

THE UNIVERSITY of EDINBURGH

# Edinburgh Research Explorer

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

#### Citation for published version:

Rafiq, MK, Yanfu, B, Aziz, R, Rafiq, MT, Masek, O, Bachmann, RT, Joseph, S, Shahbaz, M, Qayyum, A, Zhanhuan, S, Danaee, M & Ruijun, L 2020, 'Biochar amendment improves alpine meadows growth and soil health in Tibetan plateau over a three year period', *Science of the Total Environment*, vol. 717. https://doi.org/10.1016/j.scitotenv.2019.135296

#### **Digital Object Identifier (DOI):**

10.1016/j.scitotenv.2019.135296

#### Link:

Link to publication record in Edinburgh Research Explorer

**Document Version:** Peer reviewed version

**Published In:** Science of the Total Environment

#### **General rights**

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



1	<b>Biochar amendment improves</b>	alpine meadows	s growth and soil h	ealth in Tibetan

3	Muhammad Khalid Rafiq <sup>a,b,c,1, *</sup> Bai Yanfu <sup>b,1</sup> Raksanda Aziz <sup>d</sup> , Muhammad Tariq Rafiq <sup>e</sup> ,
4	Ondřej Mašek <sup>a</sup> , Robert Thomas Bachmann <sup>f</sup> , Stephen Joseph <sup>g</sup> , Maqbool Shahbaz <sup>c</sup> , Abdul
5	Qayyum <sup>h</sup> , Shang Zhanhuan <sup>b,*</sup> , Mahmoud Danaee <sup>g</sup> , Long Ruijun <sup>b</sup>
6	<sup>a</sup> UK Biochar Research Centre, School of GeoSciences, University of Edinburgh, Crew
7	Building, King's Buildings, Edinburgh EH9 3FF, United Kingdom
8	<sup>b</sup> State Key Laboratory of Grassland Agro-ecosystems, International Centre for Tibetan
9	Plateau Ecosystem Management, School of Life Sciences, Lanzhou University, Lanzhou
10	730000, China
11	<sup>c</sup> Rangeland Research Institute, National Agricultural Research Center, Islamabad 44000,
12	Pakistan
13	<sup>d</sup> Department of Environmental Science, International Islamic University, Islamabad, 44000
14	Pakistan
15 16	<sup>e</sup> Center for Interdiscplinary Research in Basic Sciences, International Islamic University, 44000 Islamabad, Pakistan
17	<sup>f</sup> Malaysian Institute for Chemical and Bioengineering Technology (MICET), University
18	Kuala Lumpur (UniKL), Lot 1988, Taboh Naning,78000 Alor Gajah, Melaka, Malaysia
19	<sup>g</sup> University of Newcastle, School of Environmental and Life Sciences, Office C325,
20	Chemistry, Callaghan, New South Wales 2308, Australia
21	<sup>h</sup> Department of Agronomy, University of Haripur, 22620, Pakistan.
22	
23	*Corresponding author's e.mail: <u>muhammad.k.rafiq@ed.ac.uk</u>
24	<u>shangzhh@lzu.edu.cn</u>
25	1. These authors contributed equally to this work.

plateau over a three year period 2

#### 26 ABSTRACT

Previous biochar research has primarily focused on agricultural annual cropping systems with 27 very little attention given to highly fragile, complex and diverse natural alpine grassland 28 ecosystems. The present study investigated the effect of biochar on the growth of alpine 29 meadows and soil health. This study was conducted in the Qinghai Tibetan Plateau over a 30 three year period to investigate the effect of three rice husk biochar application rates alone 31 and combination with high and low NPK fertilizer dosages on alpine meadow productivity, 32 soil microbial diversity as well as pH, carbon and nitrogen content at 0-10 cm and 10-20 cm 33 depth. At the end of the 3<sup>rd</sup> year soil samples were analysed and assessed by combined 34 analysis of variance. The results showed that biochar application in combination with 35 nitrogen (N), phosphorus (P) and potassium (K) fertilizer had a significant increase in fresh 36 and dry biomass during the second and third year of the study as compared to control and 37 38 alone biochar application ( $p \le 0.05$ ). 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, 39 the SEM imaging and EDS analysis of aged biochar demonstrated that the biochar has 40 undergone complex changes over the 3 years as compared to fresh biochar. This research 41 suggests that biochar has positive effect on alpine meadow growth and soil health and may be 42 an effective tool for alpine meadow restoration. 43

44

#### 45 Keywords Biochar · Alpine meadows · Soil health

46

- 48
- 49
- 50 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 58 59 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 60 and India (United Nations Environment Programme (UNEP). 2007). The plateau has the 61 largest biome plateau area on the Eurasian continent and represents a major ecological region 62 with the lowest-latitude permafrost in the globe (Wang et al. 2002). Diverse types of 63 grasslands extending from the Tibetan Plateau to Inner Mongolia and the mountains of the 64 Xinjiang province, thus constitute the third biggest grassland ecological unit on earth (Yang 65 et al. 2012). About 85 % of the plateau consists of alpine grasslands serving as a major source 66 for livestock grazing (Dong et al. 2010; Harris. 2010), predominantly yak and Tibetan sheep. 67 Alpine grasslands provide additional vital ecosystem services such as carbon capture, 68 biodiversity, soil and water conservations (Yang et al. 2004; Chen et al. 2008; Wang et al. 69 2009). The C stored in soils of the plateau  $(33.5 \times 10^9 \text{ t C})$  makes up 2.4 % of total world soil 70 C (Wang et al. 2002) but due to poor land management this carbon is being lost at an 71 increasing rate. 72

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 86 livestock and fertilizer applications have been practiced to restore these degraded grasslands 87 (Akiyama and Kawamura. 2007). However, these management practices have not been 88 demonstrated to restore the extremely degraded grasslands of the plateau (Wu et al. 2010b). 89 Fertilizer application improves grassland productivity and restores degraded grasslands. 90 Research investigations have shown that N-P-K fertilizer can enhance grassland production 91 and its forage quality. However, due to grassland degradation phenomenon, there is less 92 nutrient maintenance is grassland vegetation and nutrients are more prone to leaching. 93 Biochar, produced by thermal decomposition of organic material (Lehmann and Joseph. 94 95 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 96 applications to low fertility soils have improved yields in different cropping patterns 97 worldwide (Glaser et al. 2002; Jeffery et al. 2011; Kammann et al. 2011; Vaccari et al. 2011; 98 Spokas et al. 2012; Wang et al. 2012). Additionally, biochar application, reduce soil acidity 99 (Knowles et al., 2011), increase cation exchange capacity (CEC) of soil (Mikan and Abrams, 100

101 1996) and reduce concentrations of pollutants. Significant reduction of leaching of fertilizer
102 N from soil has been reported as a result of amendment with biochar produced from forest
103 residues (Manolikaki & Diamadopoulos, 2017). Reduction of nitrate leaching from soil
104 amended by biochar produced from pecan shells has been demonstrated over 25 and 67 days
105 (Chaplot & Cooper, 2015).

