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Biochar amendment improves alpine meadows growth and soil health in Tibetan plateau over a three year period

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ABSTRACT

Previous biochar research has primarily focused on agricultural annual cropping systems with very little attention given to highly fragile, complex and diverse natural alpine grassland ecosystems. The present study investigated the effect of biochar on the growth of alpine meadows and soil health. This study was conducted in the Qinghai Tibetan Plateau over a three year period to investigate the effect of three rice husk biochar application rates alone and combination with high and low NPK fertilizer dosages on alpine meadow productivity, soil microbial diversity as well as pH, carbon and nitrogen content at 0-10 cm and 10-20 cm depth. At the end of the 3rd year soil samples were analysed and assessed by combined analysis of variance. The results showed that biochar application in combination with nitrogen (N), phosphorus (P) and potassium (K) fertilizer had a significant increase in fresh and dry biomass during the second and third year of the study as compared to control and alone biochar application ($p \leq 0.05$). Biochar alone and in combination with NPK fertilizer resulted in a significant increase in the soil pH and carbon contents of the soil. XPS results, the SEM imaging and EDS analysis of aged biochar demonstrated that the biochar has undergone complex changes over the 3 years as compared to fresh biochar. This research suggests that biochar has positive effect on alpine meadow growth and soil health and may be an effective tool for alpine meadow restoration.

Keywords Biochar · Alpine meadows · Soil health

Introduction

Grasslands are the largest extended biome on earth and play a significant role as carbon sink (He et al. 2009). The grasslands store about 34 % of the global terrestrial carbon and are highly fragile in terms of carbon stability (Cheng et al. 2011). The carbon stocks in grasslands have been notably driven by land-use changes and management measures (Cheng et al. 2011; Sun et al. 2011; Sousa et al. 2012). Since soil carbon and nitrogen cycles closely interact, it is important to examine how anthropogenic factors such as overgrazing affect both C and N stocks and their interactions in the soil (Houghton et al. 1999; He et al. 2008).

The Tibetan Plateau is a main watershed region for China, India, and Pakistan representing a distinct cryospheric environment (Wang et al. 2007; Shi et al. 2010). The plateau is a source of usable water for nearly 40 % of the world's population, including China and India (United Nations Environment Programme (UNEP). 2007). The plateau has the largest biome plateau area on the Eurasian continent and represents a major ecological region with the lowest-latitude permafrost in the globe (Wang et al. 2002). Diverse types of grasslands extending from the Tibetan Plateau to Inner Mongolia and the mountains of the Xinjiang province, thus constitute the third biggest grassland ecological unit on earth (Yang et al. 2012). About 85 % of the plateau consists of alpine grasslands serving as a major source for livestock grazing (Dong et al. 2010; Harris. 2010), predominantly yak and Tibetan sheep. Alpine grasslands provide additional vital ecosystem services such as carbon capture, biodiversity, soil and water conservations (Yang et al. 2004; Chen et al. 2008; Wang et al. 2009). The C stored in soils of the plateau (33.5×10^9 t C) makes up 2.4 % of total world soil C (Wang et al. 2002) but due to poor land management this carbon is being lost at an increasing rate.

Similar to other ecosystems, Tibetan plateau grasslands have been experiencing considerable deposition of atmospheric N in the form of nitric acid over the past three decades (Yang et al. 2012). Persistent acidification of the soil decreased pH and increased base cation loss, resulting in enhanced aluminium toxicity and loss of soil productivity (Bowman et al. 2008). Sustained longer acidification of soil could also modify formation and function of grasslands ecologies, such as plant biodiversity loss, loss of biomass productivity, and fractional inhibition of C and N cycling (Liu et al. 2011; Yang et al. 2012).

Furthermore, raising more yaks and removal of yak dung results huge carbon and nitrogen losses in Tibetan grasslands. In 2006, 40 million tons of yak dung was produced and 60% of that was collected for household energy needs. The removal of yak dung from grasslands results a loss of 16 million tons of carbon, 0.8 million tons of N and 0.2 tons of P on annual basis, not only altering the C and N cycles on plateau but also causes grassland degradation (Cai et al.,2013; Ni, 2002; Tian et al., 2006; Lu et al., 2015).

Previous management measures including fencing of pastures, reduction in numbers of livestock and fertilizer applications have been practiced to restore these degraded grasslands (Akiyama and Kawamura. 2007).However, these management practices have not been demonstrated to restore the extremely degraded grasslands of the plateau (Wu et al. 2010b). Fertilizer application improves grassland productivity and restores degraded grasslands. Research investigations have shown that N-P-K fertilizer can enhance grassland production and its forage quality. However, due to grassland degradation phenomenon, there is less nutrient maintenance is grassland vegetation and nutrients are more prone to leaching. Biochar, produced by thermal decomposition of organic material (Lehmann and Joseph. 2009), has been shown to improve low fertility soils as well as sequester carbon to mitigate global warming (Lehmann et al. 2006; Sohi et al. 2010; Woolf et al. 2010). Biochar applications to low fertility soils have improved yields in different cropping patterns worldwide (Glaser et al. 2002; Jeffery et al. 2011; Kammann et al. 2011; Vaccari et al. 2011; Spokas et al. 2012; Wang et al. 2012). Additionally, biochar application, reduce soil acidity (Knowles et al., 2011), increase cation exchange capacity (CEC) of soil (Mikan and Abrams,

1996) and reduce concentrations of pollutants. Significant reduction of leaching of fertilizer N from soil has been reported as a result of amendment with biochar produced from forest residues (Manolikaki & Diamadopoulos, 2017). Reduction of nitrate leaching from soil amended by biochar produced from pecan shells has been demonstrated over 25 and 67 days (Chaplot & Cooper, 2015).

Yak dung clay blended biochar and yak manure biochar has been proved to enhance production of blue grass in an artificial pasture and highland barley crop in short term in Tibetan plateau (Rafiq et al., 2017 and Zhang et al., 2018), however yak manure has been used for household cooking purposes and have competitive uses for its conversion to biochar. Rice is one of the most widely cultivated agricultural crops in China. In China, approximately 54 million tons of rice husk is produced every year. The high volumes of rice husks that are considered as waste after milling are not appropriately treated. Rice husk is one of the main feedstock used to produce bio-oil by fluidized-bed reactors or other fast pyrolysis systems in China (Wang and Liu, 2018). Abundant biochar produced during the process of fast pyrolysis as by-product in China could be a potential application for grassland restoration.

Keeping in view, this study therefore aims to investigate the dosage effect of surface-applied rice husk biochar and NPK fertilizer on fresh and dry yield of grassland biomass under field conditions over a period of three years. Changes in pH, C and N content at 0-10 cm and 10-20 cm depth as well as microbial functional diversity are also elucidated.

