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Biochar improves maize growth by alleviation of nutrient stress in a moderately acidic low-input Nepalese soil

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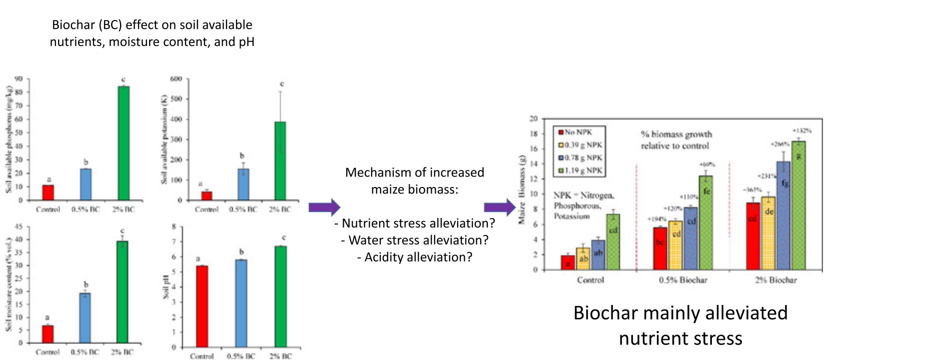
HIGHLIGHTS

- Soil limitations (moisture, nutrients, acidity) were manipulated one by one to find out why biochar improved crop growth.
- Biochar addition increased soil pH, plant available P, K and soil moisture retention in this weathered Nepalese soil.
- The biochar effect on plant growth was mainly due to alleviation of nutrient stress.

GRAPHICAL ABSTRACT



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ABSTRACT

We studied the role of biochar in improving soil fertility for maize production. The effects of biochar on the alleviation of three potential physical-chemical soil limitations for maize growth were investigated, i.e. water stress, nutrient stress and acid stress. Experiments involved soils with two dosages of biochar (0.5% and 2% w:w), as well as ones without biochar, in combination with four different dosages of NPK fertilizer, water and lime. Biochar was produced from the invasive shrubby weed *Eupatorium adenophorum* using flame curtain kilns. This is the first study to alleviate one by one the water stress, nutrient stress and acid stress in order to investigate the mechanisms of biochar effects on soil fertility.

Biochar addition increased soil moisture, potassium (K) and plant available phosphorous (P-AL), which all showed significant positive relationship ($p < 0.001$) with above ground biomass of maize. However, biochar was much more effective at abundant soil watering (+311% biomass) than at water-starved conditions (+67% biomass), indicating that biochar did increase soil moisture, but that this was not the main reason for the positive biomass growth effects. Biochar addition did have a stronger effect under nutrient-stressed conditions (+363%) than under abundant nutrient application (+132%). Biochar amendment increased soil pH, but liming and pH had no effect on maize dry biomass, so acidity stress alleviation was not the mechanism of biochar effects on soil fertility.

Abbreviations: BC, biochar; CEC, cation exchange capacity; d, days; NPK, nitrogen, phosphorous and potassium; NO_3^--N, nitrate; OC, organic carbon; P-AL, plant available phosphorous; PRS™, plant root simulators; TDR, time domain reflectometer; t ha^{-1}, tonnes per hectare.

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In conclusion, the alleviation of nutrient stress was the probably the main factor contributing to the increased maize biomass production upon biochar addition to this moderately acidic Inceptisol.

1. Introduction

Promising agronomic effects of biochar addition have been found in a wide range of latitude with low-fertile soils (Biederman and Harpole, 2013; Liu et al., 2013), due to improvements of soil biological, physical or chemical properties (Cornelissen et al., 2013; Glaser et al., 2002; Lehmann and Rondon, 2006; Yamato et al., 2006). Biological effects may include enhanced activities of mycorrhizal fungi, ameliorating nutrient uptake by plants (Atkinson et al., 2010) and increased colonization rates of arbuscular mycorrhizal fungi, which for maize plant roots have been shown to increase significantly by 26% for biochar amended soils applied at a rate of 10 l m^{-2} (around 20 t ha^{-1}) (Yamato et al., 2006).

With regard to soil physical properties, biochar addition improved soil water holding capacity (WHC) and plant available water (PAW) in both loamy and sandy loam soils (Bruun et al., 2014; Dugan et al., 2010; Martinsen et al., 2014). WHC was increased by 11% upon biochar amendment (9 t ha^{-1}) in a silty loam agricultural soil, Southern Finland (Karhu et al., 2011). Increased PAW upon biochar addition can be explained by improved porous structure (both microporosity and mesoporosity) and soil aggregation (Herath et al., 2013; Obia et al., 2016). Although it is apparent that biochar can improve soil moisture, there is a knowledge gap regarding to what extent this effect can explain the positive effect of biochar on crop growth.

In addition to soil physical properties, soil chemical properties can also be improved significantly by the addition of biochar. Besides increasing soil pH (higher Ca/Al ratios and higher PO_4^{-3} availability) and base saturation (BS) (Glaser et al., 2002; Martinsen et al., 2015) the addition of biochar increases nutrient retention capacity and soil CEC (Chan et al., 2008; Liang et al., 2006) and thus reduces nutrient leaching (Hale et al., 2013; Laird et al., 2010; Martinsen et al., 2014; Steiner et al., 2007). Low pH is commonly associated with increased Al-concentrations in soil solution, which is highly toxic to plant roots (Gruba and Mulder, 2008). The Al concentration can be reduced drastically by addition of biochar that acts as a liming agent in most of the degraded soils (Glaser et al., 2002; Major et al., 2010; Martinsen et al., 2015; Van Zwieten et al., 2010; Yamato et al., 2006). When 20 t ha^{-1} biochar was applied on highly weathered tropical soils, soil pH increased from 3.9 to 5.1, thereby reducing exchangeable Al^{3+} from 2.67 to 0.12 $cmol_c$ kg^{-1} and exchangeable H^+ from 0.26 to 0.12 $cmol_c$ kg^{-1} whereupon maize yield almost doubled (10 t ha^{-1}) compared with control soils (5 t ha^{-1}) (Yamato et al., 2006). Similar trends were observed for soil CEC, base saturation and exchangeable K upon biochar addition.

Biochar addition can have a strong influence on in-situ soil nutrient availability, emphasizing its role in soil nutrient adsorption and plant availability. Biochar produced from peanut hull at 600 °C showed reduced leaching of NH_4^+-N, NO_3^--N and PO_4^--P (35%, 34% and 21%, respectively) under ex-situ conditions (Yao et al., 2012). The main mechanism may be the absorption of NO_3^--N in biochar nano-pores (Kammann et al., 2015). PO_4^--P is tightly bound in highly weathered tropical soils that are often rich in Fe and Al oxides (Hale et al., 2013). Under such conditions, biochar addition increases soil pH which makes PO_4^--P more bio-available in soil solution (Asai et al., 2009; Hale et al., 2013). For many biochars, an increase in soil K availability may be due to high content of K in biochar ash and reduced K leaching upon biochar addition (Laird et al., 2010; Martinsen et al., 2014).Adding biochar during composting results in organic coatings being formed in the biochar pores (Hagemann et al., 2017), which retains and facilitates slow release of most important plant nutrients (Joseph et al., 2017). Similar to the effect of biochar on soil moisture, it is often unclear to what extent biochar's effect on nutrient retention explains its positive agronomic effects.

