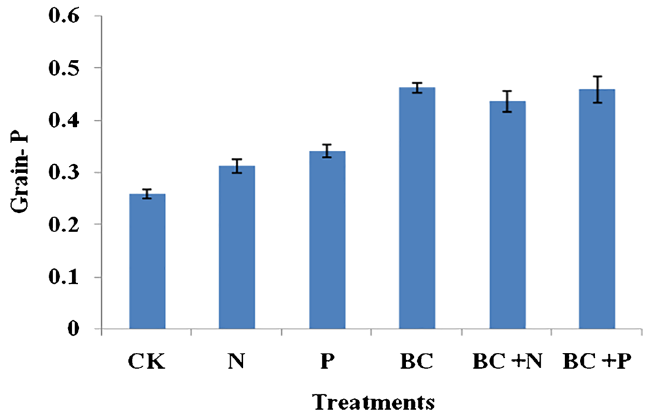

Fig. 7 Effect of treatments on mung bean grain phosphorus (%)

26%, and 17% higher in treatments BC + N, BC, P, and N respectively as compared with control. When biochar was used in combination with N, then total phosphorus was 5% higher as compared with biochar. Sika (2012) found that fertilized treatments amended with 10% biochar had higher P % in plant tissues as compared with control. Lehmann et al. (2003) also noted an increase in phosphorus concentration in plants by increasing level of biochar application.

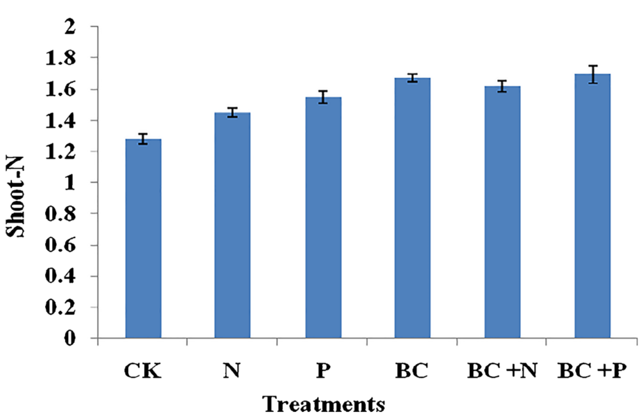

Fig. 6 Effect of treatments on mung bean shoot nitrogen (%)

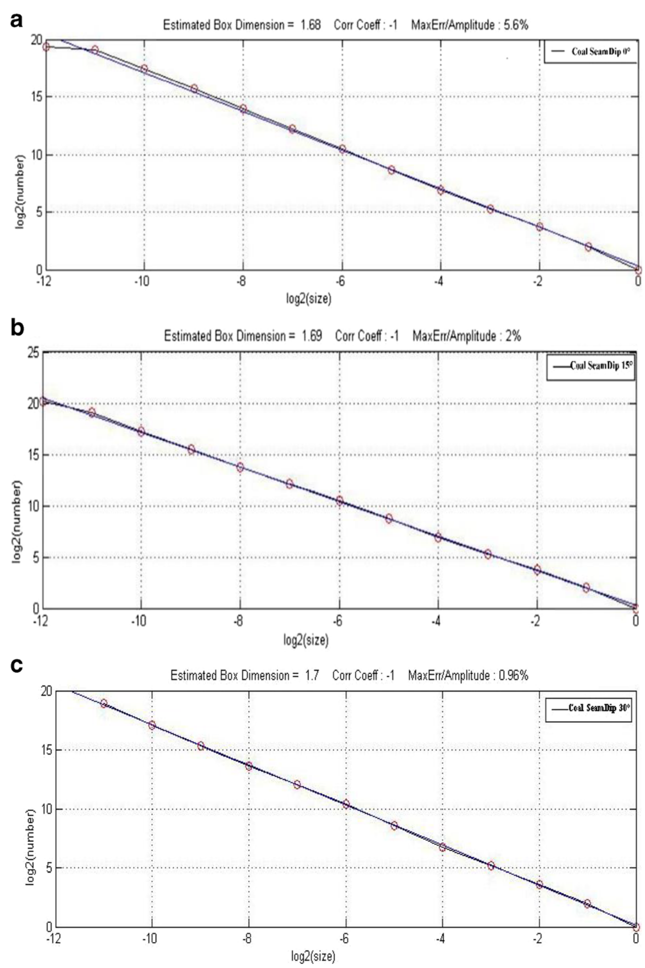

Fig. 8 Effect of treatments on shoot length (cm) and plant biomass (g)