Materials and methods

Experimental field site

The field study was carried out at Dawu village, Maqin County, of the Golou Tibetan Autonomous Prefecture of Qinghai Province, China (34° 28′11″ N, 100° 12′39″E). The alpine meadow is located at 4200 m above sea level. The soil type of the study field is silt-clay, an alpine meadow soil as declared by Chinese System for Soil Classification. The average annual temperature of the area is −0.6 °C, ranging from −10°C during the month of January to 11.7°C in the month of July. The annual mean precipitation is 513 mm occurring during the months of May to September. There is no entirely frost-free period. The primary vegetation type in the area is alpine meadows dominated by *Kobresia spp*, *Polygonum spp.* and *Poa spp.*

Characterization of experimental biochar

Rice husk was obtained from Hangzhou, Zhejiang Province (China) and converted to biochar at a pyrolysis temperature of 500˚C using a vertical furnace with continuous feeding (Jiaxing JIAHUA Animal Husbandry Co., Ltd., Zhejiang, China). The physico-chemical characteristics of the biochar such as pH, ash content, total nitrogen, total carbon, total hydrogen, total phosphorous, total potassium, calcium, magnesium, sodium were analysed. The pH of biochar was measured in deionized water at the ratio of 1:5 wt/wt with a calibrated Orion 720 pH meter (Enders et al., 2012). Ash content was analyzed by heating biochar samples at 500°C for 8 h in a muffle furnace (Dai et la., 2013). The elemental composition was determined according to Enders et al. (2012) using an elemental analyzer from Elementar Analysensysteme GmbH (varioELcube). Nutrient elements Ca, K, Mg, Na, and P were measured using an inductively coupled plasma-atomic emission spectrometer (IRIS ER/S). Before analysis, the biochar sample (about 0.05 g) was first digested by the concentrated HNO_3/H_2O_2 solutions (Dai et al.,2013).

BET (N_2) surface area, FTIR and thermo gravimetric analysis (TGA) were determined prior to field application according to techniques reported by Rafiq et al. (2016). X-ray photoelectron spectroscopy (XPS) spectra were collected from biochar powders with a thermo ESCALAB 250 spectrometer using an Al Ka monochromatized source and a multidetection analyzer under a 10^{-8} Pa residual pressure. Surface charging effects were corrected with C 1s peak at 284.6 eV as a reference. Examination of the biochars before and after the field trials was carried out using a Zeiss Sigma SEM with a Bruker X-ray dispersive spectrometer (EDS) detector.

Experimental design

The size of each experimental plot was 2×4 m. There was a distance of 50 cm between the experimental plots to serve as a buffer zone (Qi et al., 2015). There were twelve treatments in this experiment carried out in triplicate under randomized complete block design (RCBD). Biochar was applied at 3 application rates: low (2 t/ha, BC_L), medium (4 t/ha, BC_M) and high (6 t/ha, BC_H) to the grassland. Biochar application rates were selected based on the recommendations (Clare et al., 2014) that due to higher biochar production costs, it needs to apply around 1-5 t/ha to realise plant response. Furthermore, Rafiq et al., applied yak blended biochar @ 3 tons/ha on pasture areas in Tibetan plateau. Two levels of NPK fertilizer were applied (30N, 15 P and 10 K kg/ha) and (60 N, 30 P and 20 K kg/ha) and designated as NPK_L and NPK_H, respectively. Higher level of NPK fertilizer corresponds to the recommendations of (Yu li et., al 2015). The NPK fertilizer was applied in the form of urea for N, single superphosphate for P and potassium chloride for K. The detailed plan of the treatments applied include: T_1 = CK (Control, no amendment), $T_2 = BC_L$, $T_3 = BC_M$, $T_4 = BC_H$, $T_5 = NPK_L$, $T_6 = NPK_H$, $T_7 = BC_L+NPK_L$, $T_8 = BC_L+NPK_H$, $T_9 = BC_M+NPK_L$, $T_{10} = BC_M+NPK_H$, $T_{11} = BC_H+NPK_L$ and $T_{12} = BC_H+NPK_H$. The biochar and NPK were applied through surface applications. The experiment commenced at the third week of June, 2014.

Vegetation and soil sampling

At the end of August 2014, 2015 and 2016, biomass samples were collected approximately 1 cm from the ground using 50×50 cm quadrat (Qi et al.,2015) while soil samples were collected at depths of 0 - 10 and 10 - 20 cm with the help of auger and placed into plastic bags and brought to the laboratory for further analysis of pH, carbon and nitrogen. At the end of August 2016, soil samples at a depth of 0 - 10 cm were collected for selected treatments as control T_1, T_3, T_6 and T_{10} to test effect of biochar and fertilizer on microbial functional diversity. In addition, biochar samples were subjected to microscopic and XPS analysis to investigate changes on the surface.

Biomass and soil measurements

Fresh biomass of the collected grass samples was weighed and recorded in the field soon after harvesting. The fresh samples were then put into paper bags and brought to laboratory for dry weight measurements. Biomass samples were dried at 65˚C for 48 hrs in oven (Pérez-Suáre at al., 2014) and their dry biomass recorded. After cleaning and sieving with a 2 mm sieve, the air-dried soil samples (dried till constant weight) were tested for pH, C and N. The pH value of the experimental soils was tested using 1 : 2.5 soil : water suspension (Thiele-Bruhn et al. 2015) with an Orion 720 pH meter with a combination electrode. Total carbon and nitrogen of the soil was determined using elemental analyzer (Elementer Analyse systeme GmbH, varioEL-cube).

Separation of aged biochars from soils

Biochar particles present in soil were collected from the experimental fields after three years during August 2016 and brought to laboratory. Biochar samples were shaken to remove soil particles in DI water solution at a ratio of 1:10 w/v. The biochar was then washed four times with distilled water and dried at 60 ˚C (Koide et al., 2011) for further XPS and SEM analysis.

Incubation experiment for microbial functional diversity analysis

The microbial functional diversity of soil microbial population was determined using the Biolog EcoPlate™ (BIOLOG Inc., CA, USA). The soil samples were mixed with 90 mL of sterilized 0.85% (w/v) NaCl solution and shaken for 20 min followed by pre-incubation for 24 hours to initiate microbial utilization of soluble organic compound present in the soil. Samples were brought to 10^{-3} final dilutions before inoculation. Biolog EcoPlate TM has 96-

wells with three repeats, each one consisting of 31 sole carbon sources and a control with water. The consumption rate of carbon sources was tested by the reduction in tetrazolium dye which turns from color less to purple. The optical density (OD) of incubated plates was measured at 590 nm and 25°C with a plate reader and monitored every 24 hr for 7 days. The Procedure adopted by Rafiq et al., 2017 was followed to to investigate the microbial diversity and activity in this study. Average well color development (AWCD) was calculated using the equation,

$$AWCD=\Sigma(C-R)/31$$

where C is optical density (OD) of every well of carbon and R is the OD value of control with water only.