Although biochar improves soil quality, there is a knowledge gap regarding the exact mechanism resulting in this positive agronomic effect, as it varies with soil type and the most important soil constraints. Jeffery et al. (2017) conducted a meta-analysis to investigate these soil constraints, and reported 25% average crop yield increase in tropical soils upon biochar addition, mostly through liming and nutrient effects (N and K retention, P availability and direct K addition). However, their study did not allow an individual assessment of the importance of biochar amendment on soil water retention as a factor that could explain the enhanced crop production.

In Nepal, soils are often moderately acidic showing low nitrogen (N), phosphorous (P) and exchangeable base concentrations (Brown et al., 1999; Schreier et al., 1994). Such soil characteristics can have adverse effects on soil fertility and crop production (Brown et al., 1999). Biochar addition has shown positive effects on soil chemical properties in Nepal, with increased pH, CEC and organic C (Pandit et al., 2017) and crop growth (Schmidt et al., 2015). However, thus far, few studies have been published where explicit attempts were made to unravel the soil physical and chemical mechanisms responsible for the positive effect of biochar on crop production. In the present study, the effect of biochar on an acidic silty loam Inceptisol from Rasuwa, Nepal was explored. This soil can be considered representative for many moderately weathered, eroded and acidified soils of Nepal and beyond. Three possible physical-chemical limitations for crop production were anticipated for this soil: i) nutrient limitation due to insufficient nutrient retention ("nutrient stress"), ii) drought due to limited water retention capacity ("water stress"), or iii) a possible degree of aluminum toxicity due to a soil pH_{CaCl2} of 4.5 ("acid stress"). The effectiveness of biochar in alleviating these limitations under maize plantation was evaluated by individual testing of each possible limitation under controlled greenhouse conditions. Controlled conditions were chosen since the main purpose of the present study was mechanistic understanding of biochar effects on soil fertility. Small standard deviations in the biomass data were a prerequisite for the observation of significant differences. Thus, controlled greenhouse conditions were a better choice to execute such a mechanistic study than natural open field conditions. A forthcoming study will report on crop yield data for maize and mustard in exactly the same soil under natural conditions.

We explored the following three hypotheses in the present soil.

1) Biochar alleviates moisture stress through enhanced soil water retention, thus increasing plant growth.
2) Biochar alleviates nutrient stress by increased in-situ nutrient availability (increased CEC) and by the direct addition of nutrients, especially K.
3) Biochar alleviates acid stress and thus increases plant growth by increased soil pH, Ca/Al ratios and available P.

2. Materials and methods

2.1. Soil and biochar

Soil (Inceptisol-Dystrochrept order; IUSS working group WRB, 2006) was collected from agricultural land, Rasuwa district, Nepal (N 28° 00′, E 85° 10′). Soil was collected from 0 to 30 cm depth (top layer) from 20 different locations within a plot (300 m^2) to make a composite soil of approx.600 kg (30 kg each from one location), which was sufficient to carry out this pot trial experiment. The sampled soil used in this experiment was moderately acidic (pH_{CaCl2} 4.5; pH_{water} 5.1), low-CEC (6.05 $cmol_c\ kg^{-1}$ extracted with 1 M NH_4NO_3), silty loam (Supplementary Table 1).

Biochar was produced from ubiquitous, invasive and non-palatable shrubby wood "*Eupatorium adenophorum*" feedstock using a flame curtain steel-shielded soil pit "Kon-Tiki" kiln with final pyrolysis temperature of 600–700 °C. Biochar was finely ground before application (<2 mm).

Details on biochar production technology and properties of produced biochar are shown in Supplementary information files (Supplementary description 2 and Supplementary Table 1).

2.2. Experimental design

A pot trial was carried out under greenhouse conditions (11th May to 5th July 2016) at Matatirtha, Nepal (N 27° 41′ 51″, E 85° 14′ 0″). Average temperature inside the greenhouse was 28.0 ± 8.9 °C (average min. 19.5 °C and average max. 36.5 °C, n = 50) throughout the trial. Pots (top, middle and bottom diameter: 24 cm, 19 cm and 12 cm respectively; height 20 cm; volume 6 l) were filled with 3 kg of air-dried soil-biochar mixtures. Pot size was similar to our previous pot trial study carried out with maize, harvested after 50 d in the same soil (Pandit et al., 2017). Three sets of experiments were set up, each to test the alleviation of one stress factor; i.e. water stress, nutrient stress or acid stress alleviation by biochar. Nutrient stress was created by NPK fertilizer addition at four dosages ranging from very low amounts up to the recommended dosages, water stress was created by watering at four amounts below those provided by normal rainfall (calculation based on average rainfall in the rainy season in Kathmandu, and pot diameter, as in Pandit et al., 2017), and acid stress was alleviated to variable extents by liming (powdered $CaCO_3$) at four dosages to a previously tested range of pH values. Alleviation of the stresses was investigated by adding three different biochar dosages; control (0 t ha^{-1}), 0.5% biochar (10 t ha^{-1}) and 2% biochar (40 t ha^{-1}). Biochar amended at 2% dosage may be a relatively high dosage under farming conditions, but in this study, this dosage was included for mechanistic purpose, and a dosage of 0.5% was also studied for comparison. Under normal growing conditions of maize plants in the field, tillage, weather conditions and other external factors may have an impact on soil properties and crop growth, and this was not considered here. However, we managed to explore the mechanistic study under well-controlled greenhouse conditions excluding all the external abiotic and biotic disturbances that may otherwise hinder the clear illustration of biochar effects in this soil.

Each set of experiments comprised nine treatments with four replications each in completely randomized design (n = 4) resulting in 36 pots per experiment (Table 1). Three treatments receiving the highest amount of water (200 ml per pot per day), NPK (1.17 g per pot) and lime (4.7 g per pot) were added that were considered as shared treatments (common) for each of the water stress, nutrient stress and acid stress sets of experiments. Details of the experimental design and the added amounts of water, NPK and lime for three different dosages of biochar (0% biochar or control, 0.5% biochar and 2% biochar) are summarized in Table 1. After NPK (in the form of Urea, Diammonium Phosphate and Murate of Potash for N, P_2O_5 and K_2O) and lime (applied as pure $CaCO_3$, 99%, Sigma Aldrich, Norway) had been mixed into the soil (air dried not sieved), the pots were left for four days in the green house before maize was planted.