Yak dung clay blended biochar and yak manure biochar has been proved to enhance 106 production of blue grass in an artificial pasture and highland barley crop in short term in 107 Tibetan plateau (Rafiq et al., 2017 and Zhang et al., 2018), however yak manure has been 108 109 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 110 54 million tons of rice husk is produced every year. The high volumes of rice husks that are 111 considered as waste after milling are not appropriately treated. Rice husk is one of the main 112 feedstock used to produce bio-oil by fluidized-bed reactors or other fast pyrolysis systems in 113 China (Wang and Liu, 2018). Abundant biochar produced during the process of fast pyrolysis 114 as by-product in China could be a potential application for grassland restoration. 115

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.

120 Materials and methods

#### 121 *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.*

#### 131 Characterization of experimental biochar

Rice husk was obtained from Hangzhou, Zhejiang Province (China) and converted to biochar 132 at a pyrolysis temperature of 500°C using a vertical furnace with continuous feeding (Jiaxing 133 134 JIAHUA Animal Husbandry Co., Ltd., Zhejiang, China). The physico-chemical characteristics of the biochar such as pH, ash content, total nitrogen, total carbon, total 135 hydrogen, total phosphorous, total potassium, calcium, magnesium, sodium were analysed. 136 The pH of biochar was measured in deionized water at the ratio of 1:5 wt/wt with a calibrated 137 Orion 720 pH meter (Enders et al., 2012). Ash content was analyzed by heating biochar 138 samples at 500°C for 8 h in a muffle furnace (Dai et la., 2013). The elemental composition 139 was determined according to Enders et al. (2012) using an elemental analyzer from Elementar 140 Analysensysteme GmbH (varioELcube). Nutrient elements Ca, K, Mg, Na, and P were 141 measured using an inductively coupled plasma-atomic emission spectrometer (IRIS ER/S). 142 Before analysis, the biochar sample (about 0.05 g) was first digested by the concentrated 143  $HNO_3/H_2O_2$  solutions (Dai et al., 2013). 144

BET (N<sub>2</sub>) 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 dispersivespectrometer (EDS) detector.

#### 153 *Experimental design*

The size of each experimental plot was  $2 \times 4$  m. There was a distance of 50 cm between the 154 experimental plots to serve as a buffer zone (Qi et al., 2015). There were twelve treatments in 155 this experiment carried out in triplicate under randomized complete block design (RCBD). 156 157 Biochar was applied at 3 application rates: low (2 t/ha, BC<sub>L</sub>), medium (4 t/ha, BC<sub>M</sub>) and high (6 t/ha, BC<sub>H</sub>) to the grassland. Biochar application rates were selected based on the 158 159 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 160 biochar @ 3 tons/ha on pasture areas in Tibetan plateau. Two levels of NPK fertilizer were 161 applied (30N, 15 P and 10 K kg/ha) and (60 N, 30 P and 20 K kg/ha) and designated as NPKL 162 and NPK<sub>H</sub>, respectively. Higher level of NPK fertilizer corresponds to the recommendations 163 of (Yu li et., al 2015). The NPK fertilizer was applied in the form of urea for N, single 164 superphosphate for P and potassium chloride for K. The detailed plan of the treatments 165 applied include:  $T_1 = CK$  (Control, no amendment),  $T_2 = BC_L$ ,  $T_3 = BC_M$ ,  $T_4 = BC_H$ ,  $T_5 =$ 166 NPK<sub>L</sub>,  $T_6 = NPK_H$ ,  $T_7 = BC_L + NPK_L$ ,  $T_8 = BC_L + NPK_H$ ,  $T_9 = BC_M + NPK_L$ ,  $T_{10} = BC_M + NPK_H$ , 167  $T_{11} = BC_H + NPK_L$  and  $T_{12} = BC_H + NPK_H$ . The biochar and NPK were applied through surface 168 applications. The experiment commenced at the third week of June, 2014. 169

170 Vegetation and soil sampling

At the end of August 2014, 2015 and 2016, biomass samples were collected approximately 1 cm from the ground using  $50 \times 50$  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 176 control  $T_1$ ,  $T_3$ ,  $T_6$  and  $T_{10}$  to test effect of biochar and fertilizer on microbial functional 177 diversity. In addition, biochar samples were subjected to microscopic and XPS analysis to 178 investigate changes on the surface.

#### 179 Biomass and soil measurements

Fresh biomass of the collected grass samples was weighed and recorded in the field soon 180 181 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-182 Suáre at al., 2014) and their dry biomass recorded. After cleaning and sieving with a 2 mm 183 sieve, the air-dried soil samples (dried till constant weight) were tested for pH, C and N. The 184 pH value of the experimental soils was tested using 1 : 2.5 soil : water suspension (Thiele-185 Bruhn et al. 2015) with an Orion 720 pH meter with a combination electrode. Total carbon 186 and nitrogen of the soil was determined using elemental analyzer (Elementer Analyse 187 systeme GmbH, varioEL-cube). 188

189 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<sup>TM</sup> (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<sup>-3</sup> final dilutions before inoculation. Biolog EcoPlate TM has 96wells 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,

207 AWCD=Σ(C-R)/31

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

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

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

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

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

215 Data analysis

Analysis of variance was conducted and Duncan's Multiple Range Test (DMRT) at 5% level 216 of probability was employed to compare means. Computer based statistical package 217 MSTATC following Steel et al. (1997) was applied for this statistical analysis. To evaluate 218 the cumulative effect of twelve treatments over the three year period on fresh biomass (FB), 219 dry biomass (DB) and soil properties PHA, PHB are pH values at 0-10 cm and 10-20 cm soil 220 221 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 222 223 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 224

dependent variable separately at 0.05 level. The data were subjected to principal componentanalysis (PCA) in a Multivariate analysis.