Negative (C-R) values were excluded from further analysis.

Microbial functional diversity was measured with the Shannon index (H′) as follows,

$$H' = -\Sigma Pi \ln(Pi),$$

where Pi was determined by subtracting control OD from OD of every other well. After that it is divided by the total OD for all 31 substrates.

Data analysis

Analysis of variance was conducted and Duncan's Multiple Range Test (DMRT) at 5% level of probability was employed to compare means. Computer based statistical package MSTATC following Steel et al. (1997) was applied for this statistical analysis. To evaluate the cumulative effect of twelve treatments over the three year period on fresh biomass (FB), dry biomass (DB) and soil properties PHA, PHB are pH values at 0-10 cm and 10-20 cm soil depths, NA, CA are nitrogen and carbon content at 0-10 cm and NB and CB indicates nitrogen and carbon content at 10- 20 cm soil depth., were analyzed and prior to data analysis all variables were subjected to normality test and found that data for all of the variables were distributed normally. Mean comparison was done using Duncan test for each dependent variable separately at 0.05 level. The data were subjected to principal component analysis (PCA) in a Multivariate analysis.

Results

Physico-chemical characterisation of rice husk biochar

The TG curves and FTIR spectra are provided in Figure S1. Most of the carbon (94 %) in the rice husk biochar remained even when heated to 700°C, indicating a highly stable carbon in the material. Fourier Transform Infrared (FTIR) spectroscopy revealed a broad peak at 3432 cm^{-1} and 577 as well as sharp peaks at 2922, 2880, 1644, 1421, 887 and x cm^{-1}. A weakly defined peak was also detected at 1122 cm^{-1}.

Table 1 summarises the physico-chemical characteristics of rice husk biochar used in the experiment. The pH value of biochar was 10.4 with a carbon content of 40.8 wt.%. The biochar had an ash content of 39.7 wt.% and trace amounts of N, P, K and other elements necessary for plant growth. The biochar has a BET surface area of 3.19 g/m^2 and an average pore width of 10.6 nm.

Biomass responses to biochar and fertilizer application on alpine meadow

Table 2 shows the biomass productivity response of the alpine meadow in Tibetan plateau as a result of biochar application from 2014-16. It was found that an increasing biochar application rate resulted in an increase in fresh and dry biomass yield during the first year of biochar application.. However, this increase in biomass was not statistically significant at the p = 0.05 level probably.. When biochar was applied together with NPK the best yield was observed for BC_M throughout the study period. The fresh biomass yields of the treatments like BC_H+NPK_H, BC_M+NPK_H, BC_L+NPK_H were significantly greater than biochar treatments ($p \leq 0.05$). The largest dry biomass yield was measured for the NPK_H treatment in 2014. During the second year, there was no significant difference in fresh and dry biomass yield between the control and those from the biochar applications alone. In contrast, NPK fertilizer application showed a significant increase for both fresh and dry grass yield as compared to the control and pure biochar applications. However, the greatest significant increase in fresh biomass yield was measured in the BC_M+NPK_H treatment. The fresh and dry biomass productivity of the meadow in the third year was significantly greater for all treatments compared to control. However maximum fresh and dry biomass yield was observed for the (BC_M+NPK_H) treatment throughout the study period. The increase in biomass as biochar and fertilizer application together indicates that responses of alpine meadows to addition of biochar and fertilizer were additive and positive.

Amelioration effects of biochar and fertilizer application on soil pH, carbon and nitrogen content of alpine meadow

The soil pH data at two soil depth levels for a period of three years is presented in Table 3. The addition of biochar led to increase the soil pH value significantly over a three years. The data indicate that soil had a lower pH at surface level (0-10cm) as compared to 10-20 cm of soil depth. Biochar application alone or combined with NPK fertilizer showed significantly higher pH values at 0-10 cm soil depth as compared to control and alone NPK fertilizer treatments ($p \leq 0.05$) during the first year of study. However, biochar addition results higher pH levels in the both soil depth levels during the second and third years of the study. This indicates that effect remained to persistent over time. The nitrogen content of alpine meadow soil at two depth (0-10 and 10-20) during 2014-16 is provided in Table 4. Application of biochar and fertilizer led to effective addition of nitrogen in soil. Nitrogen concentrations increased in the meadow soil with biochar application. A greater nitrogen content was observed at 0-10 cm soil depth level as compared to 10-20 cm. The greatest total nitrogen content (0.55 wt.%) in year one was observed for NPK_H treatment and there were significant increases between the control and the other treatments (except BC_L) at 0-10 cm depth during the first year of study ($p \leq 0.05$). In year 2, the most significant increase in N soil content (0.66 wt.%) was measured in the NPK_H treatment for the 0-10cm soil profile and in the BC_L^+ NPK_H for the 10-20cm profile.

Changes in soil carbon content over the three years for all treatments is given in Table 5. There was little change in the C content of the control at both depths over the 3 years. The addition of BC_H^+ NPK_H and BC_H^+ NPK_L resulted in the largest increase in C in the top soil profile in year one; BC_M^+ NPK_H in year 2, and BC_L^+ NPK_H in year 3. For the samples taken at depths between10-20 cm, the greatest C content was measured in BC_M, BC_H^+ NPK_H and BC_M^+ NPK_H for year 1, BC_M^+ NPK_H in year 2 and BC_L in year 3.

Cumulative impact of different treatment on biomass and soil properties over three years.Based on a combined ANOVA over three years, results revealed that there was a significant effect of time on all dependent variables (Table 6). The results also show that all dependent variables were statistically significant in all treatments. Results of mean comparison among treatments using Duncan Multiple range test indicated that the greatest mean for fresh biomass(FB) was observed in BCM+NPKH (179 ± 18 g/m^2) which was significantly higher than other treatments and the lowest level of FB belonged to BCL, BCM, BCH which were not statistically different from the control group. For DB, results of mean comparison showed that the highest mean of BD was observed for BC_M + NPK_H (114 ± 15 g/m^2) which was higher than other treatments.

The highest level of soil pH (A) (Table 7) was measured in treatment BCH (6.99 ± 0.05), which was significantly different from other treatments and the lowest level of pH (A) was observed for control group (6.63 ± 0.04). For pH (B) results of mean comparison showed that three treatmentshad the highest level including BCH (7.02 ± 0.03), BCL+NPKL (7.02 ± 0.04) and BCL+NPKH (7.01 ± 0.04), which were not statistically different. The lowest pH (B) belonged to control group (6.76 ± 0.03) and BCM+NPKH (6.84±0.06). These results indicated that the level of N (B), NPKL (0.41±0.02) was significantly higher than other treatments except BCM, BCL+NPKH, BCM+NPKH and BCH+NPKL. Results of mean comparison for C(A) and C(B) revealed that BCM+NPKH had the highest means score for both C(A) (7.37 ± 0.39) and C(B) (4.3 ± 0.3) two variable.