Three maize seeds (*Zea mays;* Manakamana-4 variety) were sown 2 cm below the soil surface in each pot. All the pots from the three sets of experiments, including the water stress alleviation tests, were irrigated daily with 200 ml water per pot and day (corresponding to about 4 mm rainfall per day) until second leaf emergence (14 d). At this point, the smaller and least robust plants were removed, leaving the most robust plant in each pot. After 14 d, all pots were irrigated at five-day intervals with 1000 ml water per pot (20 mm rainfall), except for the pots in the water stress experiment, which received less water (Table 1). The plant water status of maize plants growing in 6 l pots is reasonably representative of that in plants growing in field conditions, since maize root systems in similar soils in the field have been found to constitute only 2–4% of total biomass (root-to-shoot-ratios of 0.02–0.04) and the root system weighed only 10–12 g (Abiven et al., 2015). No crowding of plant roots in the pots was observed. After 30 d, all the pots were top dressed with urea (0.3 g N per pot) except for the nutrient stress experiment which received less N (both basal application and top dressed) than the full dosage (Table 1). Pots were rotated every four days until harvest to ensure the homogeneity of the treatments. Manual weeding was carried out twice (30 d and 42 d) during the experiment.

2.3. In-situ soil measurement

2.3.1. Soil moisture content

Soil moisture content (% by volume) was measured in the water stress alleviation treatments only, using a hand-held Time-Domain Reflectometer (TDR; SM150 soil moisture sensor, Delta T devices Ltd.,

Table 1
Number of treatments in water stress, nutrient stress and acid stress experiment. Each experiment consisted of 9 quadruplicate treatments (n = 4) excluding common or shared treatments (for all three experiments) receiving full water (200 ml per day), NPK (1.17 g per pot) and lime (4.5 g per pot) that was mixed with three different dosages of biochar (0% BC, 0.5% BC and 2% BC) following completely randomized design.

Treatments	Irrigation (ml/pot/day)	NPK basal dose (g per pot)			N top dress (g per pot)	Total NPK (g per pot)	Lime (g per pot)
		N	P_2O_5	K_2O			
Water stress alleviation experiment							
20% water + 0% BC (control), 0.5% BC, 2% BC	40	0.32	0.32	0.23	0.3	1.17	4.5
40% water + 0% BC (control), 0.5% BC, 2% BC	80	0.32	0.32	0.23	0.3	1.17	4.5
70% water + 0% BC (control), 0.5% BC, 2% BC	140	0.32	0.32	0.23	0.3	1.17	4.5
Nutrient stress alleviation experiment							
No NPK + 0% BC (control), 0.5% BC, 2% BC	200	0	0	0	0	0	4.5
1/3rd NPK + 0% BC (control)[a], 0.5% BC, 2% BC[a]	200	0.11	0.11	0.07	0.1	0.39	4.5
2/3rd NPK + 0% BC (control), 0.5% BC, 2% BC	200	0.22	0.22	0.14	0.2	0.78	4.5
Acid stress alleviation experiment							
No lime + 0% BC (control)[a], 0.5% BC[a], 2% BC[a]	200	0.32	0.32	0.23	0.3	1.17	0
0.25 g lime + 0% BC (control), 0.5% BC, 2% BC	200	0.32	0.32	0.23	0.3	1.17	0.25
0.75 g lime + 0% BC (control), 0.5% BC, 2% BC	200	0.32	0.32	0.23	0.3	1.17	0.75
Common or shared treatments							
Full + 0% BC (control)[a], full + 0.5% BC[a], full + 2% BC[a]	200	0.32	0.32	0.23	0.3	1.17	4.5

[a] PRS™ probes treatments selected from all experiment to explore in-situ soil nutrient supply rates.

Burwell, Cambridge, England) just before each irrigation. For each pot and at each time point, three measurements were carried out and averaged to give one reading per pot. Saucers were fitted at the bottom of each pot to measure the amount of water that drained through the pot holes. Importantly, hardly any water drained during the trial from either of the treatment pots.

2.3.2. Soil nutrient availability

Plant root simulator probes (PRS™; Western Ag, Saskatoon, Canada) were used to measure the in-situ availability of cations and anions in the soil (Martinsen et al., 2014). For these measurements, eight treatments were selected; two from nutrient stress, three form acid stress and three from common/shared treatments (Table 1a). Four anion probes and four cation probes per pot were inserted on day 36 (6 days after the addition of the urea top dressing) and left in the soil for 14 days (total 12 + 12 probes per treatment). After exposure, probes were washed thoroughly with water to ensure removal all soil particles. The 12 anion and 12 cation probes per treatment were combined into triplicate anion- and cation-probe samples. PRS™ probes were stored in a cool place after sampling and shipped to Western Ag innovations (Canada) for extraction and analysis according to (Martinsen et al., 2014). Nutrient supply rates measured by PRS ™ probes are reported in μg per 10 cm^2 (sampler surface area) per 14 d (exposure period), i.e., in μg 10 cm^{-2} 14 d^{-1}.

2.3.3. Soil pH

Soil pH was measured at the start (1 d), mid-way (25 d) and end of the experiment (50 d) for soil samples from the acid stress alleviation treatments. Soil pH was measured with WTW pH 320 equipment in 0.01 M $CaCl_2$ solution (1:2.5, solid to solution ratio). Soil pH measured at 1 d, 25 d and 50 d were averaged to give one final reading per treatment.

2.4. Leaf Porometry

Stomatal conductance (mmol water m^{-2} s^{-1}) was measured as an indication of plant water stress for the water stress experiment only (Decagon SC-1 leaf porometer, Seattle, WA, USA). Stomatal conductance was determined from the measured difference in relative humidity between two sensors in the diffusion pathway through a chamber between the leaf surface and a desiccant (Decagon, Seattle, WA, USA) at relative humidity of 0%. Measurements were conducted for 30 s. Calibration was carried out using a wetted Teflon disk with a known conductance of 240 mmol m^{-2} s^{-1}. Measurements were carried out for four different leaves of each plant, giving 16 measurements per quadruplicate treatment. Measurements were carried out on day 50 of the experiment, at a temperature of 20 to 21 °C inside the greenhouse, during continuously rainy conditions at 91 to 92% relative humidity, with very little variation in light conditions during the 4 h of data collection (12 noon to 4 pm). For practical reasons, we could carry out this measurement only once, but under representative conditions for the Nepal rainy season. Measurement accuracy was 10%. During the measurement time interval, 16 background measurements were done without any leaf present in the porometer chamber. Reported values were corrected for the measured background conductance of 26.3 ± 4.7 mmol m^{-2} s^{-1}.

2.5. Plant harvest

Maize plants were harvested on day 50. Maize above ground biomass of all the treatment pots were oven dried at 70 °C for 24 h, prior to measuring dry weight. Roots systems were not considered in this study as the root constituted only a small portion of total biomass (2–4%), and the determination of root biomass is often less accurate than that of biomass because of incomplete soil/root separation and loss of roots during cleaning.