227

#### 228 **Results**

229 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<sup>-1</sup> and 577 as well as sharp peaks at 2922, 2880, 1644, 1421, 887 and x cm<sup>-1</sup>. A weakly defined peak was also detected at 1122 cm<sup>-1</sup>.

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  $3.19 \text{ g/m}^2$  and an average pore width of 10.6 nm.

240 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 241 a result of biochar application from 2014-16. It was found that an increasing biochar 242 application rate resulted in an increase in fresh and dry biomass yield during the first year of 243 biochar application.. However, this increase in biomass was not statistically significant at 244 the p = 0.05 level probably. When biochar was applied together with NPK the best yield was 245 observed for BC<sub>M</sub> throughout the study period. The fresh biomass yields of the treatments 246 like BC<sub>H</sub>+NPK<sub>H</sub>, BC<sub>M</sub>+NPK<sub>H</sub>, BC<sub>L</sub>+NPK<sub>H</sub> were significantly greater than biochar 247 treatments ( $p \le 0.05$ ). The largest dry biomass yield was measured for the NPK<sub>H</sub> treatment in 248 2014. During the second year, there was no significant difference in fresh and dry biomass 249

yield between the control and those from the biochar applications alone. In contrast, NPK 250 fertilizer application showed a significant increase for both fresh and dry grass yield as 251 compared to the control and pure biochar applications. However, the greatest significant 252 increase in fresh biomass yield was measured in the BC<sub>M</sub>+NPK<sub>H</sub> treatment. The fresh and dry 253 biomass productivity of the meadow in the third year was significantly greater for all 254 treatments compared to control. However maximum fresh and dry biomass yield was 255 observed for the (BC<sub>M</sub>+NPK<sub>H</sub>) treatment throughout the study period. The increase in 256 biomass as biochar and fertilizer application together indicates that responses of alpine 257 258 meadows to addition of biochar and fertilizer were additive and positive.

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

261 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 262 data indicate that soil had a lower pH at surface level (0-10cm) as compared to 10-20 cm of 263 soil depth. Biochar application alone or combined with NPK fertilizer showed significantly 264 higher pH values at 0-10 cm soil depth as compared to control and alone NPK fertilizer 265 treatments ( $p \le 0.05$ ) during the first year of study. However, biochar addition results higher 266 pH levels in the both soil depth levels during the second and third years of the study. This 267 indicates that effect remained to persistent over time. The nitrogen content of alpine meadow 268 soil at two depth (0-10 and 10-20) during 2014-16 is provided in Table 4. Application of 269 biochar and fertilizer led to effective addition of nitrogen in soil. Nitrogen concentrations 270 increased in the meadow soil with biochar application. A greater nitrogen content was 271 observed at 0-10 cm soil depth level as compared to 10-20 cm. The greatest total nitrogen 272 content (0.55 wt.%) in year one was observed for NPK<sub>H</sub> treatment and there were significant 273 increases between the control and the other treatments (except BC<sub>L</sub>) at 0-10 cm depth during 274

the first year of study ( $p \le 0.05$ ). In year 2, the most significant increase in N soil content (0.66 wt.%) was measured in the NPK<sub>H</sub> treatment for the 0-10cm soil profile and in the BC<sub>L</sub><sup>+</sup> NPK<sub>H</sub> 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 284 years.Based on a combined ANOVA over three years, results revealed that there was a 285 significant effect of time on all dependent variables (Table 6). The results also show that all 286 dependent variables were statistically significant in all treatments. Results of mean 287 comparison among treatments using Duncan Multiple range test indicated that the greatest 288 mean for fresh biomass(FB) was observed in BCM+NPKH (179  $\pm$  18 g/m<sup>2</sup>) which was 289 significantly higher than other treatments and the lowest level of FB belonged to BCL, BCM, 290 BCH which were not statistically different from the control group. For DB, results of mean 291 comparison showed that the highest mean of BD was observed for  $BC_M + NPK_H$  (114 ± 15 292  $g/m^2$ ) which was higher than other treatments. 293

The highest level of soil pH (A) (Table 7) was measured in treatment BCH ( $6.99 \pm 0.05$ ), which was significantly different from other treatments and the lowest level of pH (A) was observed for control group ( $6.63 \pm 0.04$ ). For pH (B) results of mean comparison showed that three treatmentshad the highest level including BCH ( $7.02 \pm 0.03$ ), BCL+NPKL ( $7.02 \pm 0.04$ ) and BCL+NPKH ( $7.01 \pm 0.04$ ), which were not statistically different. The lowest pH (B) belonged to control group ( $6.76 \pm 0.03$ ) and BCM+NPKH ( $6.84\pm0.06$ ). These results

indicated that the level of N (B), NPKL (0.41±0.02) was significantly higher than other 300 treatments except BCM, BCL+NPKH, BCM+NPKH and BCH+NPKL. Results of mean 301 comparison for C(A) and C(B) revealed that BCM+NPKH had the highest means score for 302 C(A) (7.37 0.39) C(B) (4.3 0.3) both 303 ± and ± two variable. 304

The data were subjected to principal component analysis (PCA) in a multivariate analysis. 305 Biomass productivity and soil characters have been used to define patterns on the impacts of 306 treatments applied. Results showed that three components with Eigen values more than one 307 were extracted and these three components explained 74.1 % of total variability (Figure 1). 308 This shows great variation among biomass productivity and soil characteristics under 309 investigation. The first principal component (PC1) comprising of PHB, PHA and NB 310 explained 25.0 % of total variability (Table 8). The characters with greatest positive weight 311 on PC2 were CB and CA and these components explained 24.7 % of total variance among all 312 313 data. DB, NA and FB were associated with the third principal component (PC3) which explained 24.4 % of total variance. 314

315 *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<sub>M</sub>) alone and in combination with NPK fertilizer (BC<sub>M</sub><sup>+</sup> NPK<sub>H</sub>) had higher Shannon Index values indicating that biochar addition can improve soil microbial diversity (Table 9).

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

325 XPS and SEM-EDS analysis of original and aged biochar shows that the biochar has 326 undergone complex changes over the 3 years (Table 10, Figure 3 A,B). There has been a 327 decrease in the aromatic carbon and an increase in organic compounds yielding a higher 328 content of C/O and C/N functional groups, K, Si, Ca, Mg, N, S and Fe atomic % than the 329 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.