The data were subjected to principal component analysis (PCA) in a multivariate analysis. Biomass productivity and soil characters have been used to define patterns on the impacts of treatments applied. Results showed that three components with Eigen values more than one were extracted and these three components explained 74.1 % of total variability (Figure 1). This shows great variation among biomass productivity and soil characteristics under investigation. The first principal component (PC1) comprising of PHB, PHA and NB explained 25.0 % of total variability (Table 8). The characters with greatest positive weight on PC2 were CB and CA and these components explained 24.7 % of total variance among all data. DB, NA and FB were associated with the third principal component (PC3) which explained 24.4 % of total variance.

Effect of biochar and fertilizer on functional and microbial diversity

Figure 2 shows that BC_M+ NPK_H had the highest AWCD values at 144 hours as compared to CK, BC_M and NPK. The results showed that biochar and NPK fertilizer applied in combination had positive impacts on the microbial activity as compared to control or other selected treatments.

The values of microbial diversity (***H'***) at incubation of 144 h against different treatments showed that biochar application (BC_M) alone and in combination with NPK fertilizer (BC_M+ NPK_H) had higher Shannon Index values indicating that biochar addition can improve soil microbial diversity (Table 9).

X-Ray photoelectron and electron dispersive spectroscopy of original and aged biochar

XPS and SEM-EDS analysis of original and aged biochar shows that the biochar has undergone complex changes over the 3 years (Table 10, Figure 3 A,B). There has been a decrease in the aromatic carbon and an increase in organic compounds yielding a higher content of C/O and C/N functional groups, K, Si, Ca, Mg, N, S and Fe atomic % than the control soil and stored biochar (Table 10). The – C=C- functional group constituted 63.4 mol

- % in original biochar, while biochar extracted from the soil had 51.1 mol-%. The functional groups – C-OH, C-O-C=, C-O-R and – C-N, C=O increased upon aging in soil.

SEM-EDS results show that the surface of original biochar has a relatively large content of Si, no detectable Fe and only relatively small concentrations of K, Ca, S, Al, P and Cl. The aged biochar, on the other hand, contained higher concentrations of K, Fe and Mn and Al. These images and elemental and functional group measurements are indicative of the formation of organo-mineral clusters on the surface of the biochar.

Discussion

It has been observed in several studies that biochar addition to soils due to its various properties has improved soil fertility and thus increased crop yields on agricultural lands (Marris. 2006; Chan et al. 2007). The characterization for pH, C, N and ash content were within the range reported for rice husk biochars used by Manickam et al. (2015). BET (N_2) surface area of rice husk biochar used in this experiment was lower than rice husk biochar produced in gasifiers (Manickam et al. 2012) as well as the peanut biochar used by Du et al. (2018). The observed variability is attributed to differences in process conditions primarily temperature (Rafiq et al. 2016) as well as feedstock type.

Observed FTIR peaks are in close agreement with biochars produced Sharma et al. (2004) from lignin at pyrolysis temperatures ≥ 450°C. FTIR peaksat wavelengths 3432 and 1122 cm^{-1} are attributed to -OH and C-O stretching vibration of phenolic compounds (Sharma et al. 2004; Ma et al. 2017). The appearance of peaks at 887 and 790 cm^{-1} are not only indicative of aromatic C-H but also evidence of formation of fused ring systems (Sharma et al. 2004). Sharma et al. (2004) observed a slow decrease in aliphatic CH stretch (2800-3000 cm^{-1}) with increase in pyrolysis temperature. . The presence of aliphatic CH was also observed in rice husk biochar used in this study suggesting that it originated from lignin.

The H/C molar ratio of 0.26 was well below 0.7 as required by IBI standards and EU guidelines (2012). The O/C molar ratio was 0.33 which meets the standards of the EU guidelines 2012). Similar H/C and O/C ratios have been reported for rice husk biochar in literature (Manickam et al. 2012). The molar H/C_{org} ratio can be used to predict the relative amount of organic biochar carbon that remains after 100 years incubation in soil (Budai et al. 2013). The organic carbon content in our rice husk biochar (Table 1) was assumed to be the same as total carbon since the carbonate content in wood and grass based biochars was found to be negligible (Enders et al. 2012). Hence, 91 wt.% of the rice husk biochar carbon can be expected to remain in alpine meadow soil after 100 years barring other factors such as loss due to erosion.

The application of rice husk biochar showed positive effects on alpine meadows biomass productivity over three years with and without NPK fertilizers. Crop productivity is often reported to increase with biochar application to soils but not always consistently (Jeffery et al. 2011; Subedi et al. 2016). The results from soil trials demonstrated that biochar/NPK fertilizer can assist in alpine meadow restoration. The biochar and NPK application did not show a significant impact on biomass yield during the first year of application however, in the subsequent years as in second and third years biomass yield was observed having a significant increase with the application of biochar with and without fertilizer (Table 2). Delayed impacts of biochar application on biomass improvements, till one or two years, have been reported in the literature (Haefele et al. 2011; Carvalho et al. 2016). These finding are consistent with the findings of this experiment. We observed biomass improvements during second and third years of the biochar application. Furthermore, results showed that significantly improved the biomass productivity of meadows during the second and third years. Persistent increase in crop productivity following biochar inputs are a good indicator of economic viability for scaling up the applications (Liu et al., 2013). Similar results were also reported by Adam et al (2013) and Slavich et al (2013) who observed that biochar has the ability to improve prairie growth and prairie restoration. Possible reasons for the nonsignifcant effects of biochar on forage biomass during the first year may be related to lower biochemical processes in alpine areas having lower temperatures, in the presence or absence of biochar, plus slower biochar degradation and its interaction with soil and consequently delaying its beneficial effects on soil properties and plant productivity (Verheijen et al. 2010; Fang et al. 2015). Several mechanisms for increase in biomass yield after biochar applications have been discussed in the literature. These include liming effects of biochar, improved water holding capacity of soils, nutrient use efficiency and reduced leaching, improved soils structure and porosity and increased surface area for nutrient adsorption. Many studies shown that over time aging of biochar in soils have more produced effects of biochar on coil moisture content (Paetsch et al., 2018). This increased moisture content and improvement of soil structure amended with biochar leads to effective root system development for water and nutrient supply. Perhaps, these factors contributed to the improved biomass productivity of alpine meadow after biochar application in this experiment.