2.6. Ex-situ soil parameters

Triplicate soil samples from each pot (surface layer to 8 cm depth) were collected after harvesting maize plants and pooled into one composite sample for each of the 30 treatments. Soil samples were oven dried at 40 °C for three days and passed through a 2 mm sieve prior to analysis. Particle size distribution of the soil was measured through pipette method. Soil cation exchange capacity (CEC) was measured for the three common treatments (control, 0.5% biochar and 2% biochar which received full amount of water, NPK and lime) to assess the pure effect of biochar addition on soil exchangeable ions. The soil was extracted with 1 M NH_4NO_3 and the individual exchangeable cations (Ca^{2+}, Mg^{2+}, Na^+, K^+ and Al^{3+}) were measured in the leachates using inductively coupled plasma optical emission spectrometry (ICP-OES). Exchangeable H^+ was determined by titration with 0.02 M NaOH to pH 7. Sieved samples were crushed for total C, H and N analysis with a CHN analyzer (LECO, Truspec). Plant available phosphorous (P-AL) was measured by the ammonium lactate method (Krogstad et al., 2008), where 40 ml of ammonium lactate solution was added to 2 g dry soil (sieved <2 mm) and shaken in a rotating shaker (1.5 h), and filtered, (0.45 μm). Ascorbic acid (0.4 ml) and molybdenum reagent (0.4 ml) was added to both standard solution and the extracted soil samples and measurements were done using a spectrophotometer (Gilford Stasar Spectrophotometer) at 700 nm. Plant available water (PAW % vol.) was measured for three common treatments (as for CEC above) to explore the effect of biochar addition on water retention capacity. For this purpose, hand-packed soil samples were saturated and soil water measured at different matrix potentials (pF 2, field capacity and pF 4.2, wilting point) through ceramic pressure plates (Martinsen et al., 2014; Obia et al., 2016). PAW (vol%) was calculated as the difference between field capacity (vol%) and wilting point (vol%).

2.7. Statistical analyses

Data were analyzed using R statistical software version 3.2.2. Normality and homogenous variances of all data sets were tested with Shapiro-Wilk- and Levene's test. Two factor ANOVA (fixed effect model) was used for each of the three experiment to assess the effect of the two independent fixed factors (three levels of BC dosage and four levels of either water content, NPK rate or lime rate) including interactions on selected dependent variables. For PRS™ probes datasets one way fixed effect ANOVA model was used to investigate the effect of various treatments comprised of biochar, NPK and lime addition on soil nutrient availability (NO_3^-, PO_4^{3-} and K^+) (dependent variable). Based on our relatively limited PRS™ data availability, one-way ANOVA was chosen. Basically, three factor ANOVA would be the best choice of analysis to show the main effect and interaction effect of biochar, NPK and lime on soil nutrient supply. However, our data could be analyzed only for the main effect of biochar, NPK and lime but not their interaction effect, due to lack of replications or observations for these three factor combinations. Factors showing significant effect were further explored via post hoc Tukey test ($P = 0.05$) to evaluate the significant differences between the treatment means. Analysis of covariance (ANCOVA) was endeavored to see if there is any confounding effect on biomass in each of the three set of experiment such as NPK and pH effect (covariates) under water stress experiment, water and pH effect (covariates) under nutrient stress experiment and water and NPK effect (covariates) on acid stress experiment (described in Supplementary description 1). For this purpose, each of the datasets were pooled based on the biochar effect measured on the soil factors (soil moisture content, pH, and nutrient supply rates) and carried out the ANCOVA model. Pooling the datasets from different set of experiments did not allow the precise explanation of the estimation of various explanatory soil variables on biomass production in the respective pots. In addition, there was hardly any confounding effect observed on each of the three experiments. Therefore, the ANCOVA model was reduced and two-factor ANOVA model was explored for each of the three sets of experiments. Both linear and non-linear regression analysis was included to investigate relationships between selected explanatory continuous independent variables and dependent variables (including biomass) to explain the model. With a view to assess the main effect of biochar addition (control, 0.5% biochar and 2% biochar) on soil physical and chemical properties (dependent variables), one way ANOVA, followed by a Tukey test ($P = 0.05$) were used to explore the significant differences between the biochar and non-biochar treatments (Table 1). The difference between various treatments was significant at $P < 0.05$, unless stated otherwise.

3. Results

3.1. Effect of biochar on soil properties

Biochar addition (2% w:w) significantly increased soil water retention at field capacity (from 29.8 ± 1.8% to 35.3 ± 0.2%) and plant available water (from 20.8 ± 1.9% to 25.5 ± 0.5%) in this soil (Table 2).

Biochar also increased soil CEC and pH as well as exchangeable K^+, Mg^{2+} and Ca^{2+} (Table 2). Biochar addition showed significant effect ($P < 0.001$) on plant available phosphorous (P-AL) which was increased from 11.1 mg kg^{-1} (control) to 23.4 mg kg^{-1} and 84.1 mg kg^{-1} upon 0.5% and 2% biochar addition, respectively (Table 2).

Based on a simple addition of the amount of carbon in the soil and that via the biochar amendment (that is the addition of biochar containing 70% C for 0.5% and 2% biochar dosages to the present soil organic carbon (1.35% SOC)), the resulting soil organic carbon contents should have been 1.70% and 2.75%, close to the observed values of 1.64 and 2.94%, respectively (Table 2).

Table 2
Effect of biochar on soil physical and chemical properties. Treatments with different biochar dosages (0% BC or control, 0.5% and 2% BC) receiving highest amount of agricultural inputs (water, NPK and lime) i.e. the three common treatments. Soil properties values are given as mean ± SD, n = 3. Letters a, b and c denotes significant differences between biochar vs non-biochar (control) treatments on soil properties.

Properties	Common treatments with full NPK, lime and watering rates		
	0% BC (control)	0.5% BC	2% BC
Total organic C%	1.35 ± 0.0 a	1.64 ± 0.01 b	2.94 ± 0.02 c
Total nitrogen%	0.12 ± 0.01 a	0.12 ± 0.01 a	0.14 ± 0.01 a
Total hydrogen%	0.48 ± 0.01 a	0.47 ± 0.01 a	0.48 ± 0.00 a
pH (0.01 M $CaCl_2$)[a]	5.34 ± 0.15 a	5.87 ± 0.13 b	6.58 ± 0.13 c
CEC ($cmol_c\ kg^{-1}$)	7.63 ± 0.7 a	8.69 ± 0.45 a	11.92 ± 0.24 b
Ca^{2+} ($cmol_c\ kg^{-1}$)	5.96 ± 0.24 a	6.38 ± 0.24 a	8.87 ± 0.24 b
Mg^{2+} ($cmol_c\ kg^{-1}$)	0.54 ± 0.02 a	0.67 ± 0.01 b	1.07 ± 0.04 c
K^+ ($cmol_c\ kg^{-1}$)	0.26 ± 0.02 a	0.55 ± 0.07 b	1.75 ± 0.12 c
Al^{3+} ($cmol_c\ kg^{-1}$)	0.03 ± 0.03 a	0.006 ± 0.00 a	0.006 ± 0.00 a
H^+ ($cmol_c\ kg^{-1}$)	0.81 ± 0.84 ab	1.05 ± 0.19 a	0.17 ± 0.14 b
Sand %	32.70 ± 0.49	32.1 ± 0.35	32.70 ± 0.49
Silt %	49.90 ± 0.43	50.6 ± 0.55	50.70 ± 1.05
Clay %	17.40 ± 0.11	17.40 ± 0.37	16.70 ± 0.60
Textural class	Silty loam	Silty loam	Silty loam
Soil moisture content (% vol.)[b]	6.9 ± 0.6 a	19.1 ± 1.4 b	39.3 ± 2.1 c
Field capacity (% vol)	29.83 ± 1.83 a	29.96 ± 1.34 a	35.30 ± 0.18 b
Plant available water (% vol)	20.82 ± 1.97 a	21.18 ± 0.78 a	25.55 ± 0.54 b
P-AL (mg kg^{-1})	11.10 ± 0.30 a	23.36 ± 0.28 b	84.16 ± 1.08 c
PRS™ adsorbed cations and anions			
NO_3^{-1} (μg per 10 cm^2)	304 ± 158 a	636 ± 131 a	783 ± 257 a
Ca^{2+} (μg per 10 cm^2)	1350 ± 386 a	2401 ± 645 b	2259 ± 99 b
Mg^{2+} (μg per 10 cm^2)	103 ± 45 a	223 ± 18 b	284 ± 30 b
K^+ (μg per 10 cm^2)	41 ± 11 a	156 ± 29 b	384 ± 144 c
P (μg per 10 cm^2)	1.2 ± 0.4 a	3.1 ± 0.4 b	3.5 ± 3.3 b
Fe^{3+} (μg per 10 cm^2)	40 ± 23.7 a	103 ± 4 b	86 ± 27 b
Al^{3+} (μg per 10 cm^2)	31 ± 16.6 a	54 ± 16.8 a	24 ± 6.7 a
Ca/Al (molar ratio)	32.2 ± 9.0 a	32.3 ± 17.7 a	63.8 ± 18.6 b