332 SEM-EDS results show that the surface of original biochar has a relatively large content 333 of Si, no detectable Fe and only relatively small concentrations of K, Ca, S, Al, P and Cl. The 334 aged biochar, on the other hand, contained higher concentrations of K, Fe and Mn and Al. 335 These images and elemental and functional group measurements are indicative of the 336 formation of organo-mineral clusters on the surface of the biochar.

337

### 338 Discussion

It has been observed in several studies that biochar addition to soils due to its various 339 properties has improved soil fertility and thus increased crop yields on agricultural lands 340 (Marris. 2006; Chan et al. 2007). The characterization for pH, C, N and ash content were 341 within the range reported for rice husk biochars used by Manickam et al. (2015). BET (N<sub>2</sub>) 342 343 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. 344 (2018). The observed variability is attributed to differences in process conditions primarily 345 346 temperature (Rafiq et al. 2016) as well as feedstock type.

Observed FTIR peaks are in close agreement with biochars produced Sharma et al. (2004) 347 from lignin at pyrolysis temperatures  $\geq$  450°C. FTIR peaksat wavelengths 3432 and 1122 cm<sup>-</sup> 348 <sup>1</sup> are attributed to -OH and C-O stretching vibration of phenolic compounds (Sharma et al. 349 2004; Ma et al. 2017). The appearance of peaks at 887 and 790 cm<sup>-1</sup> are not only indicative of 350 aromatic C-H but also evidence of formation of fused ring systems (Sharma et al. 2004). 351 Sharma et al. (2004) observed a slow decrease in aliphatic CH stretch (2800-3000 cm<sup>-1</sup>) with 352 increase in pyrolysis temperature. . The presence of aliphatic CH was also observed in rice 353 husk biochar used in this study suggesting that it originated from lignin. 354

The H/C molar ratio of 0.26 was well below 0.7 as required by IBI standards and EU 355 guidelines (2012). The O/C molar ratio was 0.33 which meets the standards of the EU 356 guidelines 2012). Similar H/C and O/C ratios have been reported for rice husk biochar in 357 literature (Manickam et al. 2012). The molar H/Corg ratio can be used to predict the relative 358 amount of organic biochar carbon that remains after 100 years incubation in soil (Budai et al. 359 2013). The organic carbon content in our rice husk biochar (Table 1) was assumed to be the 360 361 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 362 363 expected to remain in alpine meadow soil after 100 years barring other factors such as loss due to erosion. 364

The application of rice husk biochar showed positive effects on alpine meadows biomass 365 productivity over three years with and without NPK fertilizers. Crop productivity is often 366 reported to increase with biochar application to soils but not always consistently (Jeffery et 367 al. 2011; Subedi et al. 2016). The results from soil trials demonstrated that biochar/NPK 368 fertilizer can assist in alpine meadow restoration. The biochar and NPK application did not 369 show a significant impact on biomass yield during the first year of application however, in 370 the subsequent years as in second and third years biomass yield was observed having a 371 significant increase with the application of biochar with and without fertilizer (Table 2). 372 373 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 374 consistent with the findings of this experiment. We observed biomass improvements during 375 second and third years of the biochar application. Furthermore, results showed that 376 significantly improved the biomass productivity of meadows during the second and third 377 years. Persistent increase in crop productivity following biochar inputs are a good indicator of 378 economic viability for scaling up the applications (Liu et al., 2013). Similar results were also 379

reported by Adam et al (2013) and Slavich et al (2013) who observed that biochar has the 380 ability to improve prairie growth and prairie restoration. Possible reasons for the 381 nonsignifcant effects of biochar on forage biomass during the first year may be related to 382 lower biochemical processes in alpine areas having lower temperatures, in the presence or 383 absence of biochar, plus slower biochar degradation and its interaction with soil and 384 consequently delaying its beneficial effects on soil properties and plant productivity 385 (Verheijen et al. 2010; Fang et al. 2015). Several mechanisms for increase in biomass yield 386 after biochar applications have been discussed in the literature. These include liming effects 387 388 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 389 adsorption. Many studies shown that over time aging of biochar in soils have more produced 390 391 effects of biochar on coil moisture content (Paetsch et al., 2018). This increased moisture 392 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 393 improved biomass productivity of alpine meadow after biochar application in this 394 experiment. 395

The results showed that biochar application improved the soil pH values in the alpine 396 The plant feedstock materials that are used to produce biochar contain base meadows. 397 cations and these cations are transferred to biochars during pyrolysis of organic materials. 398 399 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, 400 as observed in our study (Table 3). Increase in soil pH values has also been reported by 401 Laird et al. (2010), where biochar with high ash content (14-56%), similar to present study 402 (37% ash), were used. The alkalinity character is enhanced with pyrolysis temperature 403 allowing rice husk biochar to act as a liming agent (Lehman et al. 2007; Wang et al. 2014). 404

Similar findings were reported in previous studies (Demirbas et al. 2004; Chan et al. 2007; 405 Revell et al. 2012; Wang et al. 2014). The application of biochar due to its ability to act as a 406 liming agent improved soil pH levels. Similar findings were reported in previous studies 407 (Demirbas et al. 2004; Chan et al. 2007; Revell et al. 2012; Wang et al. 2014). Similarly, 408 Novak et al. (2009) found that biochar enhanced soil pH in the southern United States. Wang 409 et al (2014) also showed that biochar application could increase the carbon content in soil. 410 Similarly it have be investigated that use of biochar application in prairie rehabilitation 411 initiatives and proved biochar addition not only enhances improve the growth of prairie 412 413 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 414 (Lehman et al. 2007) and accelerated the recovery of carbon pool in these soils. The AWCD 415 value in the well of an EcoPlate<sup>TM</sup> is a key indicator of microbial functional diversity, 416 because it indicates the capability of soil microorganisms to utilize various carbon substrates. 417 Previous findings have shown that the application of organic matter to soil can enhance 418 microbial populations their diversity and activities (Gomez et al., 2006). The results of this 419 experiment showed that biochar and NPK fertilizer applied in combination had positive 420 impacts on the microbial activity and diversity. The biochar upon aging has shown (table:10, 421 fig:4), that there are increased c/o functional groups in biochar. These characteristics have 422 been proved to increase the abundance of beneficial microorganisms in soil (Ye et al., 2017). 423 424 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 425 that biochar application has positive effects on soil microbial diversity. XPS results and the 426 SEM imaging and EDS analysis shows that the biochar has undergone complex changes over 427 the 3 years and these changes are similar to those describe by Joseph et al (2010), Archanjo et 428 al (2017) and Hagemann et al (2017). These images and elemental and functional group 429

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.