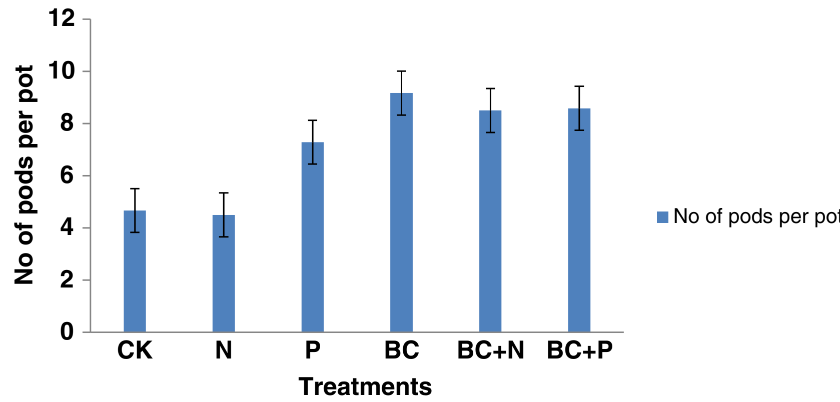

Fig. 9 Effect of treatments on the number of pods per pot

The data regarding the effect of different treatments on shoot length (Fig. 8) showed maximum shoot length and was observed in treatment in which biochar was used; it was 3.75% higher than control, while in all other treatments, shoot length was decreased as compared with control. The minimum shoot length was observed in treatment where only nitrogen was used; it might be due to immobilization of NO_3 and less availability for plant growth. Van Zwieten et al. (2009) found comparable results in a wheat pot trial using paper mill sludge biochar applied at 10 t ha^{-1} and observed 30–40% increase in plant height of wheat. The increase was primarily attributed to the liming potential of biochar. The increase in plant height might be due to ability of biochar to provide the essential nutrient to plants especially where nutrient resources are in short supply. Data regarding the effect of different treatment showed maximum plant biomass (Fig. 8) in BC treatment which was 45% higher than control. Similarly, plant biomass was 43%, 31%, and 10% higher in treatments BC + N, B + N, BC + P, and P respectively as compared with control. The results indicated that means of treatment (BC, BC + N, and BC + P) are statistically non-significant while these are highly significant as compared with control (CK). Dao et al. (2013) examined the effect of four different biochars on maize crop and observed that biochar from coconut husk, bamboo, and rice hulls increased above-ground biomass 3.4, 2.5, and 2.3 times than control treatment which received no biochar.

Statistical analysis of data indicated that different treatment (N, P, BC, BC + N, BC + P) caused significant variation in the number of pods per pot of mung bean. Biochar resulted in maximum number of pods per pot which was 96% higher than control as depicted in Fig. 9. Similarly, the number of pods per pot is 82%, 83%, 30% higher in treatments BC + N, BC, and P respectively as compared with control. It was observed that biochar used in combination with N and P were similar regarding number of pods per pot. It was observed that for number of pods per pot, biochar treatments remained statistically non-significant among themselves except control and N treatments. Arif et al. (2012) observed maximum number of grains in maize crop in the treatment in which biochar was used in combination with NPK as compared with control.

Conclusions

Biochar application alone or in combination with N and P fertilizers has a significant effect on different soil biology and fertility indices and plant growth. Combination of biochar with phosphorus (BC + P) proved superior for improving microbial biomass carbon (65.8%), alkaline phosphatase (23.9%), total shoot nitrogen (32%), total grain nitrogen (21%), and grain phosphorus (78%) over control. Moreover, alone application of biochar (BC) improved bacterial population (141%), fungal populations (131%), shoot length (3.75%), and number of pods per pot (96%) over control. It can be concluded that biochar prepared from populous leaves can be used effectively along with N and P fertilizers to improve microbial activity of soil and consequently crop yield.

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