[a] Soil pH was averaged and pooled for standard deviation from 1 d, 24 d and 50 d (in-situ and ex-situ pH measurement) to give one final reading (mean ± SD).
[b] Daily measured in-situ soil moisture percentage measurement (% vol.), n = 50.

3.2. Alleviation of water stress by biochar

3.2.1. Effect of biochar on soil moisture content

Soil moisture percentage increased up to seven-fold upon 2% biochar addition for both highest watering (200 ml per day, increased moisture content from 7% to 40% by vol.) and lowest watering rates (40 ml per day, increased moisture content from 1% to 7%) (Fig. 1, Supplementary Fig. 1).

3.2.2. Effect of biochar on maize biomass and stomatal conductance at various watering rates

A significant interaction between the effect of biochar dosages and watering rates on maize biomass production was observed (Fig. 2a, Table 3a-ii). Biochar addition showed significant effects on biomass at all watering rates (the presence of 2% biochar increased biomass by +67 to +311% dependent on watering rate; Fig. 2a), but slightly less so at the lowest water addition (40 ml per day and 80 ml per day), where only the 2% biochar dosage but not the 0.5% dosage showed significant increments on biomass production (Fig. 2a). Leaf stomatal conductance showed a positive relationship ($R^2 = 0.37$, $P = 0.03$) with soil moisture content (Supplementary Fig. 2a) and dry biomass production ($R^2 = 0.51$, $P = 0.008$) (Supplementary Fig. 2b).

3.3. Biochar and nutrient stress alleviation

3.3.1. Effect of biochar on soil nutrient availability

PRS™ probe measured K^+ and PO_4^{3-}-P rates (all in units μg 10 cm^{-2} 1 d^{-1}) were significantly higher upon biochar addition (2% biochar) for both the lowest (0.39 g NPK) and the highest amount of NPK addition (1.19 g NPK) (Table 4). At the lowest NPK rate, biochar addition strongly increased K^+ supply rates from 23.6 ± 2.5 to 667 ± 215 and PO_4^{3-}-P from 1.6 ± 0.6 to 5.5 ± 2.0 (Table 4). Other fertilizer nutrient supply rates such as NO_3^--N, Ca and Mg showed significant effects only upon NPK addition but not on biochar amendment (Table 4). Furthermore, P-AL (mg kg^{-1}) was significantly increased upon mineral nutrient (NPK) addition but the response was stronger when biochar was added (Supplementary Table 2). P-AL (mg kg^{-1}) increased approximately eight-fold in the presence of 2% biochar at all level of NPK additions (Supplementary Table 2).

3.3.2. Effect of biochar on maize biomass at various NPK dosages

A significant interaction between the effect of biochar dosages and NPK rates ($P < 0.001$) on maize dry biomass (Fig. 2b, Table 3b-ii) was observed. Both dosages of biochar increased biomass production at all levels of NPK application (Fig. 2b). The most important trends observed between various PRS™ probes soil nutrient supply rates (Supplementary Fig. 4) and maize biomass production were those for K^+ ($R^2 = 0.51$, $P < 0.001$; Supplementary Fig. 4d) and P-AL ($R^2 = 0.61$, $P < 0.001$; Supplementary Fig. 5.b).

A combination of biochar and low NPK (1/3rd NPK) revealed significantly higher biomass production compared with control (2.9 ± 1.1 g per pot); increases were +120% at 0.5% biochar (6.4 ± 0.7 g per pot) and +231% at 2% biochar (9.6 ± 1.3 g per pot) (Fig. 2b). A similar trend was observed for the combination of biochar and both the second and third NPK addition (Fig. 2b). For the highest NPK rate, biochar addition was observed to have additional but not as strong effects on biomass production (increased by +69% at 0.5% biochar and by +132% at 2% biochar compared with control) (Fig. 2b).

3.4. Biochar and acid stress alleviation

3.4.1. Effect of biochar on soil pH and plant available phosphorous

Both biochar and lime addition showed significant effects ($P < 0.001$) on average soil pH (Fig. 3a, Table 3c-i) measured at 1 d, 24 d and 50 d during the experiment (Supplementary Table 3). A similar trend was observed for the ratio between PRS™ probes extractable Ca and Al

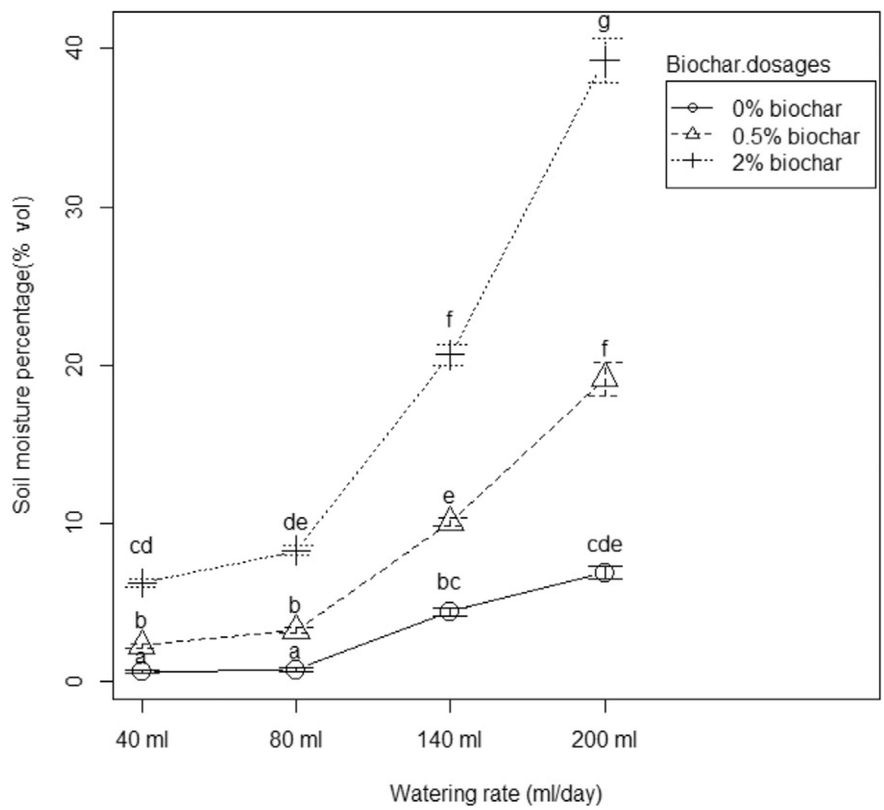

Fig. 1. Effect of biochar dosages and watering rate on soil moisture content (percentage by volume). Soil moisture percentages measured at five-day intervals after second leaf emergence at 15 d until harvest at 50 d. Each level of biochar dose combined with each level of watering rates; mean ± SE, n = 28. Different letters inside the graph denote significant differences between the treatments followed by two factor ANOVA (Post hoc Tukey test, $P < 0.05$).