435

#### 436 Conclusion

This study has demonstrated that biochar can have significant effects on biomass production, 437 soil acidification and carbon sequestration. In addition, biochar showed positive effects on 438 microbial diversity and activity. Application of biochar to natural, wasteland and degraded 439 systems could be a potential strategy to sequester carbon (Woolf et al. 2010). Further research 440 is required to evaluate the long-term effects of biochar species diversity plant and detailed 441 soil dynamics like nutrient mineralization, availability and transfer to plant. Additionally, 442 biochar application methods and biochar erosion aspects need to be investigated for its 443 appropriate testing mechanism. More research work is also required to develop and test 444 biochar from the local feed stocks and to enrich it with heterogeneous nutrient material like 445 attapulgite clay for its cost effectivity and wider acceptability. 446

447

448

449

#### 450 Acknowledgements

This study was supported by the national key research and development project (2016YFC0501906), the fundamental research funds for the central Universities(lzujbky-2017-k16), natural science foundation of China (41671508; 31870433), the second stage's research and technique extending project of Sanjiangyuan ecological protection and building 455 of 2017 in Qinghai (2017-S-1-06), Key R&D and transformation program of Qinghai (2017-

456 NK-149-2), and Qinghai innovation platform construction project (2017-ZJ-Y20).

457

#### 458 **References**

- Adams KM, Benjamin TJ, Emery NC et al (2013) The Effect of Biochar on Native and
  Invasive Prairie Plant Species. Invasive Plant Science and Management 6:197–207.
- 461 Akiyama T, Kawamura K (2007) Grassland degradation in China, methods of monitoring,
  462 management and restoration.Grassl. Sci 53(1):1–17.
- 463 Archanjo BS, Mendoza M E, Albu M et al (2017) Nanoscale analyses of the surface structure
- and composition of biochars extracted after composting and from field trials using
  advanced analytical electron microscopy. Geoderma 294:70–79.
- Bowman WD, Cleveland CC, Halada L et al (2008) Negative impact of nitrogen deposition
  on soil buffering capacity. Nature Geoscience 1:767–770.
- 468 Cai, YJ, Wang, XD, Ding WXet al (2013).Potentialshort-term effects of yak and Tibetan
- sheep dung on greenhouse gas emissions in two alpine grassland soils under laboratory
- 470 conditions. Biol. Fertil. Soils 49, 1215–1226. http:// dx.doi.org/10.1007/s00374-013-
- **471** 0821-7.
- 472 Carvalho MTM, Madari BE, Bastiaans, L et al (2016) Properties of a clay soil from 1.5 to 3.5
  473 years after biochar application and the impact on rice yield. Geoderma 276:7–18.
- 474 Chan KY, Van Zwieten L, Meszaros I et al (2007) Agronomic values of greenwaste biochar
- as a soil amendment. Soil Res 45: 629–634.
- 476 Chaplot V, Cooper M Soil aggregate stability to predict organic carbon outputs from
- 477 soils.Geoderma, 243 (2015), pp. 205-213
- Chen J, Yamamura Y, Hori Y et al (2008) Small-scale species richness and its spatial
  variation in an alpine meadow on the Qinghai–Tibet Plateau. Ecol. Res 23: 657–663.

- Cheng J, Wu GL, Zhao LP et al (2011) Cumulative effects of 20-year exclusion of livestock
  grazing on above- and belowground biomass of typical steppe communities in arid areas
  of the Loess Plateau, China. Plant Soil Environ 57: 40–44.
- Clare A, Shackley SS, Joseph J (2014) Competing uses for China's straw: the economic and
  carbon abatement potential of biochar. GCB Bioenergy (2014), 10.1111/gcbb.12220.
- 485 Dai Z, Meng J, Muhammad N, Liu X et al. (2013). The potential feasibility for soil
- 486 improvement, based on the properties of biochars pyrolyzed from different feedstocks. J.
  487 Soils Sediments. 13, 989–1000.
- 488 Demirbas B (2004) Effects of temperature and particle size on bio-char yield from pyrolysis
  489 of agricultural residues. J Anal Appl Pyrolysis 72(2): 243–248.
- 490 Dong SK, Wen L, Zhu L et al (2010) Implication of coupled natural and human systems in
  491 sustainable rangeland ecosystem management in HKH region. Frontiers of Earth
  492 Science in China 4: 42–50.
- 493 Du Z, Xiao Y, Qi X et al (2018) Peanut-Shell Biochar and Biogas Slurry Improve Soil
  494 Properties in the North China Plain: A Four-Year Field Study. Scientific Reports 8:1–9.
- 495 Enders A, Hanley K, Whitman T, Joseph S, Lehmann J (2012) Characterization of biochars
- 496 to evaluate recalcitrance and agronomic performance. Bioresource Technol 114:644–
  497 653
- Glaser B, Lehmann J, Zech W (2002) Ameliorating physical and chemical properties of
  highly weathered soils in the tropics with charcoal–a review. Biology and Fertility of
  Soils 35:219–230.
- 501 Gomez E, Ferreras L, Toresani S (2006) Soil bacterial functional diversity as influenced by
- organic amendment application. Bioresour. Technol., 97 (2006), pp. 1484-1489
- 503 Haefele SM, Konboon Y, Wongboon W et al (2011). Effects and fate of biochar from rice
- residues in rice-based systems. Field Crop Research 121: 430-440.