The results showed that biochar application improved the soil pH values in the alpine meadows. The plant feedstock materials that are used to produce biochar contain base cations and these cations are transferred to biochars during pyrolysis of organic materials. The rice husk biochar contains high concentrations of soluble oxides, hydroxides and carbonates of Ca, Mg and K (Table 1), which may have contributed to the increase in soil pH, as observed in our study (Table 3). Increase in soil pH values has also been reported by Laird *et al.* (2010), where biochar with high ash content (14-56%), similar to present study (37% ash), were used. The alkalinity character is enhanced with pyrolysis temperature allowing rice husk biochar to act as a liming agent (Lehman et al. 2007; Wang et al. 2014).

Similar findings were reported in previous studies (Demirbas et al. 2004; Chan et al. 2007; Revell et al. 2012; Wang et al. 2014). The application of biochar due to its ability to act as a liming agent improved soil pH levels. Similar findings were reported in previous studies (Demirbas et al. 2004; Chan et al. 2007; Revell et al. 2012; Wang et al. 2014). Similarly, Novak et al. (2009) found that biochar enhanced soil pH in the southern United States. Wang et al (2014) also showed that biochar application could increase the carbon content in soil. Similarly it have be investigated that use of biochar application in prairie rehabilitation initiatives and proved biochar addition not only enhances improve the growth of prairie species, but also sequestered carbon (Lehman et al. 2007) and accelerated the recovery of carbon pool in these soils improve the growth of prairie species, but also sequestered carbon (Lehman et al. 2007) and accelerated the recovery of carbon pool in these soils. The AWCD value in the well of an EcoPlate™ is a key indicator of microbial functional diversity, because it indicates the capability of soil microorganisms to utilize various carbon substrates. Previous findings have shown that the application of organic matter to soil can enhance microbial populations their diversity and activities (Gomez et al., 2006).The results of this experiment showed that biochar and NPK fertilizer applied in combination had positive impacts on the microbial activity and diversity. The biochar upon aging has shown (table:10, fig:4), that there are increased c/o functional groups in biochar. These characteristics have been proved to increase the abundance of beneficial microorganisms in soil (Ye et al., 2017). The findings are consistent with published studies that found that microbial activity enhanced with biochar application (Kolb et al. 2009; Liang et al. 2010). Liao et al (2016) also found that biochar application has positive effects on soil microbial diversity. XPS results and the SEM imaging and EDS analysis shows that the biochar has undergone complex changes over the 3 years and these changes are similar to those describe by Joseph et al (2010), Archanjo et al (2017) and Hagemann et al (2017). These images and elemental and functional group measurements are indicative of the formation of organo-mineral clusters on the surface of the biochar. Previous research (Joseph et al. 2010; Archanjo et al. 2017; Hagemann et al. 2017) has shown that these clusters with high content or redox active Fe and Mn minerals that are bonded by organic compounds that have a high concentration of C/O functional groups can increase the ability of plants to take up nutrients.

Conclusion

This study has demonstrated that biochar can have significant effects on biomass production, soil acidification and carbon sequestration. In addition, biochar showed positive effects on microbial diversity and activity. Application of biochar to natural, wasteland and degraded systems could be a potential strategy to sequester carbon (Woolf et al. 2010). Further research is required to evaluate the long-term effects of biochar species diversity plant and detailed soil dynamics like nutrient mineralization, availability and transfer to plant. Additionally, biochar application methods and biochar erosion aspects need to be investigated for its appropriate testing mechanism. More research work is also required to develop and test biochar from the local feed stocks and to enrich it with heterogeneous nutrient material like attapulgite clay for its cost effectivity and wider acceptability.

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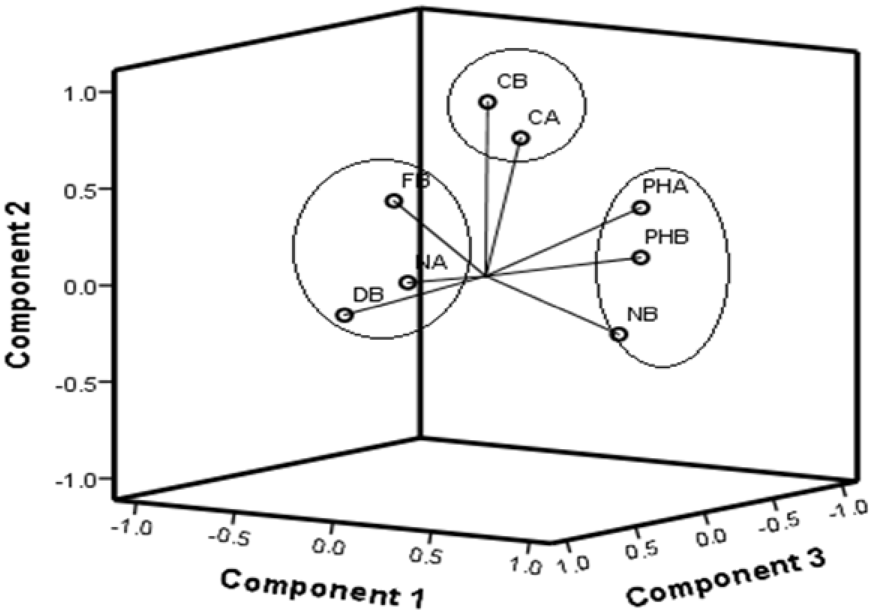