supply rates (extractable Ca/Al) (Table 4). Biochar addition (2% biochar) significantly increased Ca/Al ratio both in the absence (from 11.3 ± 0.9 to 23.7 ± 7.2) and presence of lime (from 32.2 ± 9.0 to 63.8 ± 18.6) (Table 4). In addition to improved pH and Ca/Al ratio, plant available phosphorous (P-AL) was also significantly increased upon biochar addition, probably as a result of a more favorable pH; increases in P-AL were observed from 11.0 ± 0.3 mg kg^{-1} (control) to 23.3 ± 0.2 mg kg^{-1} at 0.5% biochar and to 84.1 ± 2.0 mg kg^{-1} at 2% biochar addition for the treatments receiving full amount of liming rates (Supplementary Table 2). Improved soil pH illustrated higher plant available phosphorus ($R^2 = 0.75$, $P < 0.01$) attributed mainly by biochar amendment in this soil (Supplementary Fig. 6a).

The Al^{3+} data in Table 4 are very low compared to those of Ca^{2+}, and insignificant compared to the fluxes of base nutrients. The limed treatments also received full NPK, and NPK mineral fertilizer is acidifying. This is probably the reason that Al^{3+}, while still low, was slightly higher in the presence of full NPK (and lime; Table 4).

3.4.2. Effect of biochar on biomass production under various liming rates

Lime addition increased soil pH (Fig. 3a) and Ca/Al ratio in the PRS™ probes membranes (Table 4). However, importantly liming had no effect ($P > 0.05$) on maize biomass production (Fig. 3b, Table 3c-ii). Biochar addition was the only main factor increasing maize biomass production with respect to different liming rates in this experiment (Fig. 3b). Liming and biochar addition did increase P-AL ($R^2 = 0.63$, $P < 0.001$; Supplementary Fig. 6b) but not maize biomass.

4. Discussion

Biochar addition clearly resulted in improved soil moisture content (Fig. 1). Also, maize biomass increased with daily watering rate. However, biochar addition (2% w:w) was less effective under water-stressed conditions (+67% biomass at 40 ml water per day) than in the presence of ample water (+311% at 140 ml water per day). These observations indicate that the biochar, despite increasing soil moisture (Fig. 1), increased biomass yield in ways related to factors other than water stress alleviation. In this respect our data are similar to those of Wang et al. (2016) where biochar addition improved soil moisture but not crop growth. The most important effect of biochar in our soil was most likely nutrient stress alleviation, as biochar showed the strongest effect at the lowest fertilization rates (1/3rd NPK), with the combination of biochar and mineral fertilizer NPK showing a significant and positive effect on biomass production (Fig. 2b, Table 3b-ii).

With regard to alleviation of soil acidity, the effect of biochar on biomass production was much stronger than the effect of liming (Fig. 3b). Indeed, lime addition did not show a significant effect on biomass production (Fig. 3b, Table 3c-ii). Thus, soil acidity (pH of 4.5 in CaCl2 and 5.1 in water and reasonably low exchangeable Al^{3+} of 1.6 cmol$_c$ kg^{-1}) was not a limiting factor for crop production in this soil. An indirect effect of improved soil pH is often an increase in P-AL in the presence of biochar, so that does not seem to be the mechanism of the biochar effect on biomass. However, biochar did result in a nutrient retention effect, and a positive relationship between P-AL and biomass was observed, so it is well possible that P-AL was improved by biochar in other ways than indirectly via increasing soil pH.

Thus, hypotheses 1 and 3 were falsified with respect to water stress and acid stress respectively, whereas hypothesis 2 was not falsified by the experimental data.

In this study, we could assume the amount of water added to the pots to be constant for all the treatments, as there was no water drained during the trial for either of the biochar and non-biochar treatments. Thus, water loss from the system was mainly governed by soil surface evaporation and plant evapotranspiration. The larger amounts of soil moisture in the 2% BC treatment indicate that there was less water loss here compared to non-amended soil, despite the larger biomass and resulting larger evapotranspiration. Thus, BC probably increased