- Hagemann N, Harter J, Behrens S (2016) Elucidating the impacts of biochar applications on
  nitrogen cycling microbial communities. In: Ralebitso-Senior, T.K., Orr, C.H. (Eds.),
  Biochar Application. Elsevier, Amsterdam, pp. 163–198
- Harris RB (2010) Rangeland degradation on the Qinghai–Tibetan plateau, a review of the
  evidence of its magnitude and causes. J Arid Environ 74:1–12.
- He NP, Wu L, Wang YS et al (2009) Changes in carbon and nitrogen in soil particle-size
  fractions along a grassland restoration chronosequence in northern China. Geoderma
  302–308.
- He NP, Yu Q, Wu L et al (2008) Carbon and nitrogen store and storage potential as affected
  by land-use in a Leymuschinensis grassland of northern China. Soil Biol Biochem
  40:2952–2959.
- Hossain MK, Strezov V, Chan KY et al (2010). Agronomic properties of waste water sludge
  biochar and bioavailability of metals in production of cherry tomato
  (Lycopersiconesculentum). Chemosphere 78: 1167–1171.
- Houghton RA, Hackler JL, Lawrence KT (1999) The U. S. carbon budget, contributions in
  sustainable rangeland ecosystem management in HKH region. Front Earth Sci China 4:
  42–50.
- Jeffery S, Verheijen FGA, Velde MVD et al (2011) A quantitative review of the effects of
  biochar application to soils on crop productivity using meta-analysis. Agriculture,
  Ecosystems and Environment 144:175–187.
- Jones DL, Rousk J, Edwards-Jones G et al (2012) Biochar mediated changes in soil quality
  and plant growth in a three year field trial. Soil Biology and Biochemistry 45:113–124.
- Joseph SD, Camps-Arbestain M, Lin Y et al (2010) An investigation into the reactions of
  biochar in soil. Aust J Soil Res 501–515.

- Kammann CI, Linsel S, Gößling JW et al (2011) Influence of biochar on drought tolerance of
  Chenopodium quinoa Willd and on soil–plant relations. Plant and Soil 345(1-2):195–
  210.
- Kolb SE, Fermanich KJ, Dornbush ME (2009) Effect of charcoal quantity on microbial
  biomass and activity in temperate soils. Soil Sci Soc Am J 73(4):1173.
- 534 Koide RT, Petprakob K, Peoples M (2011). Quantitative analysis of biochar in field soil. Soil
- 535 Biol. Biochem. 43:1563–1568.
- Knowles OA, Robinson BH, Contangelo A et al (2011)Biochar for the mitigation of nitrate
  leaching from soil amended with biosolids. Sci. Total Environ., 409 pp. 3206-3210
- 538 Lehmann J, Gaunt J, Rondon M (2006) Bio-char sequestration in terrestrial ecosystems-a
- review. Mitigation and Adaptation Strategies for Global Change 11:395–419.
- Li Yu, Xiao-Long S, Jian-ning Z et al (2015), Responses of plant diversity and primary
- productivity to nutrient addition in a Stipa baicalensis grassland, China. Journal of
  Integrative Agriculture. 14(10): 2099–2108.
- Liang B, Lehmann J, Sohi SP et al (2010) Black carbon affects the cycling of non-black
  carbon in soil. Org Geochem 41: 206e213.
- Liao N, Li Q, Zhang W et al (2016) Effects of biochar on soil microbial community
  composition and activity in drip-irrigated desert soil. European Journal of Soil Biology
  72:27-34.
- Liu XJ, Duan L, Mo JM et al (2011) Nitrogen deposition and its ecological impact in China:
  an overview. Environ Pollut 159: 2251–2264.
- Liu Y, Yang M, Wu Y et al (2011) Reducing CH<sub>4</sub> and CO<sub>2</sub> emissions from waterlogged
  paddy soil with biochar. Journal of Soils and Sediments 11:930–939.
- 552 Lu X, Yan Y, Sun J (2015). Short-term grazing exclusion has no impact on soil properties

553	and nutrients of degraded alpine grassland in Tibet, China. SolidEarth 6, 1195-1205.
554	http://dx.doi.org/10.5194/se-61195-2015

- Ma Z, Yang V, Ma Q et al (2017) Evolution of the chemical composition, functional group,
  pore structure and crystallographic structure of bio-char from palm kernel shell pyrolysis
  under different temperatures. Journal of Analytical and Applied Pyrolysis 127:350–359.
- 558 Manickam T, Bachmann RT, Illani ZI et al (2012) Characterization of Local Mill Rice Husk
- 559 Charcoal and Its Effect on Compost Properties. Malaysian Journal of Soil Science 560 16:89–102.
- Manickam T, Cornelissen G, Bachmann RT et al (2015) Biochar application in Malaysian
   sandy and acid sulfate soils: Soil amelioration effects and improved crop production
- over two cropping seasons. Sustainability (Switzerland) 7:16756–16770.
- Manolikaki I, E. Diamadopoulos E (2017)Ryegrass yield and nutrient status after biochar
  application in two Mediterranean soils Archives of Agronomy and Soil
- 566 Science, 63 (8), pp. 1093-1107
- 567
- 568 Mikan CJ, Abrams AD (19969) Mechanisms inhibiting the forest development of historic
- 569 charcoal hearths in southeastern Pennsylvannia Can. J. For. Res., 26 (1996), pp. 1893570 1898
- 571 Ni J, 2002. Carbon storage in grasslands of China. J. Arid Environ. 50, 205–218. http://
  572 dx.doi.org/10.1006/jare.2001.0902.
- Novak JM, Busscher WJ, Laird D et al (2009) Impact of biochar amendment on fertility of a
  Southeastern coastal plain soil. Soil Sci 174:105–112.
- 575 Paetsch L, Mueller CW, Kögel-Knabner I (2018) Effect of in-situ aged and fresh biochar on
  576 soil hydraulic conditions and microbial C use under drought conditions.Sci. Rep., 8
- **577** (1) (2018), p. 6852