Figure.1 Scatter plot of the first three principal components of the PCA

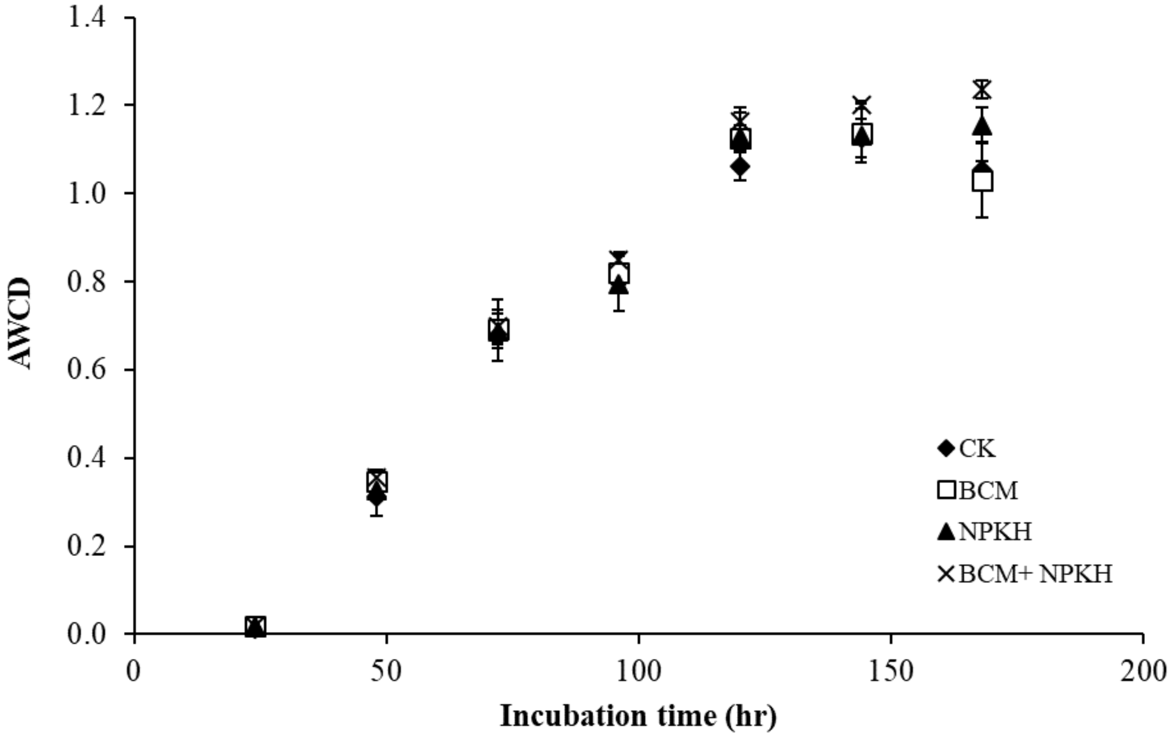

Figure.2 AWCD of metabolized substrates in Biolog EcoPlates using four different soil samples (n=3)

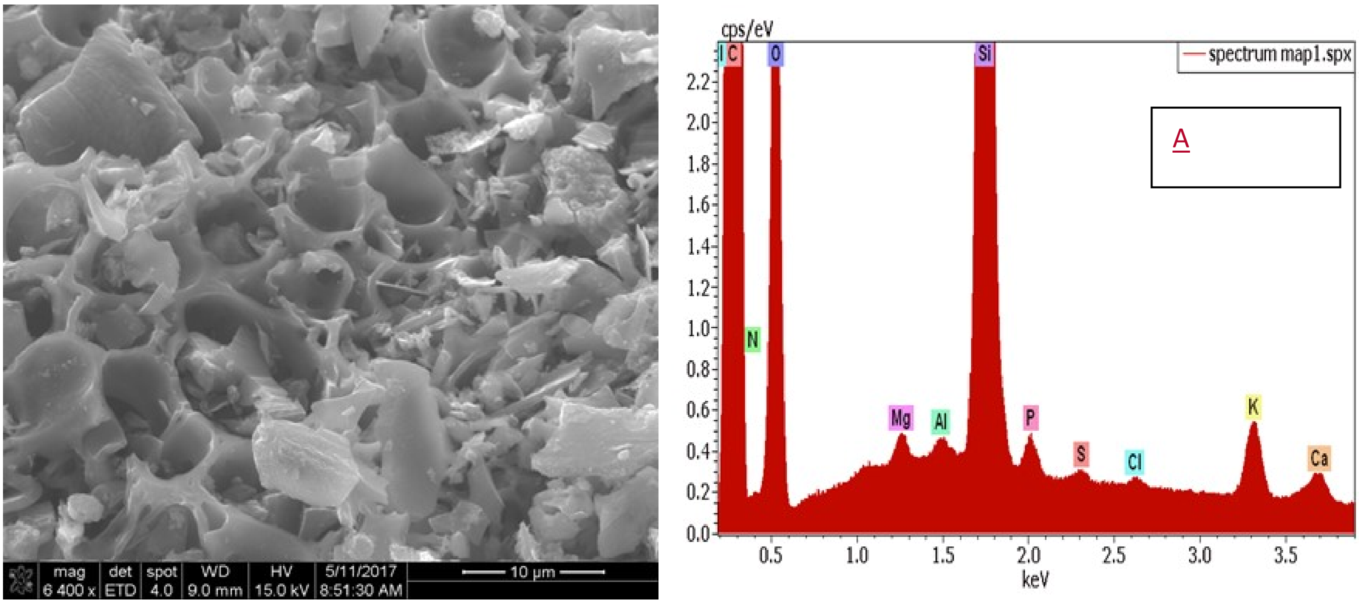

Figure 3. A. Secondary electron images and elemental analysis of the surface of fresh rice husk biochar

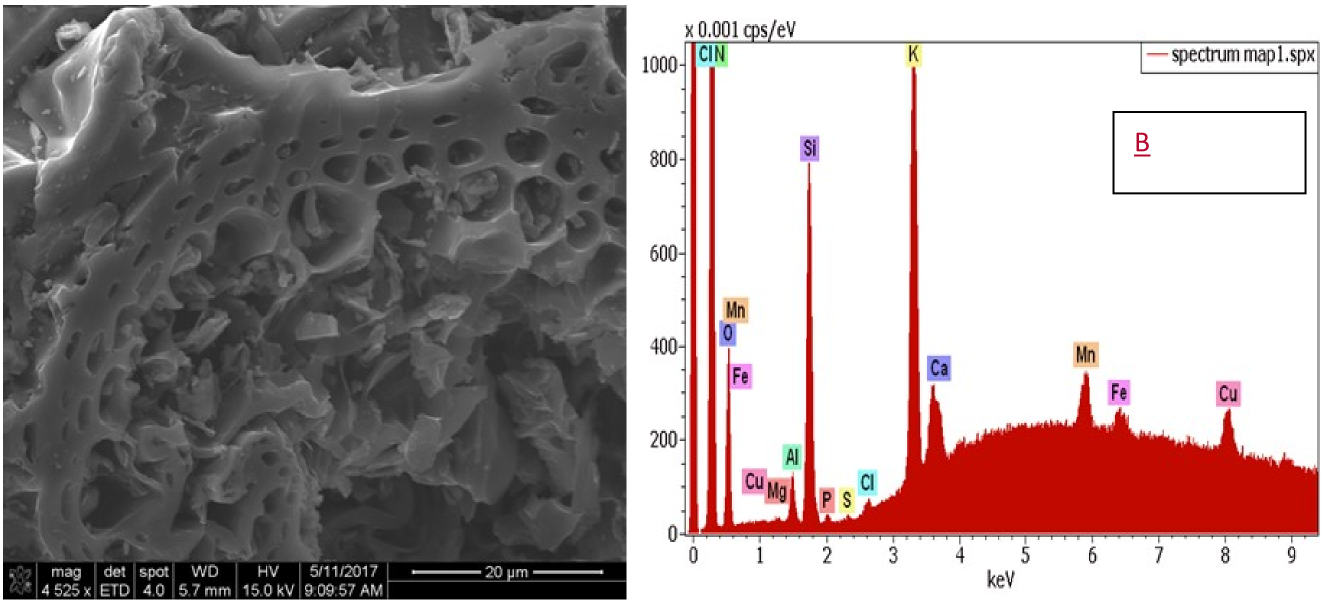

Figure 3. B Secondary electron images and elemental analysis of the surface of f 3 year aged rice husk biochar