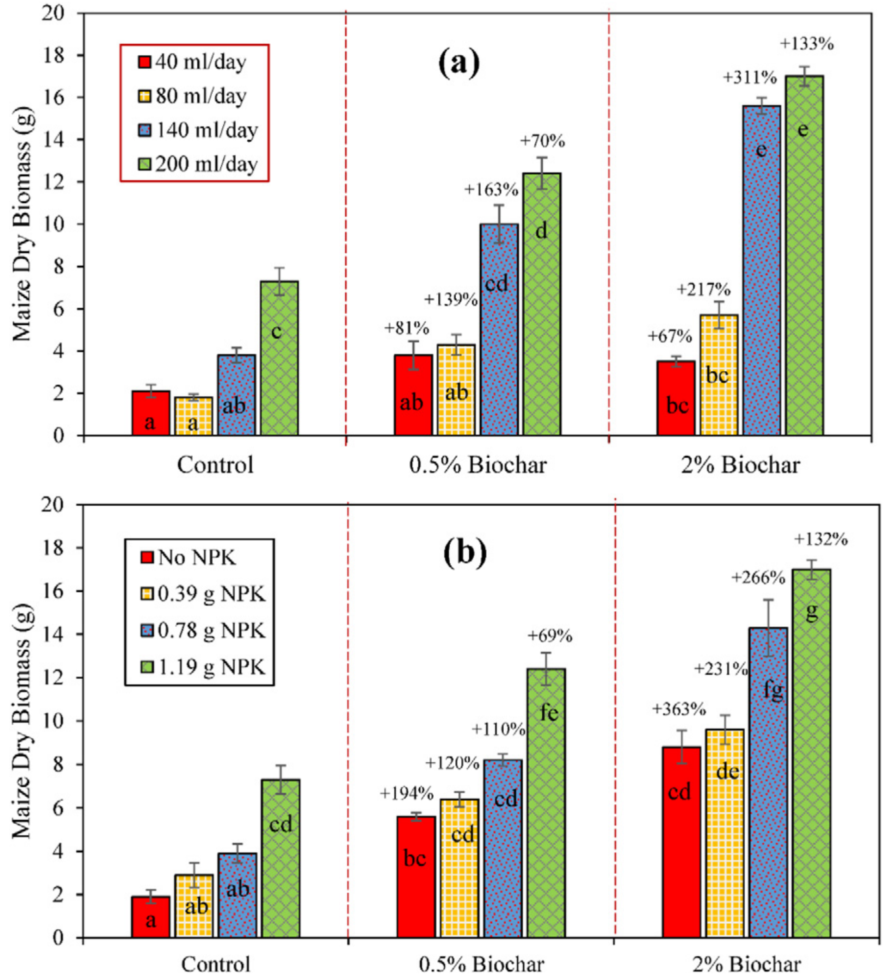

Fig. 2. Dry weight of maize above ground biomass at harvest under water stress experiment (Fig. a) and nutrient stress experiment (Fig. b); mean ± SE, n = 4. Different letters inside a bar of each treatment represents significant differences between various treatments following two way ANOVA (post hoc-Tukey test, $P < 0.05$). The percentage values above the bars denote the relative change in dry biomass production in the presence of biochar, as compared to the control receiving no biochar, at different watering (Fig. a) and NPK rates (Fig. b).

the water use efficiency (WUE), in accordance with earlier observations (Uzoma et al., 2011), and reduced evaporation from the soil surface, which was previously observed for biochar addition (3% w:w) (Basso et al., 2013). Biochar has recently been shown to form organic pore coatings that improve water retention (Hagemann et al., 2017), by reducing pore space (lowering capillary rise), and boosting hydrophilicity.

Increased PAW upon 2% biochar addition (from 21% to 26%) (Table 2), was in line with data reported by Obia et al. (2016) where biochar addition (0.2 and 4% w:w) increased PAW by 3% in Mkushi loamy soils (maize field), Zambia. Similar trend (an increase of PAW from 18.2% to 22.3%) was reported by Martinsen et al. (2014) upon biochar addition (10% vol.) in the same soil. Even stronger increases in PAW (by ~19%) have been reported for 10 t ha^{-1} biochar application on a silty loam soil of Hawera, New Zealand (Herath et al., 2013).

Stomatal conductance was on the lower end of the range previously observed for maize <100 mmol m^{-2} s^{-1} under drought conditions and 100–200 mmol m^{-2} s^{-1} under fully irrigated conditions (Medici et al., 2007), with the same trend of lower stomatal conductance under water stress (Supplementary Fig. 4).

Table 3
Statistical analysis (two factor fixed effect ANOVA model) under water stress, nutrient stress and acid stress experiments.

Factor	Response variable, *P* value		
a. Water stress experiment	i) Soil moisture content (% vol.)	ii) Maize dry biomass (g)	iii) Stomatal conductance (mmol m^{-2} s^{-1})
Biochar dosages	<0.001	<0.001	0.04
Water rates	<0.001	<0.001	<0.001
Biochar dosages X water rates	<0.001	<0.001	0.14
b. Nutrient stress experiment	i) P-AL (mg kg^{-1})	ii) Maize dry biomass (g)	
Biochar dosages (categorical)	<0.001	<0.001	
NPK rates (categorical)	<0.001	<0.001	
Biochar dosages X NPK rates	0.02	<0.001	
c. Acid stress experiment	i) Soil pH content	ii) Maize dry biomass (g)	iii) P-AL (mg kg^{-1})
Biochar dosages	<0.001	<0.001	<0.001
Lime rate	<0.001	0.8	<0.001
Biochar dosages X lime rate	0.21	0.2	<0.001

Table 4
Cations and anions concentrations adsorbed in PRS™ probes (μg 10 cm^{-2} 14 d^{-1}); mean ± SD, n = 3. Average PRS™ probes adsorbed nutrients (cations and anions) were analyzed through one way ANOVA (*levels* = 8, n = 3, *N* = 24) with subsequent post hoc Tukey test (*P* = 0.05). Different letters inside the parenthesis indicates significant differences (*P* < 0.05) between the various treatments (independent variable) on the adsorbed nutrient parameters illustrated in each column (response dependent variable). NH_4^+ supply rates not shown in the Table as these were very low.

Treatments	NO_3^-	Ca^{2+}	Al^{3+}	Mg^{2+}	K^+	PO_4^{3-}	Ca/Al[a]
0% BC + 1/3 NPK + lime	96.0 ± 51.0 (a)	703.0 ± 114.0 (a)	9.4 ± 1.2 (a)	51.6 ± 0.5 (a)	23.6 ± 2.5 (a)	1.6 ± 0.6 (a)	49.8 ± 1.8 (c)
2% BC + 1/3 NPK + lime	80.6 ± 27.3 (a)	667.6 ± 320.0 (a)	7.0 ± 1.8 (a)	55.3 ± 14.4 (a)	667.6 ± 215.0 (c)	5.5 ± 2.0 (b)	62.8 ± 19.3 (c)
0% BC + full NPK + lime	304.6 ± 158.2 (b)	1350.3 ± 386.0 (a)	30.5 ± 16.5 (ab)	103.0 ± 44.5 (ab)	41.6 ± 10.9 (a)	1.2 ± 0.4 (a)	32.2 ± 9.0 (b)
0.5% BC + full NPK + lime	636.0 ± 131.5 (bc)	2401.0 ± 644.8 (b)	54.5 ± 16.8 (bc)	223.5 ± 17.6 (bc)	155.5 ± 28.9 (b)	3.1 ± 0.3 (b)	32.3 ± 17.7 (bc)
2% BC + full NPK + lime	783.0 ± 257.7 (bc)	2259.0 ± 99.5 (b)	25.0 ± 6.7 (b)	283.3 ± 29.8 (c)	387.3 ± 154.2 (bc)	3.5 ± 3.2 (ab)	63.8 ± 18.6 (c)
0% BC + full NPK + no lime	700 ± 251.4 (bc)	882.6 ± 135.0 (a)	52.0 ± 5.0 (bc)	154.0 ± 30.3 (b)	113.0 ± 31.0 (b)	1.6 ± 0.5 (a)	11.3 ± 0.9 (a)
0.5% BC + full NPK + no lime	620.6 ± 144.8 (bc)	944.0 ± 297.3 (a)	62.7 ± 11.2 (c)	171.3 ± 67.3 (b)	329.0 ± 21.1 (c)	2.7 ± 0.1 (b)	9.8 ± 1.4 (a)
2% BC + full NPK + no lime	344.0 ± 129.6 (b)	625.6 ± 166.3 (a)	20.5 ± 13.5 (ab)	121.3 ± 37.8 (ab)	1002.0 ± 56.6 (d)	5.0 ± 2.1 (b)	23.7 ± 7.2 (b)

[a] Presented in molar ratio.

Biochar changes the soil surface albedo (Verheijen et al., 2013), which may result in an increasing variability in soil moisture. However, in controlled greenhouse conditions with less intense lighting conditions, this effect may be missed (Zhang et al., 2013), somewhat decreasing study relevance but increasing the possibilities to study the direct effects of changes in soil chemistry and soil moisture on plant growth.