- 578 Pérez-Suárez M, Castellano MJ, Kolka R et al (2014) Nitrogen and carbon dynamics in
- prairie vegetation strips across topographical gradients in mixed Central Iowa
  agroecosystems. Agric Ecosyst Environ 188:1–11.
- Qi J, Nie Z, Jiao T et al (2015) Phosphorus and Defoliation Interact and Improve the Growth
- and Composition of the Plant Community and Soil Properties in an Alpine Pasture ofQinghai-Tibet Plateau. PLoS ONE 10(10): e0141701.
- Rafiq MK, Bachmann RT, Rafiq MT et al (2016) Influence of Pyrolysis Temperature on
  Physico-Chemical Properties of Corn Stover (*Zea mays* L.) Biochar and Feasibility for
  Carbon Capture and Energy Balance. PLOS ONE 11:e0156894.
- Rafiq MK, Joseph SD, Li F et al (2017). Pyrolysis of attapulgite clay blended with yak dung
  enhances pasture growth and soil health: characterization and initial field trials. Sci.
  Total Environ. 607–608 (2017), pp. 184-194.
- Revell KT, Maguire RO, Agblevor FA (2012) Field trials with poultry litter biochar and its
  effect on forages, green peppers, and soil properties. Soil Sci 177:573–579.
- Sharma RK, Wooten JB, Baliga VL et al (2004) Characterization of chars from pyrolysis of
  lignin. Fuel 83:1469–1482.
- Shi XM, Li XG, Long RJ et al (2010) Dynamics of soil organic carbon and nitrogen
  associated with physically separated fractions in a grassland-cultivation sequence in the
  Qinghai–Tibetan Plateau. Biol Fertil Soils 46:103–111.
- Singh BP, Hatton BJ, Singh B et al (2010) Influence of biochars on nitrous oxide emission
  and nitrogen leaching from two contrasting soils. Journal of Environment Quality
  39:1224.
- 600 Slavich PG, Sinclair K, Morris SG et al (2003) Contrasting effects of manure and green waste
- biochars on the properties of an acidic ferralsol and productivity of a subtropical pasture.
- 602 Plant and Soil 366(1-2):213-227.

- Sohi S, E Krull, E Lopez-Capel et al (2010) A review of biochar and its use and function in
  soil. Advances in Agronomy 105:47–82.
- Sousa FP, Ferreira TO, Mendonca ES et al (2012) Carbon and nitrogen in degraded Brazilian
  semi-arid soils undergoing desertification. Agric Ecosyst Environ 148:11–21.
- Spokas KA, Cantrell KB, Novak JM et al (2012) Biochar: A synthesis of its agronomic
  impact beyond carbon sequestration. J Environ Qual 41:973–989 (this issue).
- 609 Steel RGD, Torrie JH, Dickey DA (1997) Principles and procedures of statistics: A
  610 biometrical approach. 3<sup>rd</sup> ed. McGraw Hill Book Co. Inc. New York: 400-428.
- 611 Subedi R, Taupe N, Pelissetti S et al (2016) Greenhouse gas emissions and soil properties
- 612 following amendment with manure derived biochars: influence of pyrolysis temperature
- and feedstock type. Journal of Environment Management 166:73–83.
- 614 Sun DS, Wesche K, Chen DD (2011) Grazing depresses soil carbon storage through changing
- plant biomass and compositionin a Tibetan alpine meadow. Plant Soil Environ 57:271–
  278.
- 617 Thiele-Bruhn S, Wessel-Bothe S, Aust MO (2015) Time-resolved in-situ pH measurement in
- differently treated, saturated and unsaturated soils. J Pl Nutr Soil Sci 178(3):425-432.
- Tian HQ, Wang SQ, Liu JY et al (2006). Patterns of soil nitrogen storage in China. Global
- 620 Biogeochem. Cycles 20, GB1001. http://dx. doi.org/10.1029/2005GB002464.
- Vaccari FP, Baronti S, Lugato E et al (2011) Biochar as a strategy to sequester carbon and
  increase yield in durum wheat. European Journal of Agronomy 34:231–238.
- Van Zwieten L, Kimber S, Morris S et al (2010) Effects of biochar from slow pyrolysis of
  papermill waste on agronomic performance and soil fertility. Plant and Soil 327:235–
  246.

- Wang CT, Long RJ, Wang QL et al (2009) Changes in plant diversity, biomass and soil C, in
  alpine meadows at different degradation stages in the Headwater Region of Three
  Rivers, China. Land Degrad Dev 20:187–198.
- Wang GX, Qian J, Cheng G D et al (2002) Soil organic carbon pool of grassland soils on the
  Qinghai-Tibetan plateau and its global implication. The Science of the Total
  Environment 291:207–217.
- Wang GX, Qian J, Cheng GD et al (2002) Soil organic carbon pool of grassland soils on the
  Qinghai–Tibetan Plateau and its global implication. Sci Total Environ 291:207–217.
- 634 Wang GX, Wang YB, Li YS et al (2007) Influences of alpine ecosystem responses to
- climatic change on soil propertities on the Qinghai–Tibetan Plateau, China. Catena70:506–514.
- Wang J, Pan X, Liu Y et al (2012) Effects of biochar amendment in two soils on greenhouse
  gas emissions and crop production. Plant and Soil 360:287–298.
- 639 Wang Y, Liu R (2018). Improvement of Acidic Soil Properties by Biochar from Fast
- 640 Pyrolysis. Environmental Progress & Sustainable Energy.Vol.37:5.1743-1749.
   641 <u>https://aiche.onlinelibrary.wiley.com/doi/epdf/10.1002/ep.12825</u>
- Wang Y, Yin R, Liu R (2014) Characterization of biochar from fast pyrolysis and its effect
  on chemical properties of the tea garden soil. J Anal ApplPyrol 110:375-381.
- Woolf, D, Amonette JE, Street-Perrott FA et al (2010) Sustainable biochar to mitigate global
  climate change. Nature Communications 1:56.
- Wu GL, Li ZH, Zhang L et al (2010b) Effects of artificial grassland establishment on soil
  nutrients and carbon properties in a black-soil-type degraded grassland. Plant Soil
  333:469–479.
- Yang YH, Ji CJ, Ma WH et al (2012) Significant soil acidification across northern China's
  grasslands during 1980s-2000s. Glob. Change Biol 18:2292–2300.

Yang YH, Rao S, Hu HF et al (2004) Plant species richness of alpine grasslands in relation to
environmental factors and biomass on the Tibetan Plateau. Biodivers Sci 12:200–205.

653 Ye J, Joseph S, Ji M et al (2017) Chemolithotrophic processes in the bacterial communities

on the surface of mineral-enriched biochars ISME J. (2017), <u>10.1038/ismej.2016.187</u>

- Zhang Y, Li B, Zheng D (2002) A discussion on the boundary and area of the Tibetan
  plateau in China. Geographical Resources 21:1–8.
- 657 Zhang J, Huang B, Chen L et al (2018) Characteristics of biochar produced from yak
  658 manure at different pyrolysis temperatures and its effects on the yield and growth of

highland barley. *Chemical Speciation & Bioavailability* 30 (1): 57–67.