Table 1. Major properties of rice husk biochar

Parameter	Value	Parameter	Value
Ash (wt.%)	39.7 ± 0.5	Fe (wt.%)	0.73 ± 0.02
pH	10.38 ± 0.02	P (mg/l)	10.3 ± 0.13
C (wt.%)	40.8 ± 1.3	K (mg/l)	47.9 ± 0.4
N (wt.%)	0.32 ± 0.03	Ca (mg/l)	11.0 ± 0.2
H (wt.%)	0.89 ± 0.21	Mg (mg/l)	6.20 ± 0.1
O (wt.%)	17.9 ± 0.7	Na (mg/l)	2.06 ± 0.06
S (wt.%)	0.41 ± 0.08	BET (N_2) surface area (m^2/g)	3.19
Si (wt.%)	11.92 ± 0.11	Average pore width (nm)	10.6

Table 2. Effect of biochar and fertilizer application on alpine meadow productivity over three years (2014-2016)

Treatments	Fresh Biomass (g/m^2)			Dry Biomass (g/m^2)		
	2014	2015	2016	2014	2015	2016
CK	101.47 abc	108.67 d	119.33 h	42.36 abc	49.73 e	58.93 g
BC_L	90.48 d	106.50 d	128.29 g	34.56 c	48.60 e	69.21 f
BC_M	97.13 bcd	119.93 d	129.92 g	37.06 bc	61.00 e	68.69 f
BC_H	96.27 cd	119.60 d	132.29 g	39.86 bc	64.87 e	70.70 f
NPK_L	101.77 abc	165.67 c	155.33 f	49.76 abc	84.30 d	75.82 f
NPK_H	104.43 abc	169.53 c	164.67 e	60.73 a	90.27 cd	84.68 e
$BC_L{}^+ NPK_L$	107.07 ab	177.67 bc	174.29 d	50.06 abc	97.47 bcd	104.31 d
$BC_L{}^+ NPK_H$	110.50 a	164.03 c	174.20 d	51.63 abc	95.20 bcd	100.62 d
$BC_M{}^+ NPK_L$	102.50 abc	170.33 c	191.21 c	45.33 abc	104.00 bc	128.66 b
$BC_M{}^+ NPK_H$	109.33 a	197.00 a	229.24 a	54.90 ab	136.27 a	152.31 a
$BC_H{}^+ NPK_L$	105.87 abc	175.80 bc	188.47 c	51.56 abc	103.80 bc	104.91 d
$BC_H{}^+ NPK_H$	108.63 a	190.37 ab	204.07 b	56.10 ab	111.97 b	118.33 c

Column means presented with different letters indicate significance differences ($p \leq 0.05$)

Table 3. Effect of biochar and fertilizer application on soil pH over three years (2014-2016)

Treatments	pH					
	2014		2015		2016	
	0-10 cm	10-20-cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm
CK	6.54 ef	6.64 d	6.62 f	6.79 e	6.72 g	6.83f
BC_L	6.77 abc	6.71 cd	6.84 de	6.87 de	6.92 ef	7.00 e
BC_M	6.87 a	6.71 cd	7.02 ab	7.02 ab	7.03 abc	7.11 ab
BC_H	6.80 ab	6.95 a	7.08 a	6.97 abc	7.09 a	7.13 a
NPK_L	6.49 f	6.72 cd	6.79 e	6.87 de	7.00 cde	7.01 e
NPK_H	6.53 de	6.75 c	6.90 bcde	6.91 cd	6.91 f	7.03 de
$BC_L^+ NPK_L$	6.77 abc	6.88 ab	6.99 abc	7.02 ab	7.08 ab	7.13 a
$BC_L^+ NPK_H$	6.75 bc	6.88 ab	6.98 abc	7.05 a	7.03 abc	7.09 abc
$BC_M^+ NPK_L$	6.69 cd	6.74 cd	6.91 bcd	6.92 cd	6.97 cdef	7.07 bcd
$BC_M^+ NPK_H$	6.78 abc	6.66 d	6.88 cde	6.81 e	6.94 def	7.05 cde
$BC_H^+ NPK_L$	6.79 ab	6.79 bc	6.87 cde	6.94 bcd	7.00 bcde	7.07 bcd
$BC_H^+ NPK_H$	6.85 a	6.93 a	6.94 bcd	6.96 abc	7.00 bcd	7.04 cde

Column means presented with different letters indicate significance differences ($p \leq 0.05$)

Table 4. Effect of biochar and fertilizer application on soil nitrogen content over three years (2014-2016) period

Treatments	Nitrogen (wt.%)					
	2014		2015		2016	
	0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm
CK	0.37 ef	0.29 b	0.35 f	0.27 e	0.39 c	0.27 f
BC_L	0.40 def	0.31 b	0.35 ef	0.31 bcde	0.44 c	0.33 cdef
BC_M	0.48 ab	0.34 b	0.50 cd	0.33 abcde	0.47 bc	0.36 bcde
BC_H	0.48 ab	0.43b	0.46 de	0.37 abc	0.44 c	0.32 def
NPK_L	0.50bcd	0.30 b	0.32 f	0.29 de	0.59 a	0.41 ab
NPK_H	0.55 a	0.42 b	0.66 a	0.39 ab	0.60 a	0.40 abc
$BC_L^{+}NPK_L$	0.44 cde	0.34 b	0.37 ef	0.31 cde	0.46 bc	0.30 ef
$BC_L^{+}NPK_H$	0.50 bcd	0.33 b	0.58 bc	0.39 a	0.55 ab	0.36 bcde
$BC_M^{+}NPK_L$	0.53 bc	0.27 b	0.34 f	0.27 e	0.47 bc	0.38 bcde
$BC_M^{+}NPK_H$	0.51 bc	0.34 b	0.62 ab	0.34 abcde	0.62 a	0.46 a
$BC_H^{+}NPK_L$	0.52 bc	0.35 b	0.48 cd	0.35 abcd	0.55 ab	0.39 abcd
$BC_H^{+}NPK_H$	0.54 a	0.35 b	0.51 cd	0.30 cde	0.45 bc	0.33 cdef

Column means presented with different letters indicate significance differences at ($p \leq 0.05$)

Table 5. Effect of biochar and fertilizer application on soil carbon content over time