Increased K^+ and P-AL supply upon biochar addition, through the 22% ash fraction of the biochar, were probably the main nutrient factors responsible for increased biomass production in this soil. A significant positive relationship between maize biomass production and K supply rates (Supplementary Fig. 4d), combined with previous observations that K is the main nutrient added by the addition of biochar (Martinsen et al., 2014), indicated that the K addition via biochar contributed to the alleviation of nutrient stress by biochar. A recent study by Gautam et al. (2017) reported increased K^+ availability upon biochar addition (5 t ha^{-1}) in silty loam Nepalese soil, the main mechanism being high content of K in biochar ash as well as reduced K leaching (Laird et al., 2010). A similar positive trend was observed between P-AL and biomass production (Supplementary Figs. 5, 6), probably due to increased P-AL, where biochar addition increased P-AL from 6 mg kg^{-1} up to a level of 70 mg kg^{-1} (Supplementary Table 2), within the range of 50–70 mg kg^{-1} required for optimal crop growth (Krogstad

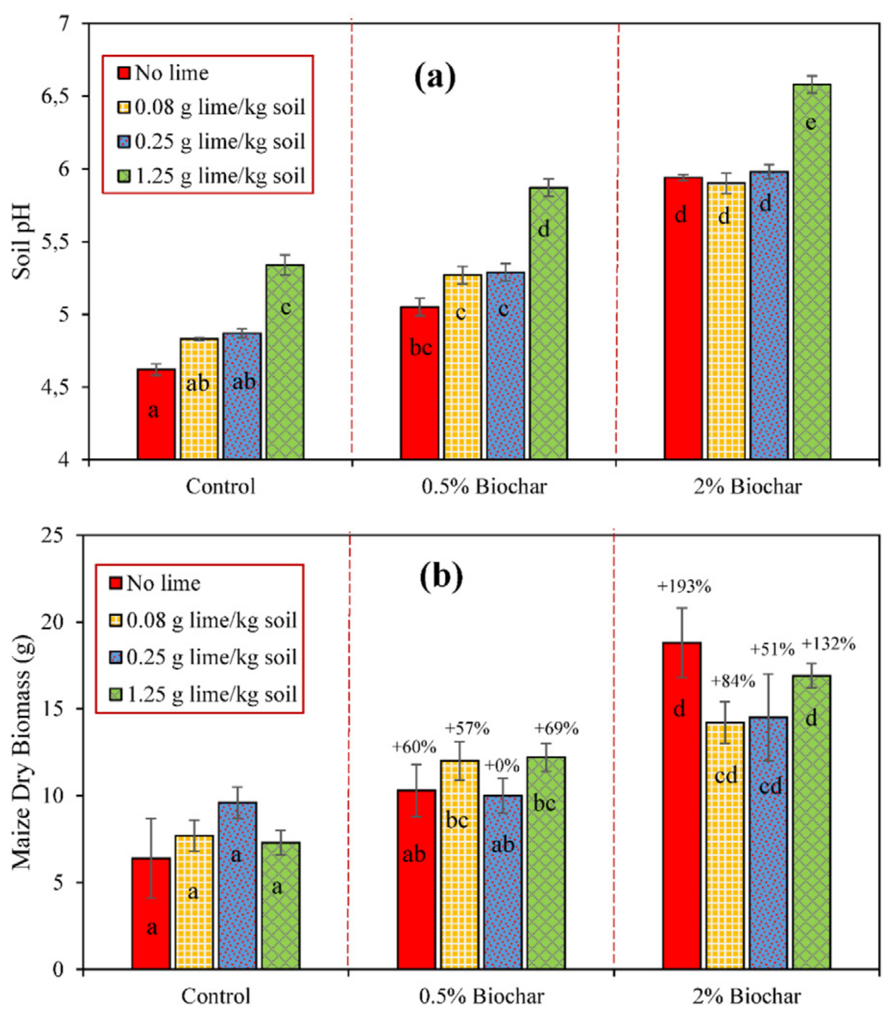

Fig. 3. Effect of the combination of biochar dosages and liming rates on average soil pH (Fig a; mean ± SE, n = 11) and maize biomass production (Fig b; mean ± SE, n = 4). Different letters inside a bar of each treatment represents significant differences between various treatments following two way ANOVA (post hoc-Tukey test, *P* < 0.05). The percentage values above the bars (Fig b) denote the relative change in dry biomass production in the presence of biochar, as compared to the control receiving no biochar.

et al., 2008). Increased P-AL in P-poor soils was reported upon biochar addition, resulting in crop production improvements (Asai et al., 2009).

Nutrient use efficiencies (NUE) were improved by biochar addition at all nutrient dosages; NUE for N was 10–15% without biochar and 30–45% with biochar (assuming the same N content of maize biomass (Martinsen et al., 2014) in the presence and absence of biochar). NUEs for P and K were 6–9% and 21–31%, respectively, in the absence of biochar, and 18–27% and 60–90%, respectively, in the presence of biochar.

A recent study from Jeffery et al. (2017) showed that biochar addition increased crop yield significantly in low fertility soils, highlighting the role of biochar in nutrient stress alleviation. However, in our study, biochar was still effective in the absence of nutrient stress, at highest NPK (132% increase) (Fig. 2b). Thus, the biochar addition in combination with NPK rate had supplementary effects on maize biomass in addition to nutrient retention/addition - probably due to other improved soil physicochemical or biological parameters.

This is the first mechanistic study to investigate the effect of biochar in alleviating some of the most important physical-chemical soil constraints (water stress, nutrient stress and acid stress) by studying the parameters one by one under controlled conditions, under maize plantation in a moderately acidic silty loam Nepalese soil.

In addition to soil moisture and nutrient availability improvements, biological properties of the soil can also be improved by biochar addition. We cannot exclude that beneficial biochar effects on soil (micro) biology, including effects on mycorrhizae, may have contributed to the observed agronomic effects. As the experiments were conducted under controlled greenhouse conditions, any effects related to the effect of biochar on pest resistance could probably be ruled out.

5. Conclusion and recommendations

Soil physicochemical properties such as soil moisture percentage, PAW, in-situ soil nutrient supply rates (PO_4^{3-}, K^+, Ca^{2+}), P-AL, soil pH and CEC were significantly improved upon biochar addition. Increased nutrient availability (K and P-AL) upon biochar addition showed beneficial effect on maize biomass production in this study, thus, alleviating nutrient stress in silty loam soil of Rasuwa, Nepal. The experiment was performed for one soil representative of low-fertility soils. However, maize is more sensitive to drought and nutrient conditions and quite tolerant to low pH conditions than other crops, thus, the results found for maize plant might not be fully representative for other plants. Repetition of the experimental design is recommended for various soils with various limiting factors for crop growth, as well as for various biochar and crop types. In addition, mechanistic field trials similar to the ones carried out in this greenhouse study are recommended.

Farmers can produce biochar themselves at low cost and labour from *Eupatorium* shrub using flame curtain pyrolysis kilns (Schmidt et al., 2015). This pest can be turned into a resource by making biochar to improve soil fertility. This will be of practical importance to identify the potential role of biochar towards sustainable, nutrient efficient agriculture, under rain-fed conditions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.01.022.

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