- 660 Zhang LH, Xu CB, Champagne P (2010) Overview of recent advances in thermo-chemical
- 661 conversion of biomass, Energy Conversion and Management 51 :969–982





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





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

686 husk biochar





689 rice husk biochar

Parameter	Value	Parameter	Value
Ash (wt.%)	$39.7\pm0.5$	Fe (wt.%)	$0.73\pm0.02$
pН	$10.38\pm0.02$	P (mg/l)	$10.3\pm0.13$
C (wt.%)	$40.8\pm1.3$	K (mg/l)	$47.9\pm0.4$
N (wt.%)	$0.32\pm0.03$	Ca (mg/l)	$11.0\pm0.2$
H (wt.%)	$0.89\pm0.21$	Mg (mg/l)	$6.20\pm0.1$
O (wt.%)	$17.9\pm0.7$	Na (mg/l)	$2.06\pm0.06$
S (wt.%)	$0.41\pm0.08$	BET (N <sub>2</sub> ) surface area ( $m^2/g$ )	3.19
Si (wt.%)	$11.92\pm0.11$	Average pore width (nm)	10.6

**Table 1.** Major properties of rice husk biochar

700	Table 2. Effect of biochar and	fertilizer application	on alpine meadov	w productivity over three
-----	--------------------------------	------------------------	------------------	---------------------------

701 years (2014-2016)

Treatments	Fresh	Biomass ( g/	m <sup>2</sup> )	Dry Biomass (g/m <sup>2</sup> )			
Treatments	2014	2015	2016	2014	2015	2016	
СК	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	
$\mathrm{BC}_{\mathrm{H}}$	96.27 cd	119.60 d	132.29 g	39.86 bc	64.87 e	70.70 f	
NPKL	101.77 abc	165.67 c	155.33 f	49.76 abc	84.30 d	75.82 f	
$\mathbf{NPK}_{\mathrm{H}}$	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	
$B{C_{H}}^{+}NPK_{H}$	108.63 a	190.37 ab	204.07 b	56.10 ab	111.97 b	118.33 c	

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

				рН		
Treatments	20	014	2	015	2	016
	0-10 cm	10-20-cm	0-10 cm	10-20 cm	0-10 cm	10-20 ci
CK	6.54 ef	6.64 d	6.62 f	6.79 e	6.72 g	6.83f
BCL	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 al
$BC_{\rm H}$	6.80 ab	6.95 a	7.08 a	6.97 abc	7.09 a	7.13 a
NPKL	6.49 f	6.72 cd	6.79 e	6.87 de	7.00 cde	7.01 e
NPK <sub>H</sub>	6.53 de	6.75 c	6.90 bcde	6.91 cd	6.91 f	7.03 d
$BC_L^+ NPK_L$	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 al
$BC_M^+ NPK_L$	6.69 cd	6.74 cd	6.91 bcd	6.92 cd	6.97 cdef	7.07 b
$BC_M^+ NPK_H$	6.78 abc	6.66 d	6.88 cde	6.81 e	6.94 def	7.05 c
$BC_{H}^{+}NPK_{L}$	6.79 ab	6.79 bc	6.87 cde	6.94 bcd	7.00 bcde	7.07 b
$BC_{H}^{+}NPK_{H}$	6.85 a	6.93 a	6.94 bcd	6.96 abc	7.00 bcd	7.04 c
$BC_{M}^{+} NPK_{H}$ $BC_{H}^{+} NPK_{L}$ $BC_{H}^{+} NPK_{H}$ Column means	6.78 abc 6.79 ab 6.85 a	6.66 d 6.79 bc 6.93 a with different	6.88 cde 6.87 cde 6.94 bcd t letters indic	6.81 e 6.94 bcd 6.96 abc cate significat	6.94 def 7.00 bcde 7.00 bcd nce difference	7.05 7.07 7.04 $es (p \le 0$

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

			Nitro	ogen (wt.%)			
Treatments	2	014	2	2015	2016		
	0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm	
СК	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	
$BC_{\rm H}$	0.48 ab	0.43b	0.46 de	0.37 abc	0.44 c	0.32 def	
NPKL	0.50bcd	0.30 b	0.32 f	0.29 de	0.59 a	0.41 ab	
NPK <sub>H</sub>	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 w	vith different	letters indic	ate significanc	e differences	at $(p \le 0.05)$	

**Table 4.** Effect of biochar and fertilizer application on soil nitrogen content over three years

733 (2014-2016) period

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

	Carbon (%)									
Treatments	2014		20	015	2016					
	0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm				
СК	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				
NPKL	4.06 e	2.51 c	5.43 d	3.07 fg	4.93 cd	2.88 d				
NPK <sub>H</sub>	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				

751 Mean values presented in columns with different letters indicate significant differences at  $p \le 1$ 

752 0.05

753

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

Source	FB	DB	РНА	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**

Treatment	FB (g/m <sup>2</sup> )	$DB(g/m^2)$	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

<b>1 able 7.</b> Cumulative impact of different treatment on biomass and soli properties over	767	rent treatment on biomass an	d soil pro	operties over t	three
---	-----	------------------------------	------------	-----------------	-------

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

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

# 774 (Varimax rotation)

Traits		Component	
Traits	1	2	3
рНВ	0.881	0.204	0.133
рНА	0.82	0.443	0.044
NB	0.604	-0.257 0.89	-0.103 -0.047
СВ	-0.024		
CA	0.391	0.797	0.305
DB NA	-0.102	-0.087	0.88
	0.162	0.098	0.798
FB	-0.017	0.479	0.642
Eigenvalue	2.00	1.98	1.95
Proportion $\sigma 2\%$	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		
СК	3.24±0.004		
$BC_M$	3.25±0.003		
NPK <sub>H</sub>	3.23±0.002		
$BC_M^+ NPK_H$	3.28±0.007		

		Biochar stored at room temperatures for three		Biochar extracted from the soil after three years	
Nama	Structure				
Name	Structure	years			
		Peak BE	At%	Peak BE	At%
C1s A	- C=C-	204.04	(2.41	204.02	51.00
	non-functionalised sp2C	284.84	03.41	284.82	51.00
C1s B	– C-OH, C-O-C=,	286.46	8.57	286.26	10.85
	C-O-R	280.40			
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

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

/ 50