Treatments	Carbon (%)					
	2014		2015		2016	
	0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm
CK	4.05 e	3.47 ab	3.89 e	3.17 efg	4.45 de	3.51 abcd
BC_L	4.78 de	3.82 ab	4.07 e	3.36 efg	5.24 c	4.12 a
BC_M	5.90 d	4.10 a	6.87 bc	3.89 cd	6.23 ab	3.51 abcd
BC_H	6.42 c	3.79 ab	5.72 d	3.19 efg	6.19 ab	2.99 cd
NPK_L	4.06 e	2.51 c	5.43 d	3.07 fg	4.93 cd	2.88 d
NPK_H	4.07 e	3.07 bc	3.67 e	2.87 g	4.17 e	3.26 bcd
BC_L+ NPK_L	5.47 d	3.14 bc	6.14 cd	3.51 def	6.31 ab	3.32 bcd
BC_L+ NPK_H	6.82 c	3.84 ab	7.54 b	4.49 b	6.92 a	3.94 ab
BC_M+ NPK_L	7.08 bc	3.72 ab	7.08 bc	3.57 cde	6.55 ab	3.93 ab
BC_M+ NPK_H	7.18 bc	4.23 a	8.65 a	5.47 a	6.29 ab	3.19 cd
BC_H+ NPK_L	8.52 a	4.15 a	7.25 b	3.31 efg	6.70 ab	3.32 bcd
BC_H+ NPK_H	8.63 a	3.56 ab	6.97 bc	4.00 bc	6.07 b	3.68 abc

Mean values presented in columns with different letters indicate significant differences at $p \leq 0.05$

Table 6. Summary of ANOVA (MS) for effect of time in years and treatments on all dependent variables

Source	FB	DB	PHA	PHB	NA	NB	CA	CB
Year	40444**	24051**	0.59**	0.63**	0.009*	8.69	1.10*	0.34**
Treatment	4917**	3614**	0.09**	0.057**	0.012**	8.70	14.23**	1.59**
Y* T	872**	549**	0.01**	0.007**	0.005*	8.70	1.27**	0.61**

*$p < 0.05$; ** $p < 0.01$

Table 7. Cumulative impact of different treatment on biomass and soil properties over three

Treatment	FB (g/m^2)	DB(g/m^2)	pHA	pHB	NA (%)	NB (%)	CA (%)	CB(%)
Control	109.82±2.91h	50.34±2.51 f	6.63±0.04 i	6.76±0.03 f	0.37±0.01 f	0.28±0.01 e	4.13±0.19 e	3.38±0.12 def
BCL	108.42±5.61 h	50.79±5.17f	6.85±0.03 fg	6.86±0.05 de	0.4±0.02 ef	0.32±0.01 cde	4.7±0.21 d	3.77±0.14 bcd
BCM	115.66±4.97 g	55.59±4.95ef	6.98±0.03 ab	6.95±0.06 bc	0.52±0.03 c	0.39±0.02 ab	7.01±0.32 ab	3.84±0.21 bc
BCH	116.05±5.64 g	58.48±4.89 e	6.99±0.05 a	7.02±0.03 a	0.5±0.04 cd	0.34±0.23bcd	6.12±0.2 c	3.33±0.17 ef
NPKL	140.92±10 f	69.86±5.32 d	6.76±0.07 h	6.87±0.04 de	0.66±0.03 a	0.41±0.02 a	5.08±0.13 d	2.82±0.11 g
NPKH	146.21±10.52 ef	74.11±6.92 d	6.8±0.06 gh	6.9±0.04 cd	0.44±0.04 de	0.34±0.02 bcd	3.97±0.13 e	3.07±0.08 fg
BCL+NPKL	148.56±10.65 e	83.95±8.69 c	6.95±0.05 abc	7.02±0.04 a	0.43±0.02 ef	0.32±0.01 cde	5.98±0.17 c	3.33±0.13 ef
BCL+NPKH	149.58±10.02 de	82.48±8.25 c	6.92±0.05 bcd	7.01±0.04 a	0.55±0.02 bc	0.37±0.01abc	7.1±0.17 ab	4.09±0.15 ab
BCM+NPKL	154.68±13.5 cd	92.66±12.45b	6.86±0.05 de	6.91±0.05 cd	0.38±0.03 ef	0.31±0.02 de	6.81±0.14 b	3.74±0.18 bcde
BCM+NPKH	178.52±18.03 a	114.49±15.2a	6.87±0.03 ef	6.84±0.06 f	0.59±0.03 b	0.38±0.02 ab	7.37±0.39 a	4.3±0.34 a
BCH+NPKL	156.71±13 c	86.76±8.92 c	6.89±0.03 ef	6.94±0.04 bc	0.52±0.02 c	0.36±0.03abc	6.99±0.12 ab	3.6±0.18 cde
BCH+NPKH	167.69±15.03 b	95.46±10.1b	6.93±0.03abc	6.98±0.02 ab	0.53±0.03 bc	0.33±0.02 cde	7.23±0.44 ab	3.75±0.12 bcde

Values are mean ± SE of three replication, Means with letters are not significantly different at $p = 0.01$ according to Duncan's multiple range test

Table 8. Principal components (PCs) for 8 traits biomass productivity and soil characteristics (Varimax rotation)

Traits	**Component**		
	1	2	3
pHB	0.881	0.204	0.133
pHA	0.82	0.443	0.044
NB	0.604	-0.257	-0.103
CB	-0.024	0.89	-0.047
CA	0.391	0.797	0.305
DB	-0.102	-0.087	0.88
NA	0.162	0.098	0.798
FB	-0.017	0.479	0.642
Eigenvalue	2.00	1.98	1.95
Proportion σ2%	25.03	24.74	24.36
Cumulative σ2%	25.03	49.77	74.13

Table 9. Impact of application of biochar on soil microbial diversity (the Shannon index)

Treatment	Shannon Index of Diversity
CK	3.24±0.004
BC_M	3.25±0.003
NPK_H	3.23±0.002
$BC_M{}^+ NPK_H$	3.28±0.007

Table 10. X-ray photoelectron spectroscopy analysis of original and aged rice husk biochar

Name	Structure	Biochar stored at room temperatures for three years		Biochar extracted from the soil after three years	
		Peak BE	At%	Peak BE	At%
C1s A	– C=C- non-functionalised sp2C	284.84	63.41	284.82	51.06
C1s B	– C-OH, C-O-C=, C-O-R	286.46	8.57	286.26	10.85
C1s C	– C-N, C=O	288.33	3.21	288.5	3.83
C1s d	– C=N, -N=C-O-	ND	ND	289.17	1.54
N1sA	Pyridne N	398.8	0.45	398.8	0.40
N1sB	N-H	400.7	0.55	400.7	0.60
Al2p		72.44	0.73	75.31	0.58
Ca2p		352.88	0.39	348.41	0.82
Fe2p		724.34	0.38	712.74	0.93
	FeOOH	711.2	0.30	711.2	0.65
	$Fe(SO_4)_3$	715.9	0.20	715.9	0.35
O1s		533.61	15.29	533.61	30.50
Mg1s		1305.35	0.37	1303.35	0.74
N1s		401.3	1.56	400.66	2.27
K2p		293.66	0.31	294.39	1.06
S2p		169.52	0.2	170.07	0.22
Si2p		104.69	5.58	103.61	11.19